Protein ligation: selenocysteine

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PROTEIN LIGATION: SELENOCYSTEINE

by

Komal Shah

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Selenium is present in human and animal body in the form of selenocysteine and selenomethionine. It is known as analogue of cysteine which contains selenium instead of sulphur. Selenocysteine which is found in many enzyme and proteins, majorly takes part into redox reaction and is more reactive than cysteine as proved from their chemical properties. This thesis includes Synthetic approach for the introduction of Sec into peptides as an alternative to recombinant methods due to the complications associated with decoding the UGA stop codon. The formation of amide bonds has been described using native chemical ligation. Further, the participation of Selenopeptides featuring an internal or C-terminal Sec residue in a transmidation reaction as well as in metathesis has been analysed. A comparative analysis of selenocysteine into peptide chain as compared to cysteine for molecular imaging has been undertaken. The application of Selenomethionine has been further discussed. Lastly, necessity of selenium in human body has been emphasised for the prevention of production of malign cell.
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CHAPTER: 1 INTRODUCTION

In 1817 Swedish chemist Berzelius discovered the basic element known as Selenium. In periodic table it is belongs to group VIA which includes oxygen, sulphur and tellurium. They all are known as chalcogen elements. The Chalcogen elements oxygen sulphur and selenium are found to be essential part of functional groups of amino acids which in related biomolecules play a unique role in terms of chemistry and structure. There is no similar structure and biological functions have been found for heavier chalcogen element i.e tellurium. Sulphur is found in various oxidation state compered to oxygen and this is the reason for its lower electronegativity and greater polarizability [1-4]. In which selenium & sulphur shares same basic chemical properties. i.e both have same major oxidation states. It has been found that selenium has six stable isotopes and a number of radionuclides with different characteristics.

Even though they could accomplish a reputedly endless variety of chemical and organic tasks, protein includes extremely limited quantity of factors. Composed mainly of carbon, hydrogen, nitrogen and oxygen with occasional incidence of sulphur, proteins depend upon facet chain diversity to generate their myriad systems and features. Selenium was first considered as a poisonous element, the attention paid to this chemical element because it shows important role in life science especially in human health as an essential trace element [5].

Selenium plays imported role in the biological system among all of them major roles are, In some plants selenium may accumulated in preference to sulphur in the form of organ selenium compound which prevents plants to being destroyed by animals, selenium is found in the
synthesis of protein specially incorporated into certain tRNA species which is basis of its essentiality for many organisms including humans.

Mainly selenium is present as a selenocysteine which is 21st proteinogenic amino acid [6-9]. Recent studies on the protein reported total 25 selenoproteins yet the biological functions of many of these protein remains unclear or poorly studied [1-4]. Selenium is found in amino acids mostly as selenocysteine (Sec) and selenomethionine (SeMet)[10]. Selenium is best known for its role as an antioxidant agent attributed to its presence in proteins as the 21st amino acid selenocysteine encoded by UGA codon [11,12]. Selenoproteins contains selenium element. Proteins containing selenium are acts as oxireductases that prevent damage to cellular components, repair this damage, and regulate redox state of proteins. Such proteins are incorporated in prevention of various disease [13, 14]. The discovery that selenium is also found in protein[15] because the amino acid selenocysteine[16] has introduced each challenges and possibilities to observe of natural and unnatural proteins. Possibly one of the greatest challenges is the mystery surrounding the human selenoproteins,[17] as a minimum half of of which have not but been fully characterised. However, as a digital cousin of cysteine (Cys, C), sec also offers scientist unheard of equipment to adjust proteins that do not incorporate the residue evidently. [18] Noteworthy, recombinant techniques to express selenocysteine-containing proteins have been described. [19,20] Likewise, the chemical synthesis of selenopeptides have been thoroughly explored by means of incorporating commercially available Sec and SeMet residues. [21–23] Sec was particularly used as a convenient synthetic platform for native chemical ligation.[24–29]
Studies on the protein in last few years by scientist showed that human selenoproteome consist of 25 selenoproteins [30] of which 18 have been describe so far. Among them the most studied proteins are glutathione peroxidase, glycine reductase, thioredoxin reductase and iodothyronine 5′-deiodinase.[31,32] Generally all the selenocysteine is replaced by cysteine residue and selenoproteins are good catalyst when it compared to sulphur protein[30]. Sulphur proteins are weaker catalyst compared to selenoproteins because selenol group (pKₐ = 5.2) having higher acidity compared to thiol group (pKₐ = 8.3) [33,34]. It has been found that selenols are present as selenolates at physiological pH in proteins and selenolates are better nucleophile than thiolates. In protein selenocysteine is observed in its reactive form i.e selenol and selenenic acid [3]. Many sulphur compounds have selenium analogs i.e disulphide (Diselenide), sulphite (selenite), Sulfide (selenide), methylated sulphur compound (methylated selemiu, analogs), sulfenic acid (selenenic acid), and so on. Sulphur is easily reacts with the selenium and form selenylsulfide bond. An activated selenium species i.e seleno (mono)phosphate is require for the incorporation of selenium into macromolecules[35]. Replacement of sulphur atom of the 2-thiouridine in to selenium is caused by the seleno (mono)phosphate and it also functions in the biosynthesis of selenocystesyltRNA⁰° from its precursor serl-tRNA⁰°. selenocysteine is also incorporated in the ribosome to determine by UGA codon and an mRNA structure.

Which in bacteria is located at the immediately 3′ side of the UGA codon. In Archaea and Eukarya, SECIS is positioned outside the reading frame in the 3′ untranslated region. In bacterial selenoprotein synthesis, the SECIS element is the target for the binding of a specific translation factor, SelB, which also interacts with selenocysteyl-tRNAsec.
Selenocysteine is incorporated in stop codon i.e UGA codon. This highly regulated process requires multiple components, including characteristics mRNA stem loop structure called selenocysteine insertion sequence (SECIS) element, a dedicated SEC-specific elongation factor (EFSec), a unique tRNA (tRNAsec), SECIS-binding protein 2 (SBP2) and other factors e.g. eukaryotes to guarantee translation fidelity[36-38]. For these reasons it is difficult to prepare sufficient amount of selenoproteins in the homogeneous forms using traditional recombinant expression systems, despite recent development in this field [39-42].

Moreover many of the Sec-to-cys mutantas exhibited decreased catalytic activity by up three orders of magnitude [43-45]. To this end in order to understand the function of natural selenoproteins such as human selenoprotein M (SELM) and selenoprotein W (SEMW) studies on their sec containing forms are essential [46].

UGA, stop codon dictated sec insertion into protein which requires the presence of conserved stem-loop structure, known as the Sec insertion sequence (SECIS) element [47]. A recent discovery of a new feature in the genetic code arise from the analysis of the coding and recoding potential of the UGA codon in various organism. UGA codes for both cysteine and Sec depending on the presence and availability of the SECIS element and on the location of UGA within selenoprotein mRNAs [48]. These evolution of amino acids raises question that one codon can code for multiple amino acids at internal position of proteins. It has been also suggest that Sec may not be inserted at any position of protein but it may inserted at specific sites of protein, depending on the availability of the SECIS element within the overall mRNA structure for translation machinery.
The highly variable sets of selenoproteins observed in the Eukaryotes. Selenoproteins in the higher plants and fungi vary from zero to 30 and more than 30 is present in some fishes and algae.

Eukaryotic and prokaryotic selenoproteomes only partially overlap. Selenoproteomes of closely related species are generally similar to each other, but at larger evolutionary distances selenoprotein use becomes sporadic wherein some selenoproteins were lost numerous times in various phyla, and some evolved only in small sets of organisms. The mosaic occurrence of selenoproteins in eukaryotes, as opposed to limited or uniform use of these proteins, provides opportunities for linking patterns of selenoproteome occurrence and composition to environmental and other factors that shape the evolution of eukaryotic selenoproteomes [49].

