N-Terminal Modification of Proteins with *o*-Aminophenols

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ⓈSupporting Information

ABSTRACT: The synthetic modification of proteins plays an important role in chemical biology and biomaterials science. These fields provide a constant need for chemical tools that can introduce new functionality in specific locations on protein surfaces. In this work, an oxidative strategy is demonstrated for the efficient modification of N-terminal residues on peptides and N-terminal proline residues on proteins. The strategy uses *o*-aminophenols or *o*-catechols that are oxidized to active coupling species *in situ* using potassium ferricyanide. Peptide screening results have revealed that many N-terminal amino acids can participate in this reaction, and that proline residues are particularly reactive. When applied to protein substrates, the reaction shows a stronger requirement for the proline group. Key advantages of the reaction include its fast second-order kinetics and ability to achieve site-selective modification in a single step using low concentrations of reagent. Although free cysteines are also modified by the coupling reaction, they can be protected through disulfide formation and then liberated after N-terminal coupling is complete. This allows access to doubly functionalized bioconjugates that can be difficult to access using other methods.
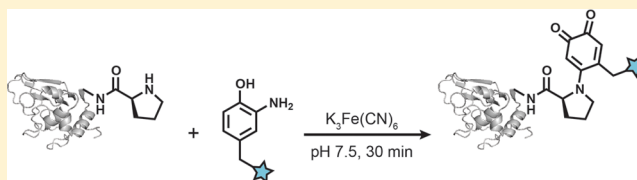
■ INTRODUCTION

The synthetic modification of proteins enables the construction of biomolecular hybrids that can be used to study protein function,[1] deliver potent therapeutics to cellular targets,[2] and build new materials.[3] The synthesis of these constructs requires a suite of chemoselective bioconjugation reactions that proceed under mild, aqueous conditions in the presence of the native functional groups that are present on protein surfaces.[4−6] The most common methods for protein modification target the nucleophilic side-chains of lysine and cysteine.[4,7,8] However, these strategies can result in complex product mixtures, as lysine is typically found in high abundance on the protein surface[9] and uniquely reactive cysteine labeling sites can be difficult to install in many instances (such as thiol proteases and proteins produced via the eukaryotic secretory pathway, for example).

Many newer approaches for the site-selective modification of proteins involve the introduction of artificial amino acids with reactivities that are orthogonal[10] to those of the native amino acids. Along these lines, a number of powerful methods have been developed for the selective modification of azide,[11−17] alkyne,[13−16] alkene,[18−21] carbonyl,[22,23] and aniline[24−27] moieties. However, the difficulty of introducing a non-canonical amino acid can limit the application of these methods. Complementary approaches rely on the site-selective modification of native amino acids by enzymes.[28−33] In addition, a reliable method for the modification of C-terminal thioesters with N-terminal cysteines, termed "native chemical ligation", has been developed by Kent and co-workers.[34,35] This method has been used for the semi- and total synthesis of complex protein substrates,[36−38] including the chemical synthesis of a single glycoform of human erythropoietin.[39,40]

As an alternative strategy, we and others have developed methods for the selective modification of the N-terminal amino group.[41−50] Methods that target the N-terminus can offer significant advantages for bioconjugate preparation, as they can be used for a wide range of protein targets produced by virtually any expression system. Conceptually powerful as they are, however, these methods can be hampered by long reaction times, often require large excesses of reagent, and/or involve at least two-steps for the attachment of synthetic molecules. We have therefore sought to develop new techniques that can achieve N-terminal modification with similarly high positional selectivity, but with significantly improved efficiency.

Herein, we report an oxidative coupling pathway that can preferentially modify the N-terminus of proteins with fast kinetics. Peptide substrates were first used to screen reaction conditions and identify the site of modification. A peptide panel with varying N-terminal residues was then evaluated to determine the sequence specificity of the reaction, leading to the identification of proline as the optimal N-terminal amino acid. The reaction was next applied to protein substrates, showing similarly high levels of conversion when an N-terminal proline residue was present. This mild bioconjugation reaction enables the facile, rapid modification of proteins to create a well-defined and stable linkage in a single position, and thus should be useful for many different applications in chemical biology and the construction of biomolecular materials.

