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Chemical Modification of Proteins at Cysteine: Opportunities in Chemistry and Biology

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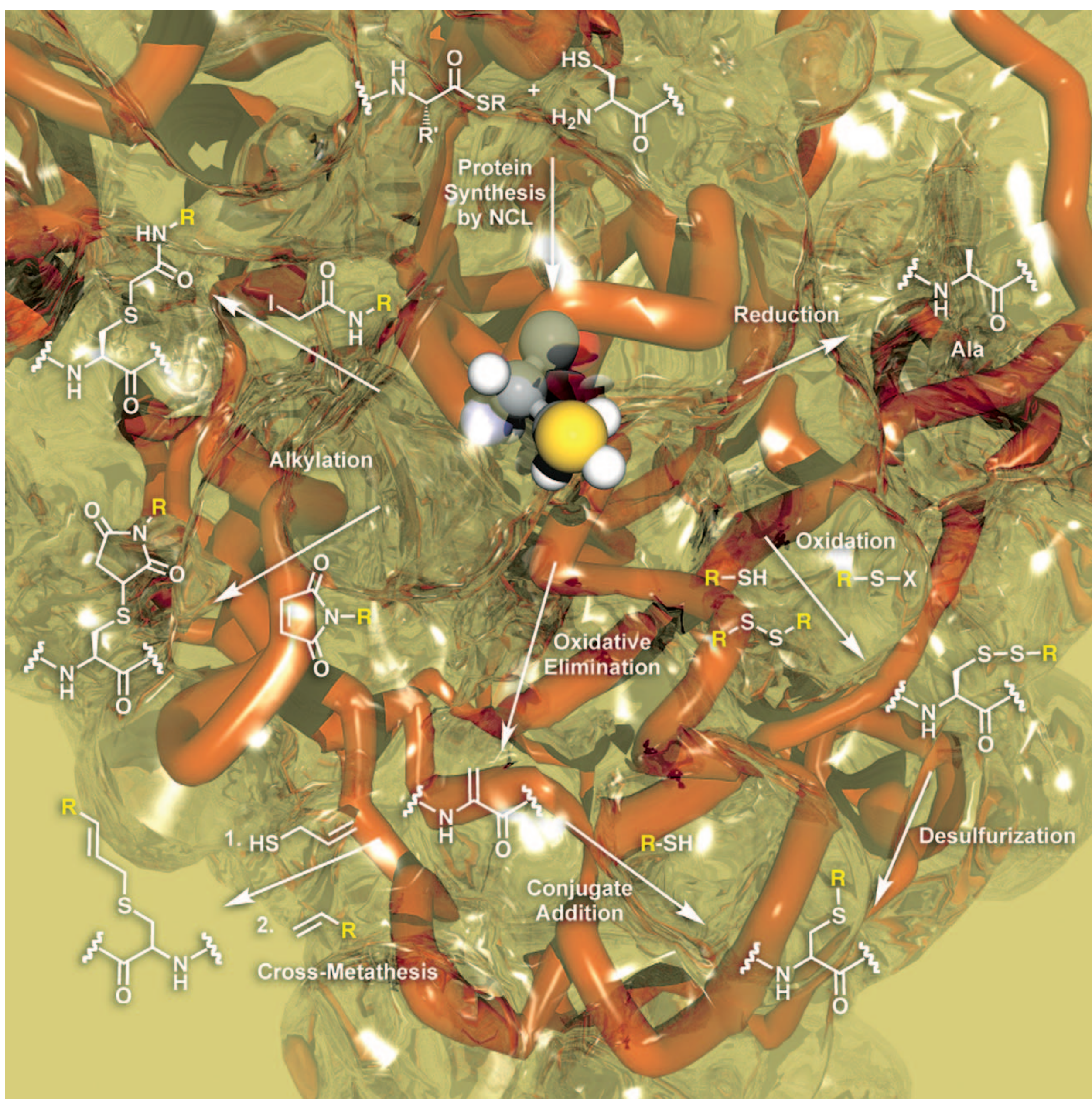

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Abstract: Chemical modification of proteins is a rapidly expanding area in chemical biology. Selective installation of biochemical probes has led to a better understanding of natural protein modification and macromolecular function. In other cases such chemical alterations have changed the protein function entirely. Additionally, tethering therapeutic cargo to proteins has proven invaluable in campaigns against disease. For controlled, selective access to such modified proteins, a unique chemical handle is required. Cysteine, with its unique reactivity, has long been used for such modifications. Cysteine has enjoyed widespread use in selective protein modification, yet new applications and even new reactions continue to emerge. This Focus Review highlights the enduring utility of cysteine in protein modification with special focus on recent innovations in chemistry and biology associated with such modifications.

Keywords: aqueous chemistry • cysteine • protein modifications • selectivity

1. Introduction to Protein Modification

1.1. Background and Significance

Covalent modification of proteins is a powerful way to modulate macromolecular function.[1–4] Nature accomplishes such alterations through a range of post-translational modifications that in turn mediate protein activity.[5,6] A selection of common post-translational modifications is shown in Figure 1. Given the ubiquity and variety of natural covalent modification of proteins, it is important to understand their precise role. Complicating the analysis of post-translational modifications is the limited availability of pure, naturally modified proteins. Chemical access to modified proteins resolves issues of homogeneity and availability, provided the reactions used to modify the protein are selective for the residue of interest and yield either the natural modification or a suitable mimic.[7] Moreover, chemical modification of proteins may be used for unnatural alterations for a desired function. For instance, fluorescent or affinity tagging allows otherwise difficult analysis and tracking of proteins both in vitro and in vivo.[1,3] Therapeutic protein conjugates[8,9] have also gained prominence in the fight against HIV,[10] cancer,[11] malaria,[12] and pathogenic bacteria.[13] The opportunity to study, understand, and influence biological processes with modified proteins prompts the development of selective reactions that yield well-defined protein constructs. As discussed next, the chemical reactions amenable to selective protein modification must satisfy a number of challenging criteria.