Selenium is a trace element which is essential for mammals and low molecular weight selenium compounds are primarily present in the human body in form of selenocysteine (sulphur replaced by selenium in cysteine) and selenomethionine [3]. Human body and mammals receive selenium from vegetables grown in selenium rich soil because plant store the elements in the form of low molecular weight methylated selenium compound as a detoxifying mechanism[50].

Many definitely excellent opinions were published on the effect of selenium and Sec in natural systems. If the reader is interested by expertise extra about the function of Se in health and disorder, we refer them to this works [51-53]. Deficiency of selenium causes disease in the human body. Such disease observed In the parts of the world where selenium levels in the soil are unusually low. One of the example of this disease is reported in the Keshan district of china where insufficient levels of selenium linked with cardiomyopathy [53]. Selenium deficiency cause
many disease like viral infection, thyroid function, reproduction dysfunction, mood disorder and cardiovascular disease [54].

The National Academy of the sciences, USA recommended daily intake of 55ug selenium for human body. Selenium is also incorporated in other pharmacological effects like protection against inflammation and cancer prevention. One reported clinical trial with 200ug selenium/day showed a remarkable decrease in cancer [55]. This study is currently followed up with the large SELECT (selenium and Vitamin E cancer prevention trial) study [56]. stimulated investigation on the use of selenium for the therapeutic uses. Methyl selenic acid and methyl selenocysteine are low molecular weight compounds of selenium which is found as antitumorigenic agent in vitro as well as in vivo in animals [57]. Selenium is present in human proteome in form of selenoprotein which is incorporated in many physiological role [58]. In human body selenocysteine considered as the twenty first amino acid being coded by the UGA codon [59].

Its presence in natural proteins indicates that Se may be introduced into proteins artificially with minimal disruption, while its relative scarcity lends it to selective amendment [60]. On account that its discovery in proteins over 40 years ago, selenium and Sec had been recruited as gear in protein folding handles for nucleophilic amendment, precursor for creation of mimics of put up-translational amendment, objectives for codon reasignment, gateways to the ligation of unprotected peptides at various amino acids and home windows to information enzyme feature. The take a look at of selenium chemistry in natural and unnatural structures keeps to offer the chemist with approaches to adjust and apprehend target proteins.

Nonetheless regardless of these similarities however clearly difference between the 2 factors and substitution for one more results in compound with particularly numerous chemical
features. The pKa value of selenium and sulphur shows that H2se is much stronger acid than H2S. One imported application of the selenium is recently reported in protein synthesis. While it is not involving selenoproteins but employing the reactivity of selenium, is the selenium dependent activation of carbohydrates enabling a chemical methods for protein gluconylation.[35]
CHAPTER: 2 Diversified NCL of Selenocysteine

Sulphur atom of cysteine is replaced by Se known as Selenocysteine which is found in the naturally occurring amino acid. Most of the selenocysteine shows participation in redox reactions i.e. as an antioxidant property. These proteins are essential as they are take part in so many biological processes such as the control of cellular redox balance.

Synthetic approach for the introduction of Sec into peptides is an alternative to recombinant methods due to the complications associated with decoding the UGA stop codon as shown in the introduction. A several synthetic peptides and proteins have been prepared by SPPS that incorporated selenocysteine by using Boc- or Fmoc-Sec (PMB)-OH.[61-66] Kent and coworkers34 developed a technique by use of NCL and they were success to introduce free selenol (or diselenide) into a peptide permits entry into larger peptides/ proteins.

TYPE-1

In the native chemical ligation of selenocysteine, two ligation positions in amino acid derivative, one a C-terminal peptide thioester and the other a peptide containing an unprotected Sec/selenocystine at its N terminus, are mixed together along with a reducing agent. After an initial Trans selenoesterification, a selenoester 1 is formed (Scheme1).
The rearrangement of an intermediate takes place via Se-to-N acyl shift to give the thermodynamically more stable native peptide bond. Studies on several recent model have shown the feasibility of this process. Several number of Sec containing analogues of the C terminus of ribonucleotidereuctase (RNR) were synthesized by using NCL of selenocysteine. [67]

RNR having four cysteines which are active in the redox reactions. Two cysteines in the active site provide the two electrons required to reduce ribonucleotides to deoxyribonucleotides. [68]

The disulfide generated in the active site is reduced back to the active form of the protein by dithiol/disulfide interchange with two Cys residues near the C terminus (Scheme 2).
These C terminal cysteines shuttle reducing equivalents from thioredoxin into the active site of RNR. The proposed disulphide intermediate 2 in which contain one of the activesite thiols and one of the C terminal thiols has never been observed, presumably because it is kinetically invisible.\cite{69} as we know sulphur and selenium possess significant difference in redox potential, replacement of one of the C-terminal cysteines with selenocysteine may allow the detection of the selenosulfide analogue of 2. On basis of above NCL of selenocysteine many studies still going
on developing selenocysteinemediated native chemical ligations with applications in RNR in mind.

The purified Sec-containing peptides were synthesized in high yields by Fmoc-based SPPS with the Sec(PMB) monomer, and the thioester coupling partner was prepared by SPPS by using 2chlorotriphenylmethyl resin.[70] At a concentration of 3mM, the reaction of the Sec-containing peptides was slower than most literature reports for ligations with cysteine even though the latter are typically performed at lower concentrations.[71] These informations are consistent with the previous observation that diselenides and selenosulfides are thermodynamically more stable than disulfides. Hence, with only the weakly reducing thiophenol present to reduce the diselenide/selenosulfides only a small equilibrium concentration of a reactive species is produced. Raines and co-workers showed that once a free selenocysteine is is incorporated, in the native chemical ligation itself actually proceeds much faster than with cysteine (Scheme 3).[72]

Glycine p-nitrophenylthioester was treated with either cystine or selenocystine in the presence of TCEP, which is a stronger and irreversible reductant. The observed rate of formation of
p-nitrophenylthiolate formation was higher with selenocysteine than with cysteine over a pH range of 5±8. At pH 5.0, for example, the selenocysteine reaction was 103 times faster than the cysteine, a fact consistent with difference between pKa of sulphides and selenols in which selenol consist lower pKa values.

Hilvert and co-workers have also studied Sec-mediated native chemical ligation in the synthesis of bovine pancreatic trypsin inhibitor (BPTI),[73] which contains six Cys residues and three disulfide bonds. Synthetic BPTI1±37 and C38UBPTI38±58 fragments were ligated in the presence of TCEP to yield C38U-BPTI, which was subsequently folded to its native structure. The mutant exhibited properties that were very similar to the wild-type protein including its stoichiometric inhibition of trypsin.

**Synthesis of dihydropeptides and their use in chemoselective ligation:**

In many natural products dihydroalanine is found and can impart interesting biological activities on polypeptides. [74, 75] Additionally, dehydroamino acids can serve as very useful precursors to peptide bond formation. Dehydroalanine-containing peptides have been prepared by various methods. The most common method is the activation and elimination of serine residues or Hofmann elimination of 2,3-diaminopropionic acid. [76±78] These methods, however, preclude the presence of other unprotected serine or threonine residues in the former case or lysine residues in the latter case. A more Adaptable approach reported recently is the pyrolytic or basic elimination of cysteine sulfoxides. [79] The drawback of this method is its incompatibility with protected cys residues.[80]
An another easy, site-specific and chemoselective method for introducing dehydroalanine into globally deprotected peptides employs Se-phenylselenocysteine. This amino acid can be accessibly incorporated into peptides by using standard SPPS (see Section 2.2.), and after cleavage from the resin the Sec derivative can be converted into Dha through a mild, chemoselective oxidation with either hydrogen peroxide or sodium periodate.[81]

The mild oxidative elimination introduces an electrophilic precursor into unprotected peptides (for example, 3 in Scheme 4), which has been exploited to prepare a number of cyclic thioether peptides called lanthionines.[81] These lanthionines are the important structural motif found in lantibiotics, a class of post translationally modified peptide antibiotics.[82] Due to intramolecular biomimetic Michael addition formation of single diastereomers takes place by stereoselective protonation of the enolate intermediates.