■ RESULTS AND DISCUSSION

We have previously reported the chemoselective coupling of aniline moieties on proteins to electron-rich aromatic rings, such as o-aminophenols, at slightly acidic pH (6.0–6.5).[25] These reactions require the addition of NaIO$_4$[25] or K$_3$Fe-(CN)$_6$[26] as a terminal oxidant, with the latter reagent exhibiting improved compatibility with glycoproteins and substrates with free sulfhydryl groups. The use of ferricyanide as the oxidant also yields a single reaction product (**1**), whereas periodate leads to the formation of a ring contracted species as a competing pathway.[25] The ferricyanide-based reactions are presumed to involve an o-iminoquinone as the reactive intermediate, as suggested in Scheme 1, or could involve the

Scheme 1. Oxidative Coupling with o-Aminophenols
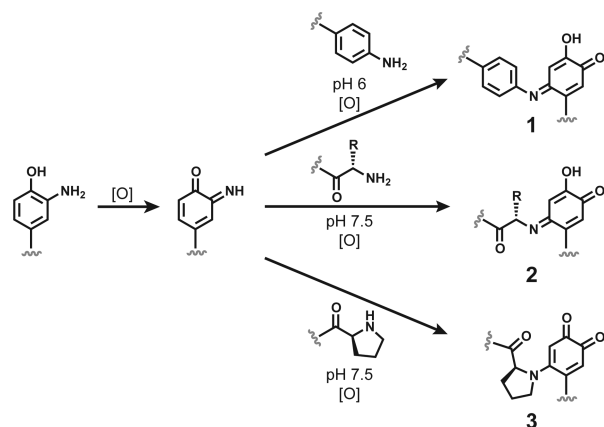
corresponding o-quinone after imine hydrolysis. Taken together, the oxidative coupling strategies have demonstrated excellent functional group compatibility and the ability to join large unprotected biomolecules at low concentrations, as demonstrated for the coupling of peptides, polymers, and nucleic acids to specific locations on viral capsids[25,26,51] and antibody Fc domains.[27]

While these coupling reactions were found to be highly aniline-selective under the conditions used, several studies have reported the reaction of o-aminophenols and o-catechols with native amino acids, dating back to 1949.[52–57] In addition, recent work by Messersmith has shown the ability of proteins to be coupled to o-quinone moieties present on polydopamine-coated surfaces.[58,59] These reports suggested that secondary coupling pathways could be developed to achieve the modification of native amino acids with o-aminophenols (Scheme 1), and thus initial experiments were designed to identify the optimal reaction conditions for achieving this with complex molecules.

Screening Reactivity on Peptide Substrates. In our previous work, we noted that low amounts of background reactivity could be observed in aniline-based oxidative coupling reactions when higher pH conditions (>pH 6.5) were used.[26] In an initial effort to characterize this alternative reaction pathway, conditions and reaction times were first screened to increase the reaction yields for peptides that did not contain aniline groups. Angiotensin I and melittin were used as substrates, as they contain many reactive amino acids, including Lys, Arg, His, Trp, and Tyr. The peptides were exposed to 2-amino-p-cresol using K$_3$Fe(CN)$_6$ as the oxidant. The reaction pH was varied from 5.5 to 8.5, and the reaction mixtures were