1.2. Challenges in Selective Protein Modification

For a reaction to be of general use in protein modification, it must selectively modify a residue of interest in the presence of hundreds of competing side chains of the unprotected polypeptide. This selectivity must also be realized in conditions required to prevent protein denaturation: aqueous media, low to ambient temperature, and at or near neutral pH. Additionally, the reaction must tolerate salts and surfactants often needed for protein stability.[14] Finally, since proteins are often only available in low concentrations, the reaction must be rapid to achieve full conversion. These requirements present a considerable chemical challenge. The reaction will be most useful if it is *specific* for the residue of interest. One solution to the issue of selectivity is to incorporate unnatural amino acids[15,16] that contain a side chain with a chemical handle that is "bio-orthogonal"; that is, the residue, reagents, and reaction are not compromised by the heterogeneous milieu of the protein solution or living cell. At the same time, the residue, reagents, and reaction do not alter the biological system in any way other than intended. Many remarkable labelling techniques have been reported on these unnatural residues.[1,3,17,18] For the purposes of this review, focus will remain on selective modification of the innate protein residue cysteine. Of the proteogenic amino acids, cysteine is perhaps the most convenient target for selective modification owing to the strongly nucleophilic side chain sulfhydryl. Furthermore, cysteine's relatively low natural abundance,[19] combined with standard site-directed mutagenesis, allows access to protein constructs with a single

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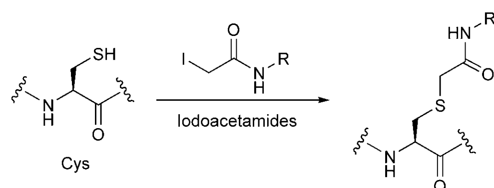
cysteine at a predetermined site. This Focus Review will highlight recent developments in chemical modification of cysteine with emphasis on novel and biologically relevant transformations at this amino acid.

2. Alkylation of Cysteine

Direct alkylation with suitable electrophiles is a common technique for cysteine modification. By careful control of pH, cysteine can sometimes be alkylated in preference of other nucleophilic residues such as lysine and histidine. Protocols for these alkylations have been described,[20] and recent investigations have delineated considerations in electrophile selection.[21] Here we juxtapose some of the earliest examples of cysteine alkylation alongside recent conjugation strategies to highlight the enduring utility of this chemistry in protein modification.

2.1. α-Halocarbonyls

α-Halocarbonyl electrophiles such as iodoacetamides were among the first reagents used for the direct alkylation of cysteine. As early as 1935, iodoacetamide was used to modify and study the cysteines of keratin (Scheme 1).[22]



Scheme 1. Modification of cysteine using iodoacetamides.

This derivative of cysteine has since been widely used in mass spectral analysis and peptide mapping of cysteine containing proteins.[23] Undesired reaction with lysine is known, but this can be resolved by the use of chloroacetamide.[24] Functionally relevant modifications have been realized by Clark and Lowe using α-bromoacetophenone derivatives to alkylate the active-site cysteine of papain. In their seminal application of synthetic chemistry to protein modification, the alkylated cysteine was photochemically converted to formyl glycine which was in turn reduced to serine and glycine.[25,26] Similarly, an α-halocarbonyl was used for the covalent attachment of a flavin to the active cysteine of papain,

Abstract in Chinese:

蛋白質的化學修飾在化學生物學是個蓬勃發展的領域。經由選擇性的生化探針，我們對於蛋白質的天然修飾與功能有了更深入的了解。在某些例子，化學修飾更可以完全地改變蛋白質的功能。除此之外，鍵結具治療功能的載體於蛋白質上，已被證實在醫療方面是非常有價值的。對於如何有選擇性地調控並利用這類修飾蛋白質，需要一種獨特的化學方法。半胱氨酸，因為其顯著的親核性，長久以來都被廣泛地用在選擇性蛋白質修飾上。然而新的應用和反應層出不窮，本回顧論文概括半胱氨酸在蛋白質修飾的應用，並著重於此類蛋白質化學修飾在化學及生物學上最新的進展。

__Justin M. Chalker__ was born in Kansas City, Kansas in 1983. He graduated from the University of Pittsburgh with a B.S. in Chemistry and a B.A. in the History and Philosophy of Science in 2006. Justin carried out undergraduate research in the laboratory of Prof. Theodore Cohen where he investigated the Zn-ene cyclization and its use in total synthesis. As a Rhodes Scholar, Justin is pursuing his Ph.D. at the University of Oxford under the direction of Prof. Benjamin G. Davis where he is exploring novel aqueous chemistry for selective protein modification.

__Gonçalo J. L. Bernardes__ was born in Torres Vedras, Portugal in 1980. He received his M.Chem. from the University of Lisbon in 2004. After receiving a scholarship from the Portuguese Science Foundation, he has been working on reaction engineering for site-selective modification of proteins under the supervision of Prof. Benjamin G. Davis at the University of Oxford. In the summer of 2008, he completed his Ph.D. and joined the laboratories of Prof. Peter H. Seeberger as a Postdoctoral Research Fellow.

__Yuya A. Lin__ was born in Tainan, Taiwan in 1985. She completed her M.Chem. in 2008 at the University of Oxford. As an undergraduate, she studied aqueous olefin metathesis and its use in protein modification under the supervision of Prof. Benjamin G. Davis. With a Ph.D. scholarship from the EPSRC, Yuya is continuing her work in the Davis laboratory on site-selective protein modification.