Bradley and co-workers reported similar stereoselective cyclizations and assigned the stereochemistry of the products as the natural meso-lanthionines by using NMR spectroscopy methods.[79] More recently, Studies on this investigated biomimetic cyclizations with dehydrobutyrine-containing peptides obtained by oxidative elimination of (2R,3S)-3-methyl-Se-phenylselenocysteine residues (for example, 4 in Scheme 4). Of the four possible stereoisomers that could be formed in the Michael addition to form the bring of the lantibiotic subtilin, only the natural (2S,3S,6R)-methyllanthionine was obtained; this demonstrates that, in the context of a constrained peptide, the local chiral environment can bias the stereoselectivity of these Michael additions.[83]
Scheme 4. Biomimetic formation of meso-lanthionines and methyllanthionines by stereoselective intramolecular cyclization.
To demonstrate the use of dehydroalanines, a heptapeptide (5) corresponding to the C terminus of N-Ras was synthesized with Sec (Ph) installed at the site of a Cys residue (Scheme 5).

Since the same dehydroalanine precursor was used in the preparation of a variety of analogues, this method can also be a useful technique for the synthesis of glycopeptides. These structures are particularly suited for convergent ligation strategies since they are not readily prepared by either traditional synthetic or recombinant techniques. Furthermore, the well-known heterogeneity caused by the presence of various isoforms of glycoproteins hampers structure-reactivity studies. Compared with the various non-native linkages that have been used to prepare O-linked glycopeptide mimics through chemoselective ligations, [84-86] the isosteric S-linked conjugates...
are perhaps the closest analogues. Another interesting feature of these structures is their reported higher chemical stability compared with their O-linked counterparts.[87] The potential of Michael additions to dehydroalanines for the assembly of S-linked glycopeptides was estimate with a series of tripeptides and 1thiosugars (Table1).[88]
<table>
<thead>
<tr>
<th>Entry</th>
<th>aa(^1)</th>
<th>aa(^2)</th>
<th>RSH</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ala</td>
<td>Ala-OtBu</td>
<td>![RSH structure]</td>
<td>62</td>
</tr>
<tr>
<td>2.</td>
<td>Ala</td>
<td>Ala-OtBu</td>
<td>![RSH structure]</td>
<td>62</td>
</tr>
<tr>
<td>3.</td>
<td>Ala</td>
<td>Ala-OtBu</td>
<td>![RSH structure]</td>
<td>74</td>
</tr>
<tr>
<td>4.</td>
<td>Pro</td>
<td>Val-OtBu</td>
<td>![RSH structure]</td>
<td>88</td>
</tr>
<tr>
<td>5.</td>
<td>Pro</td>
<td>Val-OtBu</td>
<td>![RSH structure]</td>
<td>91</td>
</tr>
<tr>
<td>6.</td>
<td>Gly</td>
<td>Gly-OtBu</td>
<td>![RSH structure]</td>
<td>79</td>
</tr>
</tbody>
</table>

Table-1
Protected or unprotected -1-thiosugars resulted in the generation of exclusively linked glycopeptides in good % of yields (entries 1±5). Use of 1-thioN-acetyl--D-galactose afforded the desired -linked glycopeptide, which is an important similar of the core structure of O-linked glycoproteins (entry6). Importantly, no special protecting group used for Fmoc-based SPPS is required for either the generation of the dehydroalanine precursor or the Michael addition. Moreover, all steps used to synthesized and derivatize dehydroalanines are compatible with solid-phase chemistry. For instance, resin-bound peptide 8 was synthesized by Fmoc-based SPPS, followed by oxidative elimination, and Michael addition with unprotected thioglucose moiety (Scheme 6).

Scheme 6
Subsequent cleavage from the resin, and HPLC purification provided glycoconjugate 9 in 45% yield based on the loading of the Wang resin.[88]

The major advantage of this methodology involves the defined stereochemical integrity at the anomeric center and the accessibility of both and anomers. However, an obvious current drawback is the lack of diastereoselectivity at the peptidic carbon atom that provides two diastereomers, even though these are readily separated by HPLC. The lack of substrate- or reagent-controlled stereoselectivity is undoubtedly due to the inherently fast rate of protonation of the enolate intermediate in protic solvents. Consequently, to overcome this impediment, the barrier for the stereodetermining step must be raised. One potential avenue to accomplish this would be to exploit radical conjugate additions. Owing to the high bond dissociation energy of O-H bonds, protic solvents are poor hydrogen-atom donors whereas the thio sugars are much better reductants. The energies of the diastereomeric transition states for hydrogen-atom transfer generating either the L- or D-configuration at the carbon atom may therefore be sufficiently different that selectivity can be achieved. In fact, Kessler and coworkers have shown the utility of dehydroalanines for addition of anomeric glycosyl radicals providing C-linked glycopeptides.[89]

Furthermore, the feasibility of stereoselective radical additions to dehydroalanines has been demonstrated in a nonpeptidic context.[90±92] Efforts are currently underway in our laboratory to combine these approaches for the development of a chemo- and stereoselective radical ligation. An alternative approach that would assure formation of only one isomer at the position features the ligation of 1-thiosugars to cyclic sulfamidates derived from L-serine. The feasibility
of this strategy has been shown at the amino acid level[93] and more recently with small peptides.[94]

**TYPE-2 Rapid Additive-Free Selenocystine–Selenoester Peptide Ligation**

The formation of amide bonds is one of the most important synthetic transformations. While various reagents and methods have been developed and researched for amide synthesis within small molecules, large polypeptides and proteins are most commonly formed by use of native chemical ligation methodology.[95,96] This reaction takes place between a peptide bearing an N-terminal cysteine (Cys) residue and a peptide functionalized as a C-terminal thioester (Scheme 1) and, mechanistically, proceeds through an initial intermediate step transthioesterification (facilitated by the nucleophilic Cys thiol) to covalently join the two fragments, followed by a fast intramolecular S→N acyl transfer to generate the native peptide bond. Usually a large excess of a thiol additive is required to generate a reactive thioester from less reactive alkyl thioester precursors,[97,98] and reactions are normally supplemented with an additional reductant to prevent disulfide bond formation.

Scheme 1
To increase the repertoire of this era to amino acid residues aside from Cys, current efforts have focussed on thiol-derived amino acids [99] for the various class of peptides and proteins via ligation–desulfurization chemistry (Scheme 1).[100-104] While Native chemical ligation and the related ligation–desulfurization technologies have revolutionized artificial protein chemistry, [105] the strategies suffer from shortcomings: 1) ligation charges at sterically hindered C-terminal thioesters are very gradual, main to extended reaction time (>48 h) [106] and, therefore, tremendous thioester hydrolysis, and 2) desulfurization reactions are incompatible with Cys residues because it cause desulfurization of all the cysteine molecule present in the sequence of amino acids.