analyzed using MALDI-TOF MS (Supporting Information Figure S1). The level of modification increased with the basicity of the reaction, with near quantitative modification of angiotensin I after 20 min at pH 7.5 and higher. Throughout these initial investigations, it was noted that angiotensin and melittin showed significant differences in reactivity, with angiotensin consistently demonstrating better conversion. MS/MS analysis of the angiotensin product was used to identify the participating residue, and revealed that the N-terminal amino group was responsible for the observed reactivity (Supporting Information Figure S2). As further confirmation of the site-selectivity, several peptide substrates were screened for reactivity (Figure 1a and Supporting Information Figure S3). Consistent with the N-terminal reaction selectivity, the only peptide that did not react had a pyroglutamate in this position, and therefore no free amino group.
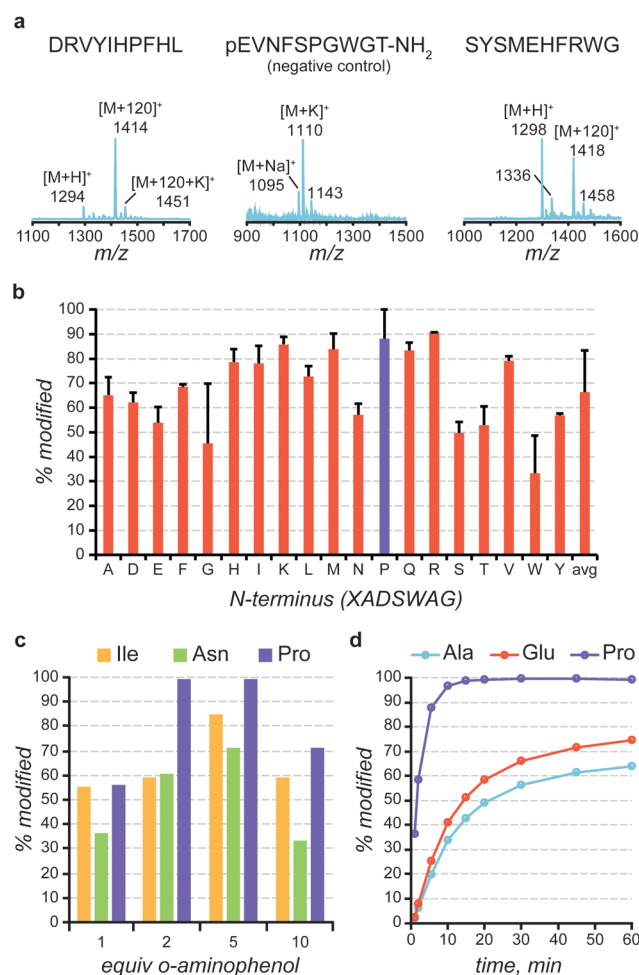
Figure 1. Peptide modification with o-aminophenols. (a) Modification of commercially available peptides was monitored by MALDI-TOF MS. The product can be observed at [M + 120] m/z. (b) A positional scan of the N-terminal amino acid was evaluated. Peptides with the sequence XADSWAG were tested for reactivity with 2-amino-p-cresol. The reactions were run with 100 μM peptide, 200 μM aminophenol, and 5 mM ferricyanide at pH 7.5 and analyzed by LC−MS. Shown is the average percent modification with error bars representing the standard deviation of three reactions. The same peptides were used for a screen of coupling partner equivalents (c) and a time screen (d).

The oxidative coupling conditions were next optimized using the peptide substrates. Several buffer salts were screened for compatibility with the reaction. Most buffers did not alter the reactivity, but imidazole and buffers containing a morpholine or piperazine ring (PIPES, HEPES, HEPPS, and MOPS) significantly impeded the reaction (Supporting Information Figure S4). This is possibly due to small amounts of buffer impurities that react competitively with the oxidized intermediate. The time course of the reaction was also investigated (Supporting Information Figure S5). The reaction reached its maximum conversion after only 20 min. In addition, it was found that the peptides could be modified using $NaIO_4$ as the oxidant, or 4-methylcatechol as the coupling partner (Supporting Information Figures S6−7). Both of these reactions showed the same dependence on pH; however, moderate levels of modification were still observed at acidic pH (5.5−6.5) when using these alternative coupling conditions. These observations suggested that the peptides were reacting with the o-quinone intermediate formed in situ from either the catechol or the iminoquinone precursor (after imine hydrolysis). The effect of the N-terminal residue on reactivity was next investigated.