__Benjamin G. Davis__ completed his B.A. (1993) and D.Phil. (1996) at the University of Oxford. During this time he learned the beauty of carbohydrate chemistry under the supervision of Professor George Fleet. He is now a full Professor at the University of Oxford and Pembroke College. His group's research centers on chemical biology with an emphasis on carbohydrates and proteins. In 2008, he was awarded the Wain Medal for Chemical Biology and was the first U.K. recipient of the American Chemical Society's Horace S. Isbell Award.

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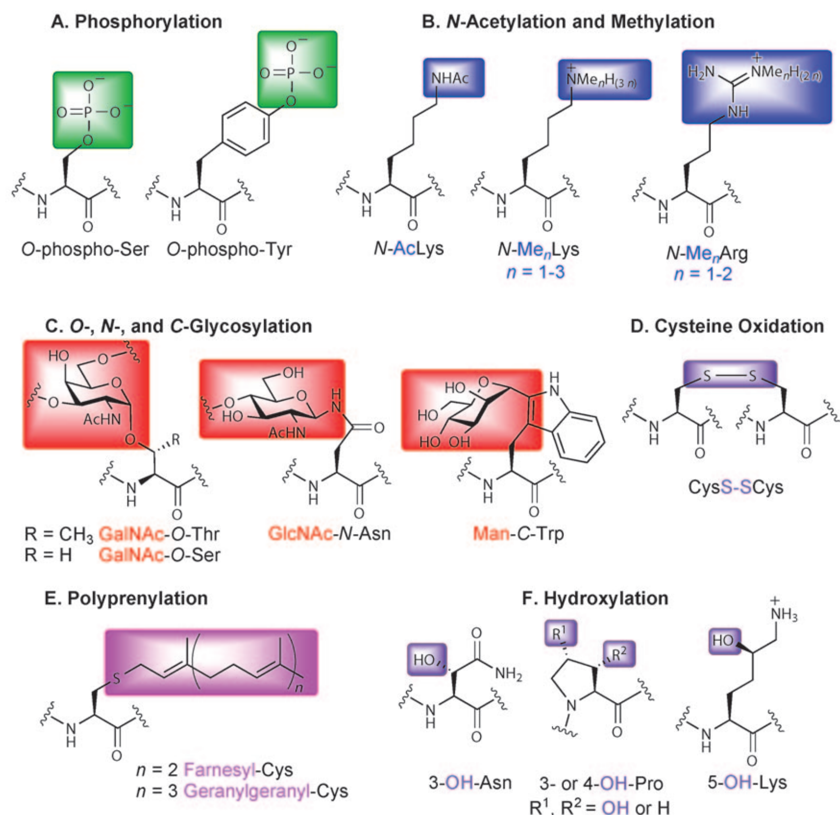


Modification	Function	Residue
Phosphorylation	Control of cellular function and signalling	Ser, Thr, Tyr, Asp, His, Cys
N-Acetylation *N*-Methylation	Transcriptional regulation	Arg, Lys, His
Glycosylation	Cellular differentiation, Fertilization, Immune response	Ser, Thr, Asn, Trp
Oxidation	Protein stability, Redox regulation	Cys, Met
Polyprenylation	Membrane localization, Protein-protein interactions	Cys
Hydroxylation	Oxygen sensing, Collagen maturation	Lys, Pro, Asn

Figure 1. Examples of post-translational modifications.[5,6]

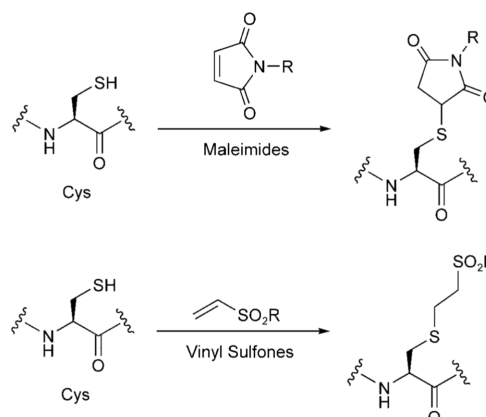
thereby functionally altering the cysteine protease to an oxidoreductase.[27] Finally, carbohydrate-tethered iodoacetamides have been used to alkylate cysteine, affording mimics of the natural asparagine-linked glycoproteins.[28] This method has been used in accessing homogeneously glycosylated samples of both human erythropoietin[29] and dihydrofolate reductase.[30]

2.2. Michael Acceptors

The conjugate addition of cysteine to Michael acceptors is also a reliable way to selectively alkylate the cysteine side chain. Maleimides,[18,20] vinyl sulfones,[31] and related α,β-unsaturated systems are the most widely used Michael acceptors for cysteine (Scheme 2). In an early example, Moore and Ward reduced disulfide bonds and then replaced the cross-link with the adduct of bis-maleimides.[32] The use of maleimides has continued to the present day as a widely used method in cysteine alkylation.[18,33] Recently, glycosylation of myoglobin using a maleimide construct imparted higher oxygen affinity to the protein.[34] Another testament to maleimide utility is their use in leading synthetic cancer vaccine constructs.[35] α,β-Unsaturated ketones are also useful in cysteine alkylation. A bifunctional Michael acceptor of this variety was recently used for the site-specific PEGylation at the reduced disulfides of human interferon α-2b as well as a human CD4 receptor-blocking antibody fragment.[36] Vinyl sulfones have also maintained use in protein modification. For instance, a vinyl sulfone handle was used to conjugate enzymes to a ubiquitin-like protein.[37]

2.3. Aminoethylation

Aminoethylation of cysteine has been used for over half a century for the conversion of cysteine to lysine mimics.[38] The resulting residue was validated as a synthetic substrate for trypsin, which recognizes the positive charge of the lysine side chain as a site for proteolytic action. Aminoethylation has since developed into a chemical approach to study the role of active site lysines.