To deal with these boundaries, ligations among selenocysteine (Sec) [108-110] or selenol-derived amino acids [111,112] and thioesters through a native chemical ligation pathway have been explored (Scheme 1). [113] Owing to the low redox capacity of Sec (−381 mV),[114] selenopeptides exist as the corresponding diselenide dimers below standard situations and do no longer take part in ligation chemistry inside the absence of an external reductant. [109] Aryl thiol catalysts are generally added for reduction of the diselenide to the corresponding selenol. [108,109,115] Despite the improved nucleophilicity of selenols relative to thiols, the weak reductive efficiency of aryl thiols leads to a low steady-state concentration of selenol which frequently slows the fee of Sec ligations in comparison with Cys. [108,112] Unfortunately, using more potent reducing reagents, which includes phosphines, promotes homolysis of the susceptible C–Se bond of Sec, a change that has been exploited for the chemoselective deselenization of Sec to Ala in the presence of free Cys.[113,115] Rates of native chemical ligation at Cys may be better by way of altering the acyl donor, particularly thru the use of alkyl
selenoesters [116] in vicinity of thioesters. Therefore studies on this reasoned that if the improved nucleophilicity of Sec might be effectively harnessed and blended with the enhanced electrophilicity of a selenoester acyl donor, the speed of ligation should be dramatically improved. Somewhat exceedingly this reaction has no longer been explored so far. To avoid the undesired phosphine-mediated deselenization pathway, scientist found research alternative chemical and electrochemical methodologies for the resduction of the diselenide to ‘liberate’ its latent reactivity. During the discover of such investigations, it was fascinated to observe that an experiment involving peptide dimer 1 bearing an N-terminal selenocystine [(Sec)2] moiety and peptide 2 containing a C-terminal Ala phenylselenoester in denaturing buffer at pH 7.0 formation corresponding diselenide 3 takes place as the major product (together with 10% of unsymmetrical diselenide 4, Scheme 2). For understanding, this modification represents remarkable reactivity and, remarkably, proceeds at room temperature in much less than 60 s without thiol or reductive additives (see Scheme 2B–2C). The reaction also proceeded at concentrations as low as 250 μM of one (reaching completion in 60 min, see Supporting Information).

Scheme 2

Additive free ligation:
6M Gdn-HCl, 0.1M Na2HPO4
pH 7.0, 25°C, 60 s
72% isolated (3+4)

Scheme 2
Based on these discrete computational predictions, it could be found that the impact of electron density in the aryl ring of the selenoester on the rate of peptide ligation. Indeed, solubility of the aryl diselenide, in place of electronics, remained the most outstanding predictor of selenoester reactivity. As such, in spite of some of additional experimental and computational studies, presently unable to distinguish between the two pathways and, moreover, cannot definitively rule out the opportunity of an alternative, associative mechanism, wherein neither intermediate A nor B are involved (see Supporting Information for details). Further mechanistic exploration of the additive-free ligation platform will therefore be the issue of future research work on it. Scheme 3

1. Additive free ligation: 6M Gdn-HCl
   0.1 M Na₂HPO₄, pH 6.2, 5 min
2. DPDS removal: hexane extraction
3. Deselenization: TCEP (50 eq.)
   DTT (5 eq.), pH 5.0, 16h
4. Folding: dialysis into 50mM tris
   0.1 M NaCl, pH 7.5, 16h 4 C

Scheme 3
Having very well explored the scope of the additive-free ligation on a number of model systems and interrogated the mechanism of the reaction, An interest of developers subsequent moved to assess the performance of the reaction for the chemical synthesis of proteins. They've first targeted an enzyme, specifically the intracellular chorismate mutase (CM) from Mycobacterium tuberculosis (Mtb) 10, the etiological agent of TB (Scheme 5A). This 83 residue enzyme is accountable for the conversion of chorismate to prephenate through a Claisen rearrangement (see Supporting Information), and is a important enzyme en path to fragrant amino acid synthesis in Mtb.33 Reaction of Mtb CM 1–forty phenylselenoester (eleven) and Mtb CM 41–83 bearing an N-terminal (Sec)2 moiety (12) beneath the additive-free ligation situations proceeded to of entirety in 5 min to completely afford the symmetrical diselenide, which following in situ deselenization, supplied full duration Mtb CM with great crude purity. [117]

**TYPE-3 Selenopeptide transmidation and metathesis**

**(A) TRANSMIDATION**

There were several researches done and also are going on the selenopeptides which are not fully explored about their features [118]. Scientist from the university of Lille Nord de France reported important class of biomolecules by showing that selenopeptides featuring an internal or C-terminal Sec residue can participate in a transmidation reaction (scheme-1). Transmidation of amide requires very difficult conditions (>250 °C) Or metal as a catalyst in organic solvent to occur due to the high stability of the amide bond [119]. Selenoproteins shows exactly same reactions with exactly opposite conditions i.e. it shows transmidation and metathesis at 37.c and in reaction takes place in water at pH-5.5 in presence of 4mercaptophenylacetic acid (MPPA) [120].
Scheme 1. Transmidation or Metathesis of Selenopeptides

(A) Transmidation

peptide 1 $\rightarrow$ peptide 2

selenopeptide I $\rightleftharpoons$ transient selenoester II

transient arylothioester III $\rightarrow$ transient thioester V

peptide 3 $\rightarrow$ peptide 1

S,N-acyl shift

peptide VI $\rightarrow$ peptide 2

peptide 3 + peptide 2 $\rightarrow$ peptide 7

MPPA
Previous reports have validated the metathesis of peptidomimetics featuring a reversible thioester bond [121–123] or a very few me ago an α-aminoacyl N-alkylcysteine [142] reversible latent thioester.

Reversible covalent bonds have a best potential in dynamic covalent chemistry (DCC) [124-126]. Nathalie and co-workers reported the field of DCC can be potentially extended to native peptide structures by exploiting the reversibility of the peptide bond to selenocysteine in aqueous media.

The first steps of the transmidation reaction illustrated in Scheme 1 proceed through reverse native chemical ligation (NCL).[127] in this step N,Se-acyl migration generates transient selenoester II intermediate [128] which further reacts with MPAA and the cysteinyl peptide to ultimately produced transient thiooester V.

A corresponding N, S acyl shift of peptides Proposing c terminal Cys residue and the subsequent replacement of the transient thioester by an excess of an alkylthio [129] or hydrazine [130] has been shown to occur under forcing conditions. An interesting results obtained for the displacement of c-terminal residue by an excess of an alkylthiol could be performed under very
mild conditions (40 °C).[131] This is because of that incubating the selenopeptides in water in the presence of MPAA would permit the formation of the transient arylthioesters of variety III (Scheme 1), which are identified to be a lot better acyl donor accessories in the NCL response than alkylthioesters.[132] Once produced, the transient thioester V was expected to rearrange spontaneously into peptide VI by means of an S,Nacyl shift mechanism. The last step is poorly reversible in water at 37 °C at mildly acidic pH and for this reason drives the reaction toward the formation of peptide VI.15 total, the transamidation reaction proven in Scheme 1 proceeds by way of making use of the mild experimental conditions designed for the NCL response.

The selenopeptide 3 was used in their study which is produced by reaction of cyclic disulfide form of bis(2sulfanylethyl)amido (SEA) peptides-1 [133] with selenocystine 2 in the presence of MPAA (200 mM) (Scheme 2).