Screening N-Terminal Residues. Given the differential reactivity observed on peptide substrates, we synthesized peptides with varied N-terminal residues (XADSWAG) to determine the specificity of the reaction. The base sequence was selected to increase the mass of the peptide, impart water solubility, and include a tryptophan residue for quantitation using UV monitoring. The peptides were synthesized on the solid phase using standard Fmoc synthesis, cleaved from the resin, and purified by HPLC. After purification, the peptides were resuspended in phosphate buffer, pH 7.5, adjusted to a concentration of 1 mM, and stored at −20 °C until use. To assess the effect of the N-terminal residue on reactivity, the peptides (100 μM) were reacted with 2 equiv of 2-amino-p-cresol (200 μM) in the presence of $K_3Fe(CN)_6$ (5 mM) in phosphate buffer, pH 7.5 (Figure 1b). The reactions were performed in triplicate and the modification was monitored by LC−MS (see Supporting Information Figure S8 for representative MS data for the modified peptides). Most N-terminal amino acids showed good-to-high levels of conversion (60−90%), but proline stood out as the only residue that showed nearly complete modification (90−100%). A second observation of this screen was the fact that tryptophan, tyrosine, and methionine residues were not oxidized by the ferricyanide reagent, consistent with our previous report of oxidative coupling with this oxidant (see Supporting Information Figure S8). However, free cysteine residues can be oxidized to various species, potentially including disulfides and sulfenic acids, and thus it is recommended that they be protected as disulfides before oxidative coupling is attempted (vide infra).

To optimize the reagent ratios (specifically the equivalents of o-aminophenol), the peptides (100 μM) were reacted with 1−10 equiv of the o-aminophenol (100−1000 μM) in the presence of ferricyanide (10 mM). After 30 min, the reactions were quenched with excess tris(2-carboxyethyl)phosphine (TCEP). It was demonstrated that conversion was highest using 2−5 equiv of the coupling partner (Figure 1c). Using more than 5 equiv of the aminophenol resulted in lower levels of peptide modification. This was most likely due to the ability of the aminophenol to react with itself at higher concentrations (∼1 mM). Consistent with this, when using 10 equiv of the o-aminophenol, a byproduct was observed with a mass that

corresponded to the condensation of 3 aminophenols (344 Da).

We also investigated the differences in coupling rates for representative N-termini. The reaction of 2-amino-p-cresol with three different peptides was monitored over the course of 1 h (Figure 1d). The peptides (100 μM) were reacted with 2 equiv of the o-aminophenol (200 μM) in the presence of ferricyanide (5 mM), and aliquots were quenched with excess TCEP at the indicated time points. The proline terminal peptide not only reached the highest level of conversion, but also did so in a significantly shorter time than the other termini. Despite efforts to optimize conditions for all N-termini, proline still stood out as the most reactive species.

Product Characterization with Small Molecule Analogues. The reaction of N-terminal amines with o-aminophenols was characterized using small molecule mimics. The methyl esters of phenylalanine (H-Phe-OMe) and proline (H-Pro-OMe) were coupled to 2-amino-p-cresol using ferricyanide at pH 7.5. The crude products were characterized using two-dimensional NMR and high-resolution mass spectrometry. The primary amine of H-Phe-OMe formed p-iminoquinone product **2**, which was analogous to the one formed with aniline coupling partners (Scheme 1, Supporting Information Figure S9). However, the secondary amine of proline prevented the formation of the p-iminoquinone tautomer, and thus favored o-quinone product **3** (Scheme 1, Supporting Information Figure S10). Given the different linkage obtained with proline, we verified the stability of the product to a variety of conditions. The proline terminal peptide, PADSWAG, was first modified with 2-amino-p-cresol. After purification, the modified peptide (100 μM) was exposed to reductants, nucleophiles and acidic and basic pH (10 mM additives or buffer). After 8−18 h of treatment, the peptides were analyzed by LC−MS. No loss of product was observed under any of the conditions tested, demonstrating the hydrolytic stability of the product (Supporting Information Figure S11). The ability of the linkage to withstand these conditions renders this method quite useful for the construction of biomolecular materials for a variety of applications. With a view toward in vivo applications, current efforts are examining the stability of the linkage in blood plasma, as well as evaluating the intrinsic immunogenicity of the o-quinone group.