Scheme 2. Modification of cysteine with maleimides and vinyl sulfones.

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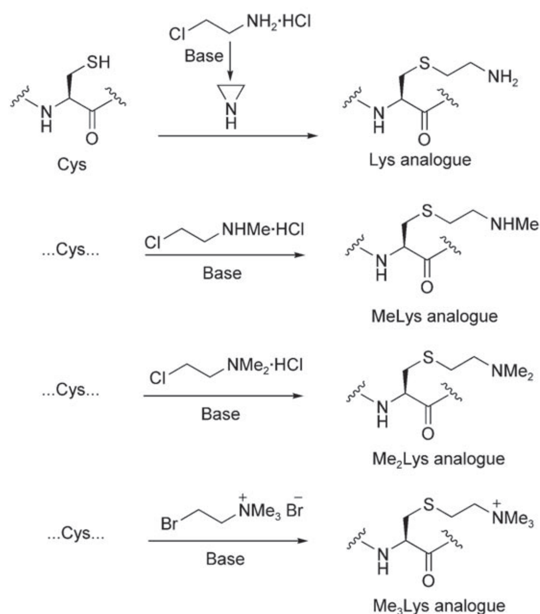
The inactive cysteine mutant is aminoethylated, chemically recovering the catalytic role of lysine.[39] Recent access to methylated lysine analogues was provided by this method (Scheme 3).[40] These modifications allow access to analogues of an important, but poorly understood, class of post-translation modifications of histone proteins that control the epigenetic status of the cell.[41] This final example is a testament to the reliability of simple alkylation reactions in protein modification that continue to offer solutions to complex biological problems.

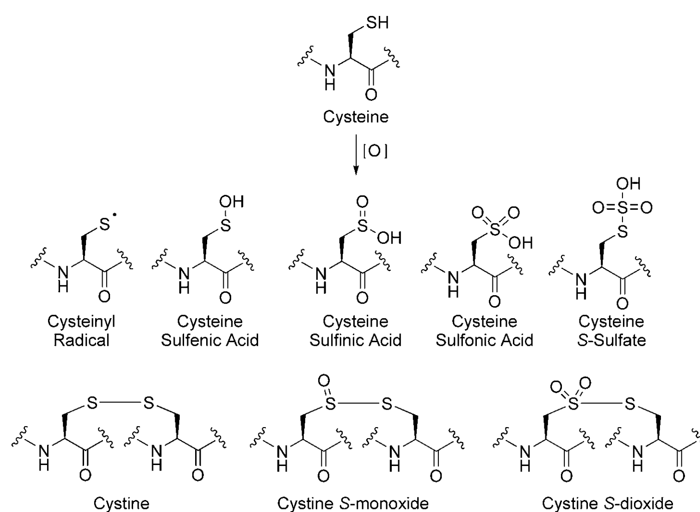


Scheme 3. A) Aminoethylation of cysteine allows chemical access to lysine analogues. B) Incorporation of methyl lysine analogues in histone H3 by aminoethylation.[40]

3. Oxidation of Cysteine

The facile interconversion between several oxidation states accounts for much of cysteine's versatile chemistry and is essential in cellular redox regulation (Scheme 4).[42]

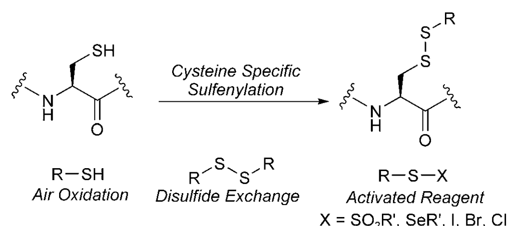
For the purpose of chemical modification and especially chemoselective ligation, the formation of disulfides has dominated chemical oxidation of cysteine. It is to this modification that we now turn.



Scheme 4. Several natural oxidation states of cysteine.[42]

3.1. Disulfides

Disulfides impart essential, and often resilient, structural stability in many proteins. It is perhaps not surprising that one of the most common natural modifications of cysteine has been adopted as a chemical strategy for the modification of proteins (Scheme 5). One of the simplest methods to form disulfide at cysteine is air oxidation. In many cases, simply mixing the thiol and cysteine containing protein in basic buffer open to air will generate the desired disulfide. This method is used both to form internal disulfides when refolding proteins as well as a method for conjugation of small molecules.[20] Disadvantages of this method include long reaction times and limited control of product distribution (e.g. mixed disulfide vs. dimer). To remedy these shortcomings, many alternative protocols have been developed.



Scheme 5. Methods for disulfide formation at cysteine.