**Scheme 2. Synthesis Of Model Selenopeptides 3 or 5**

![Scheme 2](image)

a X=Phe; b X=Ser; c X=Thr; d X=Arg; e X=Ala; f X=Glu; g X=Cys; h X=Gly;
Latent thioester must be activated and the Sec component required to perform the reaction in the presence of reducing reagents i.e. disulphide and diselenide. In a first technique, the reaction of peptide 1a (X = Phe) with selenocystine 2 within the presence of tris(2carboxyethyl)phosphine (TCEP, 200 mM) furnished the selenopeptide 3a along with the corresponding deselenized product (Sec → Ala, ∼10% by way of HPLC). The TCEP triggered deselenisation of selenopeptides reminds us to the desulfurization of thiols by phosphites.[134,135] many authors reported that side reactions were observed during the study of selenopeptides in the presence of TCEP [136,137]. Alternatively in NCL reaction of C-terminal peptide thioesters with N-terminal Sec peptides [138,139] generates alanine residue [140,141] The problem of Deselenisation by TCEP solved by using dithiothreitol (DTT, 200 mM).

It is noticed that during the reaction of diselenide 3 or 5 an excess of DTT was found to be gradual and most often incomplete after a number of hours.8 Moreover, the recent use of DTT with reversible tertiary amide thioester surrogates resulted in acyl-DTT thioester and/or Oester side-product formation.[142] Accordingly, using TCEP, a strong irreversible reducing agent for disulfides and diselenides, was predicted as a substitute. During the reaction MPAA inhibit the process but it can’t protect Sec residue against deselenization. A solution to prevent Sec residue is use of selenophosphine derived from TCEP i.e. TCEP=Se.
Metallic Se reacts with the TCEP in one step and produced TCEP=Se in good yield. It also inhibits the desulfurization of Cys residue. Analogue of TCEP=Se with sulphur element i.e. TCEP=S does not shows any inhibitory actions. There is may be many other valuable properties of TCEP=Se exist which have to be investigate in future.

(B) **METATHESIS**
Studies on the NCL of selenocysteine shows the possibility to perform a metathesis reaction using selenopeptides 5e (X = Ala) and 3d (X = Arg) and MPAA as catalyst (Scheme 5). Luckily, the reaction showed the synthesis of two identical selenopeptide products, peptides 5d and 3e (Figure 1a), which coeluted with authentic samples by HPLC. They were identified by extensive mass spectrometry fragmentation analysis. It was observed formation of two other products, i.e., peptides ILKEPVHGA-OH and ILKEPVHGR-OH, arising from the X-Sec peptide bond hydrolysis (peptides 8d,e, Figure 1a). The level of hydrolysis products 8d and 8e remained below 10% after 100 h.
Importantly, the reversibility of the metathesis reaction was showed by performing another metathesis experiment starting from selenopeptides 5d and 3e, which leads to the formation of peptides 5e and 3d (Figure 1b). After \(~95 \text{--} 100\) h, both forward and reverse metathesis reactions showed similar proportions for selenopeptides 3d and 3e but not for peptides 5d and 5e. The equilibrium is probably perturbed by the concomitant hydrolysis of the selenopeptides. The hydrolysis proceeded through C-terminal Sec peptides 3. Unquestionably, peptide 8d was the major hydrolysis product in the first metathesis reaction starting from 5e and 3d (Figure 1a),
whereas peptide 8e was formed preferably in the second metathesis reaction starting from 5d and 3e (Figure 1b). [143]
CHAPTER: 3 Oxidative Deselenization to Serine

The introduction of native chemical ligation,[143] a way for the mild and chemoselective condensation of peptide fragments to generate larger peptides and proteins, has enabled entry to numerous protein goals through chemical synthesis.[144] The methodology entails the response of a peptide bearing an N-terminal Cys residue with a C-terminal peptide thioester in an initial transthioesterification reaction, accompanied with the aid of a rapid S-N acyl shift to generate a native amide bond in aqueous media and at impartial Ph. The transformation takes location within the presence of unprotected amino acid side chains and is tolerant of extra capability, such as the presence of a various array of post-translational modifications. [144,145]

Efforts focused at the extension of native chemical ligation to include choose non-Cys ligation junctions have these days converged on ligation–desulfurization chemistry,[146] wherein an amino acid bearing a reactive thiol auxiliary allows the ligation of peptide fragments, with a next desulfurization generating a native amino acid residue. First validated in the conversion of Cys to alanine (Ala),[147] synthetic get entry to thiol-derived proteinogenic amino acids has lately facilitated the instruction of an expansion of objectives through non-Cys ligation disconnections.

A major drawback of overdue-level global desulfurization protocols is the want to protect native and structurally critical Cys residues within the target collection,[148] which might be concomitantly desulfurized to Ala if left unprotected. The recent studies of peptide ligation at selenocysteine (Sec)[149] coupled with selective deselenization (within the presence of unprotected Cys residues) through treatment with tris-(2carboxyethyl)phosphine (TCEP) and dithiothreitol (DTT),[150] supplied, for the primary time, a completely chemoselective method to
Ala residues at the ligation junction (Scheme 1A). Mechanistically, the selective deselenization is thought to continue via a radical mechanism (thru an alanyl radical intermediate), taking benefit of the not strong nature of the carbon–selenium bond as well as the ability to shape selenium-centered radicals preferentially over sulfur-centered radicals.[150] This key discovery laid the intellectual framework for the development of ligation–deselenization chemistry at unnatural selenol-derived amino acids, which includes at phenylalanine (Phe).[151]

During the various investigations into the chemoselective deselenization of β-selenol Phe-containing peptides, it was observed that the formation of minor hydroxyl byproducts constant with the formation of diastereomeric 34-hydroxy Phe (Scheme 1B).[151,152] Invoking a similar mechanism to that proposed for the chemoselective deselenization of Sec,[150] scientists were presumed that this interesting byproduct resulted from reaction of a benzylic radical with dissolved oxygen within the media. Although an undesirable by way of product in the context of ligation–deselenization chemistry at Phe, we hypothesized that the development of an equal reaction at Sec residues may want to offer a programmed method to access serine (Ser) residues at the ligation junction via a ligation, followed by Sec to Ser transformation (Scheme 1C).
It will have to be noted that different methods for ligation at Ser junctions have lately been explored, including the usage of thiol auxiliaries linked by way of the Ser side-chain,[153] ligation at Ser through the rearrangement of O-acyl isopeptides[154] or direct ligation with activated salicylaldehyde (SAL) esters.[155] Even as these protocols enable ligation, there are boundaries associated with every, namely long reaction time[153] and a reliance on high substrate concentrations in non-aqueous media.[153,154,155] Recently, a system for the conversion of Cys to Ser has been developed by Okamoto and Kajihara.[156] although this is a powerful transformation, the requirement for multiple steps (including intermediary HPLC purifications)
and the use of poisonous reagents (e.g. CNBr)[156] to promote the desired substitution are enormous drawbacks. Concurrent to this work, Bode and colleagues described an robust ligation at Ser by way of application of an oxazetidine amino acid spinoff in ketoacid–hydroxylamine ligation chemistry. [157] Right here latest work describes the progress of an operationally easy Sec to Ser conversion below mild conditions in a single post-ligation transformation. This disclosure is an fundamental addition to the currently to be had instruments for peptide and protein ligation and underscores the designated chemical diversity supplied by means of the strategic incorporation of Sec residues into target peptides and proteins

Having attributed the minor hydroxylation pathway in our b-selenol Phe work (Scheme 1B) to dissolved oxygen in the reaction media, In this reaction preliminary examination of the proposed Sec to Ser conversion began with the treatment of selenopeptides with TCEP in oxygen-saturated buffer (see determine S2, assisting knowledge). Beneath these conditions, studies on such reaction determined the desired transformation in conjunction with Sec to Ala conversion, equivalent to typical deselenization, as good as a number of backbone cleavage by products. Difficulties in optimizing using oxygen gas to preserve targeted manage of stoichiometry brought on the exploration of an substitute oxidant. Ultimately the recent focus for the source of oxygen is Oxone,[158] a commercially available, bench steady and water-soluble oxidant. Importantly, there’s also priority for using Oxone in peptide chemistry and the compatibility of the reagent with proteinogenic amino acid side-chains.[159]
CHAPTER: 4 Future Prospects of Selenocysteine and Selenomethionine