In the process of characterizing the reaction products, it was observed that the colored products absorbed light at wavelengths greater than 500 nm (with λ_{max} between 505 and 525 nm depending on the amine coupling partner). As the starting coupling partners and ferricyanide did not absorb at these wavelengths, this unique absorbance provided a means to monitor the reaction progress. The different amine coupling partners (p-toluidine, H-Pro-OMe, and H-Phe-OMe) were reacted with 4-methylcatechol in the presence of 10 mM ferricyanide, and the absorbance of the resulting solution was monitored at 520 nm to determine the relative rates of reactivity (Figure 2a; for unnormalized data see Supporting Information Figure S12). The catechol substrate was used for these studies to simplify the reaction pathway by eliminating the imine hydrolysis step. The reactions were run under pseudo-first order conditions with 0.1 mM catechol and 1 mM amine coupling partner. When the reaction was carried out at pH 6.0, only the aniline coupling partner exhibited rapid coupling with the catechol. However, at pH 7.5 all three amines reacted efficiently. The aniline coupling partner demonstrated the fastest coupling (<30 s). The reaction with the proline
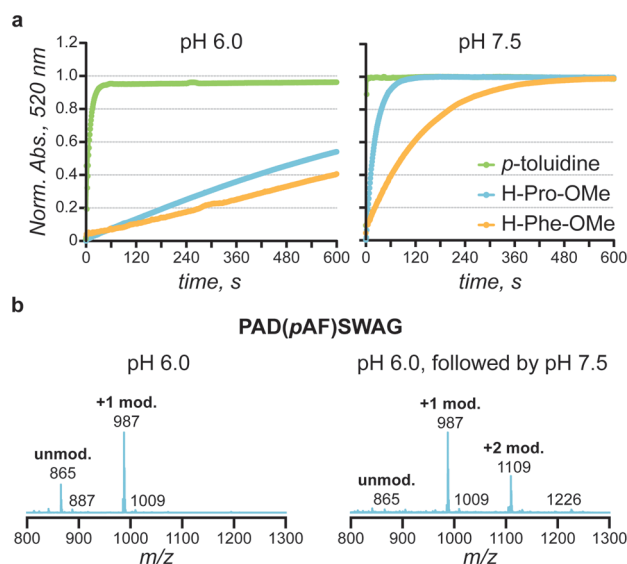
Figure 2. Characterization of the oxidative coupling reactions using small molecules. (a) Amine coupling partners were reacted with 4-methylcatechol as a model substrate. The reaction was followed by monitoring the product absorbance at 520 nm. Reactions were run under pseudo-first order conditions with 100 μM catechol, 1 mM amine, 10 mM ferricyanide in 50 mM phosphate buffer. (b) A peptide containing both an N-terminal proline and a *p*-aminophenylalanine residue (PAD(*p*AF)SWAG) was tested for reactivity with 2 equiv of 2-amino-*p*-cresol at pH 6. An aliquot of the reaction was quenched and analyzed by LC−MS. The remainder of the reaction was purified and then reacted with 2 equiv of the aminophenol at pH 7.5 and analyzed by LC−MS.

analogue reached completion nearly as rapidly (∼2 min), but the reaction with the primary aliphatic amine of phenylalanine required longer reaction times (∼10 min). This demonstrates how the reaction can have very high selectivity for aniline residues.

By quantifying product formation by absorbance, we were also able to measure the second-order rate constant for the proline-based coupling (Supporting Information Figure S13). The reaction of 1 equiv of H-Pro-OMe (100 μM) with 1 equiv of 4-methylcatechol (100 μM) and 100 equiv of $K_3Fe(CN)_6$ (10 mM) was performed in triplicate at 25 °C. The second-order rate constant for the coupling was determined to be 44 ± 4 M^{-1} s^{-1}. While proline reacted rapidly with the electron-rich coupling partner, the small molecule studies indicated that aniline should react faster. The rate for the aniline reaction was too fast under these conditions to determine the second-order rate constant accurately.