The formation of disulfides on a protein can be accomplished by disulfide exchange, provided there is a thermodynamic preference for the disulfide formation on the protein. A well-known example is the reaction of 5,5-dithiobis(2-nitrobenzoate) (DTNB or Ellman's Reagent) with cysteine.[43] This reagent has been used extensively to measure protein sulfhydryl content. Synthetically, thiopyridyl disulfide has been used to activate cysteine for conversion into an imidazole containing disulfide adduct that was a semi-synthetic catalytic antibody.[44]

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The most general method for disulfide formation is the use of activated reagents. Iodine has been used as an oxidant, activating cysteine for the formation of mixed disulfides.[45] Sulfenyl halides have also been demonstrated as suitable reagents for mixed disulfide formation.[46] These methods served largely analytical purposes, and it was with the development of methanethiosulfonate reagents[47,48] and related activated thiols[49–51] that disulfide modification for functional purposes became common. These methods have been used for the installation of several post-translational modifications or mimics thereof, with protein glycosylation the most developed.[51,52] The convenience of these methods for disulfide formation is the rapid, selective reaction. The high rate also means a large excess reagent is not necessary, an important consideration for valuable materials. In certain cases, kinetic control may also give enhanced selectivity.[51] The control offered by this approach has even allowed the synthesis of a protein trisulfide glycoconjugate.[53]

The ease of disulfide formation has driven methodology in the synthesis of useful thiols. Our own efforts have capitalized on the use of Lawesson's reagent in these syntheses. Glycosyl thiols were synthesized from reducing sugars[54] and allylic thiols were synthesized from the corresponding alcohols. The product thiols were used to site-specifically glycosylate and prenylate a model protein.[55,56]

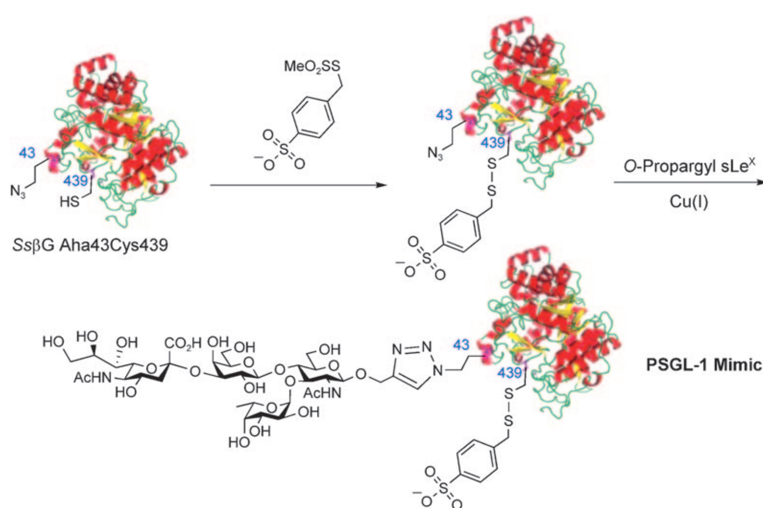
A recent and notable use of disulfide linkages in the mimicry of post-translational modifications was in the chemical synthesis of a mimic of P-selectin-glycoprotein-ligand-1 (PSGL-1).[57] PSGL-1 possesses two modifications: an *O*-linked sialyl Lewis^X (sLe^X) containing carbohydrate moiety and a sulfated tyrosine. A galactosidase reporter enzyme (*Ss*βG) displaying appropriately spaced azide and cysteine tags was doubly modified to generate a mimic of PSGL-1. The sulfated tyrosine mimic was installed as a disulfide at cysteine and sLe^X through a triazole linkage at the azidohomoalanine (Aha, Scheme 6). Importantly the disulfide remained intact during the second modification. The modified protein displayed strong binding to human P-selectin and the innate galactosidase activity was exploited as a sensitive reporter for brain inflammation. This example highlights the utility of chemical modification of proteins and insight into biology and disease.

It is undeniable that disulfides have proven useful for in vitro assays—as in the PSGL-1 mimic described above. Yet, disulfides may be unstable in reducing environments, limiting their use in certain in vivo applications where intracellular concentration of glutathione can reach 15 mM.[58] The disulfide lability may be used to one's advantage in cases where the tethered modification is designed to be released in a reducing environment, as in recent developments in drug delivery.[59] If the modified protein is to remain intact in the cell, it is worth investigating the conversion of these conjugates to a more stable derivative. The conversion of disulfides to thioethers is discussed next.

4. Desulfurization at Cysteine

4.1. Disulfide Contraction to Thioether

The conversion of disulfides to thioethers upon treatment with hexamethylphosphorous triamide (HMPT) was first studied in detail by Harpp and Gleason.[60–62] Their work was motivated, at least in part, by the need to understand and manipulate biologically relevant disulfides[61] and thioethers.[62] With an eye for the conversion of disulfide modified proteins into the corresponding thioether conjugates, our lab has adapted Harpp and Gleason's transformation. With such a transformation, one can benefit from the ease of synthesis of disulfides (see above), and render them resistant to reduction. Our preliminary efforts successfully gave a variety of glycoaminoacids and glycopeptides, as well as a thioether-linked glycoprotein.[63] During the course of this investigation, it also became clear that at least one productive mechanism for this transformation on cysteine disulfides proceeded by attack of HMPT on the sulfur of cysteine, followed by elimination of the resulting phosphonium ion. Dehydroalanine was observed by ^{1}H NMR, providing direct evidence for this mechanistic course. Michael addition of the resulting thiol gave the corresponding thioether (Scheme 7). As a consequence of this mechanism, an epimeric mixture at the α-center of cysteine is formed. Nevertheless, application of this method to a disulfide-linked glycoprotein enabled the first conversion of cysteine into a thioether-linked glycoprotein.