1. Chemical tags for labelling proteins

To build on the last century’s tremendous strides in understanding the workings of individual proteins in the test tube, we now face the challenge of understanding how macromolecular machines, 37isulphide pathways, and other biological networks operate in the complex environment of the living cell. The fluorescent proteins (FPs) revolutionized our ability to study protein function directly in the cell by enabling individual proteins to be selectively labelled through genetic encoding of a fluorescent tag. Although FPs continue to be invaluable tools for cell biology, they show limitations in the face of the increasingly sophisticated dynamic measurements of protein interactions now called for to unravel cellular mechanisms. Therefore, just as chemical methods for selectively 37isulphid proteins in the test tube significantly impacted in vitro biophysics in the last century, chemical tagging technologies are now poised to provide a breakthrough to meet this century’s challenge of understanding protein function in the living cell.

With chemical tags, the protein of our interest is attached to a polypeptide rather than an FP. Then the polypeptide is subsequently modified with an organic fluorophore or another chemical probe. The FlAsH peptide tag was first introduced in 1998. Since then, more refined proteintags, exemplified by the TMP-andSNAP-tag, have improve dselectivity and enabled imaging of intracellular proteins with high signal-to-noise ratios. Further improvement is still required to
achieve direct incorporation of powerful fluorophores, but enzyme-mediated chemical tags show promise for overcoming the difficulty of selectively 38isulphid a short peptide tag.

In this Account, we focus on the development and application of chemical tags for studying protein function within living cells. Thus, in our overview of different chemical tagging strategies and technologies, we emphasize the challenge of rendering the labelling reaction sufficiently selective and the fluorophore probe sufficiently well behaved to image intracellular proteins with high signal-to-noise ratios. We highlight recent applications in which the chemical tags have enabled sophisticated biophysical measurements that would be difficult or even impossible with FPs. Finally, we conclude by looking forward to (i) the development of high-photon-output chemical tags compatible with living cells to enable high-resolution imaging.

Previously, organic fluorophore and other biological probes were used for the site specific labelling of proteins which is also useful for the fundamental studies of proteins in vitro. (160) The chemical probes designed to react selectively with Cys and Lys residues that are so effective at 38isulphid purified proteins in vitro, but they do not provide perfect selectivity to label an individual protein from the large number of other species.

**Protein Labelling via Chemical Tags**

The first report of a chemical surrogate to FPs for 38isulphid proteins with organic fluorophores in living cells was FlAsH from Tsien and co-workers in 1998.[161] In design, FlAsH is the ideal chemical tag, a short 15 amino acid polypeptide tag with a tetra cysteine core (CCXXCC) that is covalently 38isulphi with a fluorogenic bisarsenical fluorescein ligand whose fluorescence increases upon binding to the polypeptide tag (Figure 1a).
This biarsenical fluorescein ligand was proved effective among tetra cysteine for labelling. Recent chemical biological research reported selenocysteine as 21st amino acid. A total function and its presence is various proteins is still unknown. Selenocysteine is proved more reactive than cysteine because it contains selenium rather than sulphur in cysteine. Therefore, this byarsenical tag may react faster with selenocysteine. It is imported to notice when protein contains both selenocysteine and cysteine than this ligand chooses which one of them? This biarsenical ligand contains sulphur in same manner it may be prepared with selenium. Figure 2a
To date, a number of bisarsenical fluorophores and corresponding tetracysteine (TC) tags have been reported,[162 163] biarsenical ligand with selenium may give some useful or different results compared to this ligand along with sulphur. Figure 3a Despite its elegant design, the FlAsH technology suffers practically from nonspecific isulphid of thiol-rich biomolecules in the cell and toxicity of the bisarsenical ligands and 40isulphid conditions.[164] Biarsenical ligand with selenium may do not cause toxic effect of arsenic because selenium is used to prevent toxicity of arsenic. While, thiols cannot prevent arsenic toxicity in this way biarsenical ligand along with selenium is more useful and effective than previously reported ligands.
Remarkably, the Snap-tag now has also been rendered covalent by installing a unique Cys residue on via a nucleophilic addition of sulphide.\textsuperscript{36} Figure 4a as we know from the chemical properties of cysteine and selenocysteine pKa of selenocysteine is more than the cysteine. Which shows that selenocysteine is more nucleophilic than cysteine. Figure 4b This property exist evidence that SNAP tag may be more effective and reactive with selenocysteine.
2. Use of Inorganic Selenium & Selenomethionine for heavy metal Toxicity

Although heavy metals such as cadmium, mercury, platinum, arsenic, thallium and lead may have a physiological role, it is their toxicity which has attracted most attention. Thus cadmium giving rise to itai-itai disease (itai means pain); Thallium is considered a cumulative poison that can cause adverse health effects and degenerative changes in many organs. For example, impaired glutathione metabolism, oxidative stress, and disruption of potassium-regulated homeostasis may play a role. Chronic arsenic toxicity results in multisystem disease. Arsenic is a well-documented human carcinogen affecting numerous organs.

2 (a): Arsenic Toxicity

Chronic exposure to arsenic from groundwater has been recognized to cause the largest environmental health disaster in the world, putting more than 100 million people at risk of
cancer and other arsenic-related diseases[165,166]. Because of its prevalence in the environment, potential for human exposure, and the magnitude and severity of health problems it causes, the United States Agency for Toxic Substances and Disease Registry (ATSDR) has ranked arsenic as No. 1 on its Priority List of Hazardous Substances for many years.[167,168]

The toxicity of trivalent arsenicals likely occurs through the interaction of trivalent arsenic species with sulfhydryl groups in proteins. Arsenic binding to a specific protein could alter the conformation and function of the protein as well as its recruitment of and interaction with other functional proteins. Therefore, there has been much emphasis on studies of arsenic binding to proteins, for the purpose of understanding arsenic toxicity and developing arsenic-based therapeutics.

**CHEMICAL BASIS AND BIOLOGICAL IMPLICATIONS OF ARSENIC BINDING TO PROTEINS**

Trivalent arsenicals have high affinity for sulfhydryl groups and can bind to reduced cysteines in peptides and proteins.[169] Figure 1 illustrates schematically the binding of inorganic arsenite (iAsIII), monomethylarsonous acid (MMAIII), and dimethylarsinous acid (DMAIII) to cysteine residues in proteins.
Figure 1. Binding of inorganic arsenite (iAs\textsuperscript{III}), monomethylarsonous acid (MMA\textsuperscript{III}), and dimethylarsinous acid (DMA\textsuperscript{III}) to cysteines in a protein.

Arsenic binding to a specific protein could alter the conformation of the protein, resulting in loss of its function, and affect its recruitment of and interaction with other proteins.