Scheme 6. Dual differential modification of *Ss*βG using disulfide formation at Cys 439 and Cu^I-catalyzed azide-alkyne [3+2] cycloaddition at Aha 43.[57]

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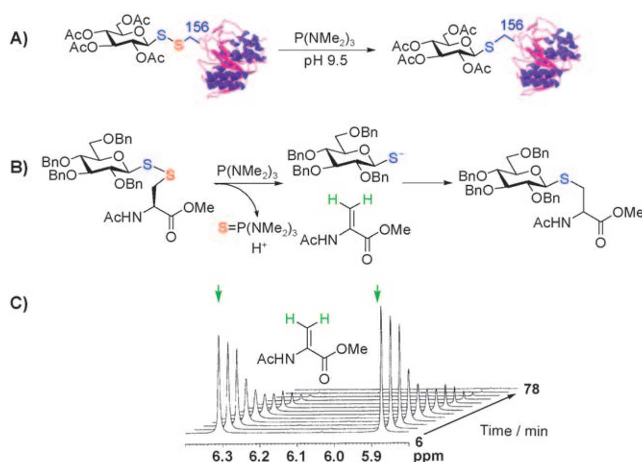
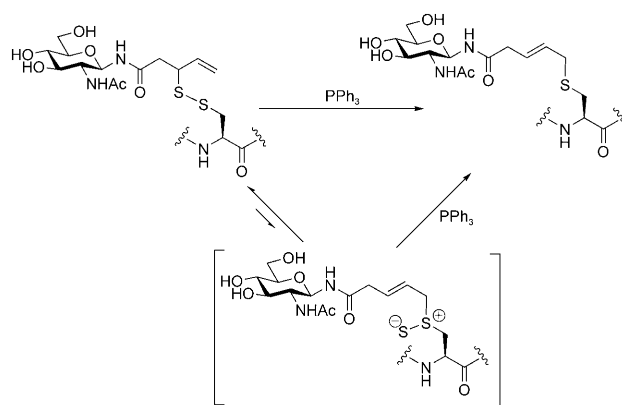


Scheme 7. A) Thioether-linked glycoprotein by desulfurization. B) Model desulfurization and C) ^{1}H NMR observation of dehydroalanine formation and consumption during the desulfurization in MeOD.[63]

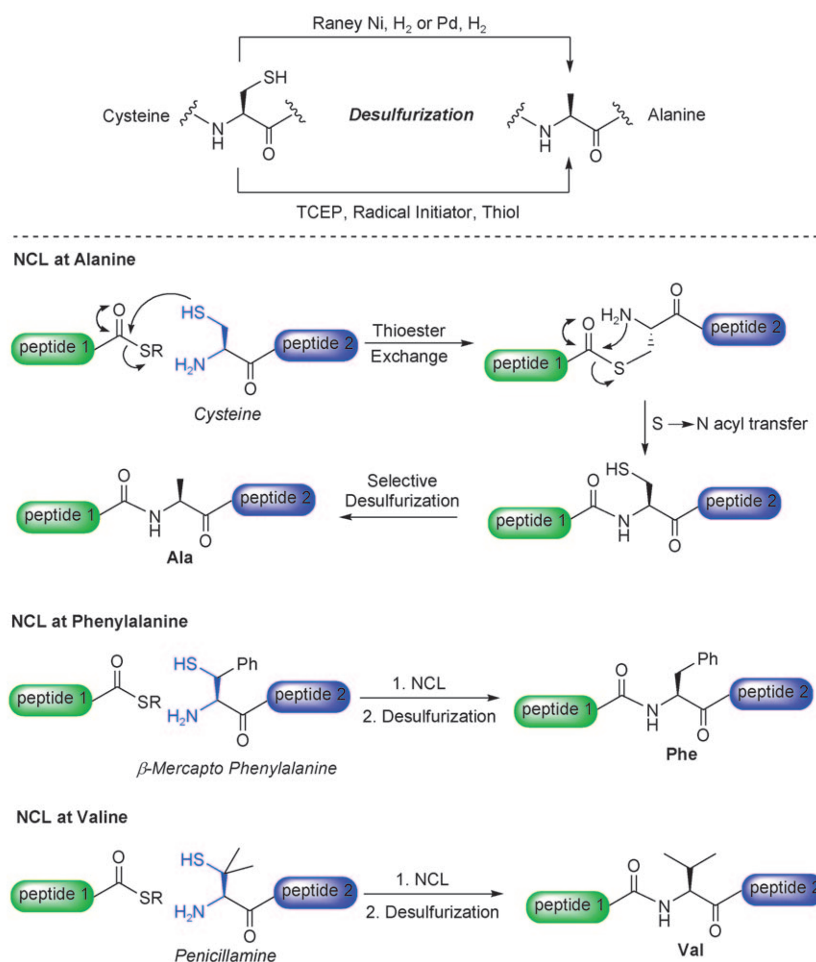


Scheme 8. Desulfurization of allylic disulfides following an allylic rearrangement.[67]

Related desulfurization reactions have been reported by Crich et al. on model peptides.[64–67] In these examples allylic disulfides or allylic seleneosulfides are dechalcogenized by a suitable phosphine after allylic rearrangement (Scheme 8). Notably, in these instances the stereochemistry is retained and may resolve the overall epimerization that complicates the HMPT transformation described above. The successful ligation of various modifications to peptides in aqueous media bodes well for future application of this methodology to protein substrates.

4.2. Reductive Removal of Cysteine Thiol

A key reaction in cysteine modification is the reductive removal of the side chain sulfhydryl. This modification yields alanine. The transformation is most widely used in the desufurization of cysteine after native chemical ligation (NCL),[68] the now widespread strategy for the total chemical synthesis of proteins based on seminal ligations developed by Wieland and co-workers in 1953.[69] The post-ligation reduction of cysteine to alanine constitutes a formal ligation at the latter residue. Alanine is an abundant residue so this desulfurization is one strategy to overcome the reliance on cysteine for NCL. Dawson first reported hydrogenolytic desulfurization of cysteine using various palladium sources or Raney nickel (Scheme 9).[70] This method was used to synthesize cysteine-free peptides by NCL. A related nickel



Scheme 9. Desulfurization of cysteine (top). Formal native chemical ligation (NCL)[68] at alanine,[70,72] phenylalanine,[71] and valine[73,74] (bottom).

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boride reduction was applied to β-mercapto phenylalanine for formal NCL at phenylalanine (Scheme 9).[71]

Prompted by concerns of chemoselectivity in the metal-mediated reduction of cysteine to alanine, Danishefsky and Wan have developed a mild radical based desulfurization that is specific for cysteine.[72] The reduction is mediated by the water-soluble phosphine tris(2-carboxyethyl)phosphine (TCEP). This mild reduction has been adopted in not only formal NCL at alanine, but also at valine.[73,74] The latter is accessed by performing NCL with penicillamine followed by desulfurization (Scheme 9).

The utility of cysteine and β-substituted cysteines in sequential NCL and reductive desulfurization is two-fold: first, the side chain is used to mediate the ligation of peptide fragments, yielding a native amide bond. Second, reductive removal of the side chain thiol converts cysteine or the derivative into the final residue. These transformations also owe their efficiency to the versatile chemistry of the thiol side chain.

5. Oxidative Elimination of Cysteine

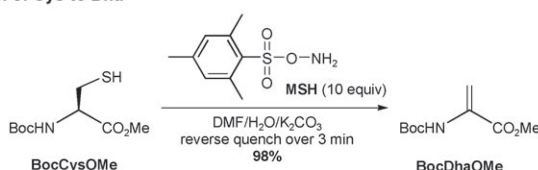
5.1. Cysteine as a Precursor to Dehydroalanine

The oxidative elimination of cysteine to dehydroalanine (Dha) is essentially a desulfurization. One of the earliest reports of the conversion of cysteine into dehydroalanine was by Holmes and Lawton.[75] Here the reactivity of cysteine as a potent nucleophile was used in a double alkylation—first a conjugate addition and then an intramolecular annulation—to give a sulfonium salt that underwent β-elimination to give dehydroalanine. The dehydroalanine residue was then hydrolyzed under acidic conditions with the overall effect of a specific chemical digest at cysteine.

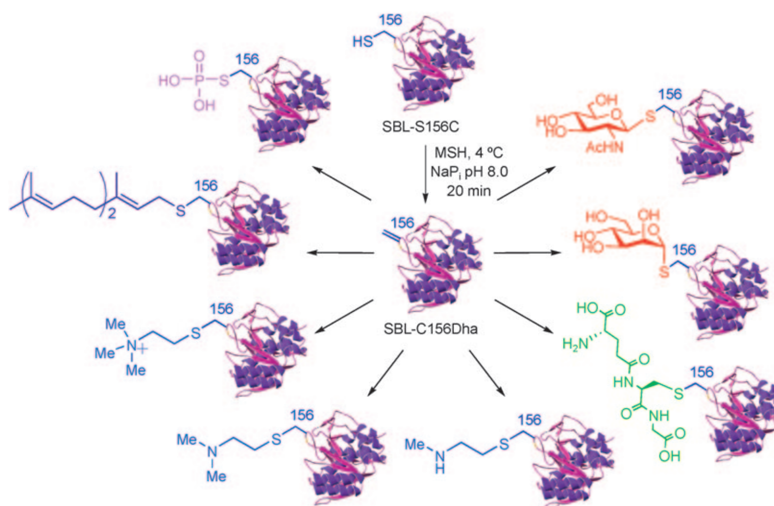
Dehydroalanine is perhaps most useful as a Michael acceptor for thiol nucleophiles. The resulting thioether is an alkyl cysteine derivative, and the overall transformation is a strategic alternative to the direct alkylation procedure that was discussed in Section 2. Access to dehydroalanine is realized by the oxidative elimination of alkylcysteine[76] and selenocysteine[77,78] derivatives in peptides. These methods of elimination have been applied to biosynthetic peptides[79] and proteins[80] by Szostak and Schultz, respectively. In the latter case, the dehydroalanine-containing protein—accessed from the oxidative elimination of phenylselenocysteine—was then converted into lipidated and mannosylated cysteine derivatives by conjugate addition of the corresponding thiol. It is worth noting that diastereoselectivity of thiol additions to dehydroalanine is generally quite low,[81,82] but it is dependent on the sequence and local stereochemical environment.[83]

While the methods of Szostak and Schultz are powerful in their own right, we considered it would be useful and complementary to find a mild way to eliminate the *natural* residue cysteine to dehydroalanine.[56] Motivated by the oxidative elimination of thioethers upon treatment with *O*-mesitylenesulfonylhydroxylamine (MSH),[84,85] we discovered that when cysteine is added to excess MSH under basic conditions, rapid formation of Dha is observed. Methionine also reacts with MSH, but the resulting sulfilimine is unstable in basic conditions and decomposes back to methionine. The sulfilimine can also be rapidly reduced to methionine when treated with base and dithiothreitol (DTT). MSH compatibility with methionine resolves chemoselectivity issues of oxidants like H_2O_2 that result in the sulfoxides and/or sulfones of methionine.[79,80] With ready access to dehydroalanine-containing proteins, a variety of modifications were installed by conjugate addition of thiols (Scheme 10).[56] Farnesylated, phosphorylated, and glycosylated cysteine were all

Oxidative Elimination of Cys to Dha



Dha as Chemical Handle for Protein Modification



Scheme 10. Oxidative elimination of Cys to Dha. Post-translationally modified proteins were accessed by conjugate addition of thiol nucleophiles.[56]