**Arsenic Biomethylation**

Biomethylation is the major metabolic pathway for inorganic arsenic (iAs) in nearly all organisms, including humans and most animal species.\[171\] Arsenic biomethylation involves specific reductases\[27,30,69–73\] and methyltransferases.\[170-176\] The major methyltransferase responsible for arsenic biomethylation is arsenic (+3 oxidation state) methyltransferase (AS3MT, previously named Cty19). This enzyme catalyzes transfer of the methyl group of S-adenosylmethionine (SAM) to trivalent arsenic.\[170-173\]
Arsenic Binding to Hemoglobin and Accumulation of Arsenic in Rat Blood There are noticeable differences in the half-life of arsenic in blood between animal species. Most arsenic can be rapidly eliminated from blood in humans, with a half-life of about 1 h. Studies on hemodialysis patients indicate that part of the arsenic is bound to transferrin.[177] However, arsenic is retained in rat blood considerably longer than in blood of other species. The accumulation of arsenic in rat red blood cells was reported more than 50 years ago.[178] The cat can also accumulate arsenic in blood, albeit to a lesser extent than does the rat.168 Binding of arsenic to 45 isulphide45 in red blood cells was proposed as the site of accumulation in rat blood.[179]

2 (b): Thallium toxicity

Thallium is included in US Environmental Protection Agency list of priority toxic pollutants as it has caused a number of accidental and occupational poisonings. Thallium toxicity has not been studied extensively in all living organism because it has already been established that this metal is extremely toxic. For this reason, the Commission of the European Communities finds that there is no need for further investigation. However, for the lack of experimental animal, epidemiological, mutagenicity, and genotoxicity data, the Commission could not classify thallium with respect to its (potential) carcinogenicity (180).

Thallium appears as monovalent thallo- and trivalent thalli-compound. It tends to form stable complexes such as sulphur-containing compounds. The chemical properties of the monovalent compound are similar to alkali metals whereas the trivalent compound behaves more like aluminium. As a nonessential element, it plays no role in human, plant, or animal metabolism.
MECHANISM OF TOXICITY

The exact mechanisms that mediate thallium toxicity are still poorly understood and not known since thallium interacts with cells at different levels.

Another possible mechanism of toxicity is thallium’s capacity to react with thiol groups (181). Thallium seems to inhibit a range of enzyme reactions and to interfere with a variety of vital metabolic processes, disrupting cell equilibrium, which in turn leads to generalised poisoning (182). Due to the presence of empty d-orbitals in electronic configuration, thallium has a high affinity for sulphur ligands. It can form complexes with and thus inactivate sulphhydryl groups of proteins which are usually involved in reactions catalysed by enzymes (183). Inhibition of enzymes with active sites containing cysteine residues may increase oxidative stress as a result of glutathione modification (183).

When bound to membrane phospholipids, especially to the anionic headgroups, thallium changes membrane rheology, lipid packing, lipid arrangement in the lateral phase of the bi-layer, and the hydration of the polar headgroups (184). This in turn could affect the activity of membrane-associated enzymes, intracellular transport, and the function of receptors that could contribute to the neurotoxicity associated with thallium poisoning (185).

Some evidence implies that thallium triggers oxidative stress in the cell. In fact, thallium deposits in the brain and a local increase in lipid oxidation products have been reported in rats poisoned with thallium (186, 187). Moreover, the ability of thallium to oxidise membrane lipids and alter their fluidity, could ultimately disrupt membrane-associated metabolic processes (187).
Thallium affects the metabolism of glutathione, a non-protein thiol whose major role is to maintain plasma oxidant homeostasis by defending against reactive oxygen species. In addition, glutathione plays a crucial role in heavy metal toxicity by binding heavy metals through its SH group, which ends in their excretion. The impairment of this protective system can result in the accumulation of oxidant species, that could adversely affect different molecules and their related cellular processes (188). Hanzel et al. (185) investigated the effects of thallium (III) hydroxide on glutathione metabolism in vitro using rat brain cytosolic fractions. Thallium hydroxide decreased inhibited glutathione peroxidase and glutathione reductase activity (187).

Prevention of Arsenic & Thallium Toxicity:

Recent research work have focused on the interaction between arsenic, thallium and sulfhydryl moieties, including cysteine, homocysteine, s-adenosylmethionine, metallothionine, and glutathione. Arsenic and thallium undergo react with cysteine moiety just like mercury does. Methylat ion of mercury occurs when mercury reacts with cysteine and methionine. Same way arsenic also undergo biomethylation by using methyltransferase. Methyltransferase is an enzyme which transfer methyl group from of S-adenosylmethionine to trivalent Arsenic.

Suppose we assume that arsenic & thallium toxicity follow path same as mercury toxicity by reactiong readily with cysteine, s-adenosylmethionine, metallothionine, and glutathione and undergo methylation. The prevention of mercury toxicity is demethylation of it by seleno-proteins or by inorganic selenium. Therefore, it is assumed that demethylation of arsenic & thallium can be possible via interaction with a selenoaminoacid (e.g., L-selenoglutathione or
selenomethionine), ultimately resulting in demethylation and formation of a stable insoluble and inert As:Se and Tl:Se complex. Inorganic selenium as H2Se may also lead to demethylation of Arsenic & thallium as well.

We can try sodium selenite, Diphenyl diselenide and Selenomethionine for the prevention of arsenic toxicity. These substances were found to be active for the demethylation of mercury and reduce its toxic effects. Regular dosage of this substances may reduce level of arsenic and prevent from disease caused by this.
A new supposed pathway to prepare selenomethionine in laboratory:

methionine

\[ \text{HO} \quad \text{OH} \]

hydrogenperoxide

A

Heat

B

HBr

C

\text{Major product}

Se

NaBH}_4

O
3. Thiol Reactive Probes

Thiol-reactive dyes are principally used to label proteins for the detection of conformational changes, assembly of multisubunit complexes and ligand-binding processes. In the case of proteins and peptides, the primary targets of thiol-reactive probes are cysteine residues.

In proteins with multiple cysteine residues, the multiplicity is often small enough that it is practicable to obtain single-cysteine variants by site-directed mutagenesis without significant disruption of the structure or function of the native protein. Site-specific modification is particularly important for 50isulphid small proteins in applications where the activity or binding affinity of the conjugate is paramount; thiol-reactive 50isulphid is the preferred approach over amine-reactive 50isulphid in such cases.

Reducing Diselenide with DTT or TCEP

In proteins, cysteine contains thiol group. It can be also generated when cysteine disulphide reacts with dithiothreitol or 2-mercaptoethanol. These reagents can be removed by gel filtration before reaction with thiol reactive probes. Though new research found 21st amino acid i.e. selenocysteine which contains selenium instead of sulphur in cysteine and selenium is more reactive than sulphur. Therefore it catches attention towards its reactivity with thiol reactive probes. Future aspects of this reactivity suggest that thiol reactive probes may reacts with selenocysteine more readily than cysteine or thiol reactive probes failed to reacts with selenocysteine.

There are some disadvantages of use of DTT or 2-mercaptoethanol i.e. sometimes accompanied by air oxidation of the thiols back to the disulfides. Reformation of the 50isulphide bond can
often be avoided by using the reducing agent tris-(2-carboxyethyl) phosphine (TCEP, T2556), which usually does not need to be removed prior to thiol modification because it does not contain thiols. DTT or 2-mercaptoethanol may showing disadvantage with thiols but it may does not show this disadvantage when it reacts with diselenide or either it will react in same manner as it reacts with sulphur. Then it also gets oxidized by air and form diselenide therefore to avoid such oxidation some reducing reagents like TCEP reacts with diselenide as it is reactive for disulphide. As we know TCEP reduce disulphide bond it may reduce S-Se bond. S-Se bond may found in the human or mammalian proteins.
Reduction of diselenide and S-Se bond by using TCEP may use in proteomic analysis because may be some of unknown or known proteins have diselenide or S-Se bridges and are therefore particularly susceptible to reductive denaturation.