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accessed by this method. Methylated lysines analogues were also synthesized. The methyl lysine and related acyl lysine analogues were later installed in the same manner in the H3 histone by the Schultz laboratory.[86] Importantly, the acyl lysine mimic was validated as a substrate for histone deacetylase 3. These developments are an addition to a growing interest in deconvoluting the "Histone Code" that is implicated in control of the epigenetic status of a cell.[87]

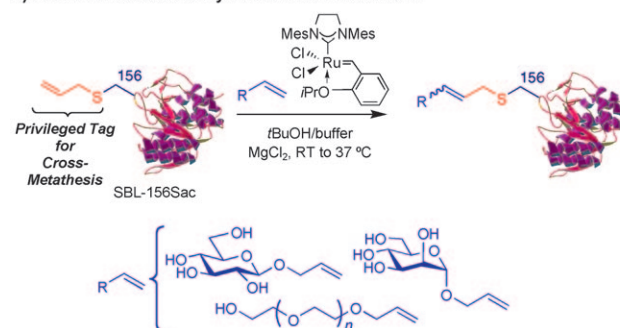
6. Metal-Mediated Modifications at Cysteine and Cysteine Derivatives

The use of metals to mediate the chemistry of cysteine is motivated, at least in part, by the natural affinity of cysteine to various metals.[42] The affinity of sulfur to soft metal centers can be used to the protein chemist's advantage. For instance, as already discussed for desulfurization, nickel and palladium can both be used to mediate the selective reduction of cysteine to alanine.[70] Complexes with zinc have also been used to mask the reactivity of cysteine until needed.[88] The affinity of cysteine and related sulfur centers for metals can also be used for bond-forming transformations as discussed next.

6.1. Olefin Metathesis at *S*-Allyl Cysteine

The biosynthetic incorporation of alkenes into proteins is well documented,[89,90] but olefin metathesis at these residues remained an outstanding problem for the last decade. Despite its vast utility in organic synthesis,[91,92] olefin metathesis has rarely been applied to biomolecules owing to the comparatively limited scope of aqueous metathesis. Novel, water-soluble catalysts and other strategies for aqueous olefin metathesis have emerged,[93–96] yet aqueous cross-metathesis is largely limited to simple, reactive olefins such as allyl alcohol.

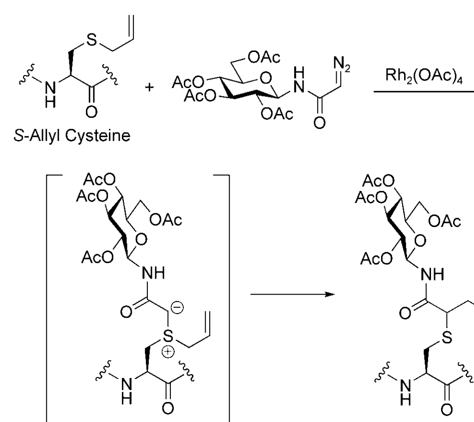
Our own interest in aqueous cross-metathesis was piqued by the observation that allyl sulfides undergo rapid cross-metathesis in aqueous media with Hoveyda–Grubbs second-generation metathesis catalyst.[97] This result was striking given the "… general incompatibility of ruthenium based metathesis catalysts with substrates containing sulfur(II) donor sites,"[98] and the sensitivity of the catalyst to water. Allyl sulfides have been used in metathesis in organic solvents, but the apparent rate enhancement we observed went unnoticed or did not manifest itself with the catalysts used.[99,100] Allyl sulfides are not just compatible with metathesis catalysts, they *enhance* the rate of metathesis relative to their carbon and heteroatom counterparts. Taking advantage of the allyl sulfide reactivity and its easy installation at dehydroalanine,[56] glycosylation and PEGylation were accomplished on a protein surface. These modifications are the first examples of cross-metathesis on a protein (Scheme 11).[97]



Scheme 11. Aqueous cross-methathesis in site-selective protein modification.[97]

6.2. Kirmse–Doyle Reaction at *S*-Allyl Cysteine

Cross-metathesis is not the only transition-metal-mediated reaction that has been used at allyl cysteine. The allyl sulfide has also been used in the Kirmse–Doyle reaction to functionalize peptides (Scheme 12).[101] A sulfur ylide is generated upon attack of a rhodium carbenoid. Subsequent rearrangement affords the ligated product. This reaction has been used in the synthesis of several peptide conjugates. With multiple routes to allylated cysteine,[56,64,65] this method is likely to be explored in protein modification.



Scheme 12. Kirmse–Doyle strategy for *S*-allyl cysteine modification.[101]

Conclusions and Prospects

The utility of cysteine in protein modification cannot be overstated. The versatile reactivity of this residue has enabled access to a range of modified proteins that have al-

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lowed insight into complex biological problems. Moreover, this utility in biology has driven chemical methodology and reaction engineering for mild, selective reaction at cysteine and cysteine derivatives. It is likely that efforts in aqueous chemistry for modification at cysteine will continue to emerge as more sophisticated techniques for the precise alteration of proteins are needed. Indeed modification of cysteine has extended beyond that of a simple nucleophilic substution or conjugate addition. For instance, conversion of cysteine to dehydroalanine demonstrates the use of cysteine as a latent *electrophile*. Additionally, transition-metal-catalyzed carbon–carbon bond-forming reactions can be directed and even enhanced by cysteine derivatives, as in olefin metathesis and Kirmse–Doyle ligation at *S*-allyl cysteine. These innovations in aqueous chemistry augment an already powerful arsenal of chemistry at cysteine. It is clear that reaction engineering has expanded and improved access to homogenous modified proteins. These chemically tailored proteins are essential for both fundamental studies in biology and therapeutic strategies. Such progress in chemical biology indeed owes much to cysteine.

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