**Thiol-Reactive Reagents**

The primary thiol-reactive reagents, including iodoacetamides, maleimides, benzylic halides and bromomethylketones, react by S-alkylation of thiols to generate stable thioether products. Arylating reagents such as NBD halides react with thiols or amines by a similar substitution of the aromatic halide by the nucleophile. Because the thiolate anion is a better nucleophile than the neutral thiol, cysteine is more reactive above its pKa (~8.3, depending on protein structural context). Now, as we know pKa of selenium is higher than the sulphur therefore thiol reactive reagents like iodoscetamides, maleimides, benzylic halides reacts more readily with selenite and generate stable selenoether which may be more stable than thioether. In addition to insertion of selenocysteine residues by site-directed mutagenesis, several reagents have been developed for introducing selenium into proteins, nucleic acids and lipids. Because the selective introduction of selenium is particularly important for crosslinking two biomolecules.
4. Iodoacetamide

Iodoacetamides readily react with all thiols, including those found in peptides, proteins and thiolated polynucleotides, to form thioethers. Therefore it will react more readily with all selenol found in proteins. (fig-3.2) Now, it will become important reaction when protein contains both thiol as well as selenol group. From the comparison of chemical properties of cysteine and selenocysteine my assumption is when a protein contains both then iodoacetamide first reacts with selenol group as it is more reactive than thiol group because this reaction is followed by nucleophilic attack of selenol group.

\[
\begin{align*}
\text{R}^1\text{CH}_2\text{X} + \text{R}^2\text{SH} & \rightarrow \text{R}^1\text{CH}_2\text{SR}^2 + \text{HX} \\
\text{Alkyl halide} & \quad \text{Thiol} & \quad \text{Thioether} \\
\text{or} & \quad & \\
\text{Haloacetamide} & \quad & (\text{X} = \text{I, Br, Cl}) \\
\text{R}^1\text{CH}_2\text{X} + \text{R}^2\text{SeH} & \rightarrow \text{R}^1\text{CH}_2\text{SeR}^2 + \text{HX} \\
\text{Alkyl halide} & \quad \text{Selenol} & \quad \text{Selenoether} \\
\text{or} & \quad & \\
\text{Haloacetamide} & \quad & (\text{X} = \text{I, Br, Cl})
\end{align*}
\]

Figure 3.2 Reaction of Thiol and selenol with an alkyl halide.
B. Maleimides

Maleimides are excellent reagents for thiol-selective modification, quantitation and analysis. Applications of these fluorescent and chromophoric analogs of N-ethylmaleimide (NEM) strongly overlap those of iodoacetamides, although maleimides apparently do not react with methionine, histidine or tyrosine. As maleimides produce thioether with thiols it may reacts with selenol and produce selenoether. Generation of thioether form via insertion of nucleophile thiol in double bond as we know selenol is more nucleophilic therefore reaction of selenol with maleimide will be more faster than reaction with thiols.

![Chemical reactions](image)

Figure 3.3 Reaction of thiol and selenol with Maleimide
4. The connection between selenium and soil

The amount of selenium in foods depends on geographical location, climate conditions, the presence of organic matter in soil, geological conditions. Ultimately these variables all contribute to the level of selenium in soil, which is how plant foods receive this mineral. Food processing methods also greatly affect selenium content.

The world’s supply of selenium is very small compared to other minerals because it’s a byproduct and cannot be mined directly.

Plant foods are the most popular source of selenium around the world. However, areas with low selenium levels in their soil, often result in deficiencies for people in that geographic location. An example of this would be in Russia or China, where selenium is absent from the region’s soil. Due to the residing population eating mostly local foods, selenium deficiencies are much more common in those areas. In contrast, certain parts of America have very high levels of selenium in their soil, which results in healthy levels of this trace mineral among local populations.

It has been found that there was some disease like cancer and other caused due to the insufficient micronutrient called selenium. The best way to take selenium is from plants of vegetables. Selenium is found as selenocysteine in plants as well as in biological body of human and animals. Selenocysteine is found as a part of protein or enzyme.

Nowadays soils do not have sufficient amount of selenium therefore humans and animals suffers from insufficient amount of selenium inside the body. To overcome this issue we have
to add chemically prepared materials which contains selenium. This chemical compound containing selenium will form a matrix along with fertilizer in this way it will be incorporated into soil and then soils will become rich in selenium. Plants from selenium rich soils can up take selenium and then it will be converted into selenocysteine which humans and animal can take up inside the body.

Selenium proves effective for deduction of heavy metal toxicity therefore if we add selenium with fertilizer than some amount of selenium will prevent plant as well as soil from the toxicity of heavy metals. This will be natural way for up take selenium and it will be better than any medicines containing selenium as a drug agent. To make soils selenium rich we have to found chemical compounds of selenium and how can we form matrix of Se based chemical compound with fertilizer. Amount of selenium based compound must be decided before mixing because it may cause toxic effect. It is also important to notice that plants will grow as usual when selenium containing fertilizer will be added. For various plants various amount of selenium must be
decided before addition for this study a botanical perspective of plant should be known of each plant. According botanical data of plant we can decide their pre and post effects of selenium based fertilizer.
CHAPTER: 5 CONCLUSION

Selenocysteine can be introduced in to the protein by various methods as discussed in diversified NCL of selenocysteine. Selenocysteine is more reactive than cysteine because of pKa value difference between selenium and sulphur. Deselenization of selenocysteine has wide scope in the preparation of alanine and serine.

High-Resolution Imaging Enabled by Chemical Tags. In very recent results, TMP-tag has been exploited in combination with the SNAP-tag to enable single-molecule imaging. There were certain chemical tags available for cysteine residue now as we know cysteine and selenocysteine having similar chemical properties than it is important to check reactivity of selenocysteine towards such chemical tags. Study in such direction may lead to some of important results with selenium analogue of chemical tags. Such tags may be future of molecular imaging and it may take imaging technology to the next level.

Recent health study shows large number of heavy metal toxicity in human beings. Such heavy metals are arsenic, mercury, lead, thallium and some of caused by silver. Selenium containing another amino acid i.e. selenomethionine proves effective towards cure of mercury toxicity. In such a way it may be effective towards arsenic and thallium toxicity because arsenic and thallium undergone reaction mechanism inside the body as mercury toxicity does. Therefore some amount of oral selenomethionine may prevent from arsenic and thallium toxicity.

In proteins, cysteine contains thiol group and selenocysteine contains selenol group. Inside the body cysteine undergone to redox reactions and some time it forms disulphide bond which can
be reduced by various chemicals and free thiol group. This free thiol group is now reactive toward some of thiol reactive probes. In this way we may generate free selenol group and we can check reactivity of selenol group towards thiol reactive probes. Either it will be more reactive or not reactive with selenol group. There were some of proteins in which cysteine and selenocysteine both are present in such a case it is important to choose perfect probe for selectivity among them.

Most of basic micro nutrients are obtained from the food we eat. Insufficient amount of nutrients will lead to the disease or disorder in protein manifold. Selenium is important micro nutrient for good human health. Selenium levels in soil is decreasing day by day therefore we need to keep our soil selenium rich to prevent from disease like cancer. Selenium up take for cancer patient shows positive result in progression of health. Precaution is better than cure means let us keep maintain selenium level inside the body by plants. We may make our soil selenium rich by adding chemical compound of selenium along with fertilizer which will be taken by plants and converted in selenocysteine. This will cure disease before it caused.

In this way selenocysteine may have so many future aspects as some of function of selenocysteine is unknown inside the body. Selenocysteine leads to diversity into the field of molecular imaging. A very serious disease of this century i.e. cancer may cure from biosimilar drugs containing selenocysteine and its analogue. Unique characteristics of selenocysteine make difference from other amino acid. A wide research on selenocysteine and its incorporation to human body gives important and useful result in the field of drug discovery.
**CHAPTER: 6 REFERENCES**


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