Preferential Reactivity on Anilines over N-Terminal Amines. Given the differences in reactivity observed at pH 6.0 and 7.5, we hypothesized that it would be possible to modify the aniline side chain of *p*-aminophenylalanine (*p*AF) and the N-terminal proline amine sequentially. To test this hypothesis, we synthesized a peptide containing both reactive moieties (PAD(*p*AF)SWAG). Only one modification was observed when the peptide was reacted with 2-amino-*p*-cresol at pH 6.0 (Figure 2b). MS/MS analysis of the modified peptide confirmed that the single modification occurred on the aniline side chain (Supporting Information Figure S14). After this step, the peptide was purified and subsequently reacted with 2-amino-*p*-cresol at pH 7.5. Reaction at the higher pH enabled a

second modification of the peptide substrate, at the N-terminal proline residue.

The preferential reactivity with aniline side chains was also probed using protein substrates. The differential reactivity was investigated by comparing the reactivity of a protein containing a *p*AF residue to proteins without the artificial amino acid. The *p*AF residue was introduced into the coat protein of bacteriophage MS2, which self-assembles into a spherical, hollow protein shell. Myoglobin and a mutant of the tobacco mosaic virus (TMV) coat protein were used as native protein substrates. Reactions with 2-amino-*p*-cresol were either performed on the isolated, individual proteins or with the aniline containing protein mixed with the native protein substrate (Supporting Information Figure S15). Addition of the aniline containing protein to the native protein decreased the N-terminal reactivity, indicating that the aniline residues react more rapidly than N-terminal residues with the *o*-aminophenols. In addition, MS2 showed significantly higher reactivity at all of the pHs tested, confirming preference for aniline residues.

Application of N-Terminal Oxidative Coupling to Proteins. The oxidative coupling reaction with N-terminal amino groups was first tested on proteins with native N-termini. Several proteins were reacted with *o*-aminophenol-functionalized 5 kDa PEG under the optimized reaction conditions (Figure 3). The native proteins showed moderate
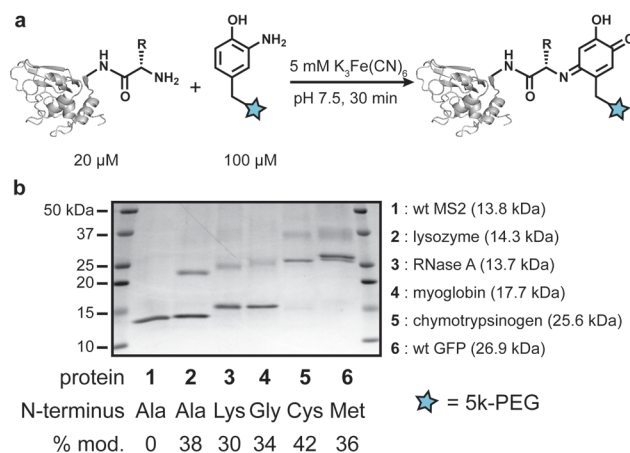
Figure 3. Protein modification with *o*-aminophenols. (a) The N-terminus of several proteins was PEGylated using *o*-aminophenol-functionalized 5 kDa PEG and ferricyanide. (b) Modification of wild type proteins with 5 kDa *o*-aminophenol-PEG was monitored by SDS-PAGE. The products appear as higher MW bands in the lanes.

levels of reactivity, which could be attributed to inaccessible N-termini or simply to the less reactive N-terminal residues. To test if proline terminal proteins were more reactive, a proline residue was introduced to the N-terminus of GFP and the tobacco mosaic virus (TMV) coat protein. The N-terminus of TMV was also slightly extended from the native sequence (addition of PAG). The proline-GFP was treated with a variety of conditions to determine the specificity of the reaction (Figure 4a). Only at basic pH in the presence of both the *o*-aminophenol substrate and the oxidant was modification observed. Additionally, the proline-terminal variant showed significantly improved reactivity compared to that of the wild-type N-terminus. These high levels of modification were maintained even when only 1−2 equiv of the *o*-aminophenol

a

b

N-terminus : **PAG**SYS TMV   N-terminus : **AG**SYS TMV
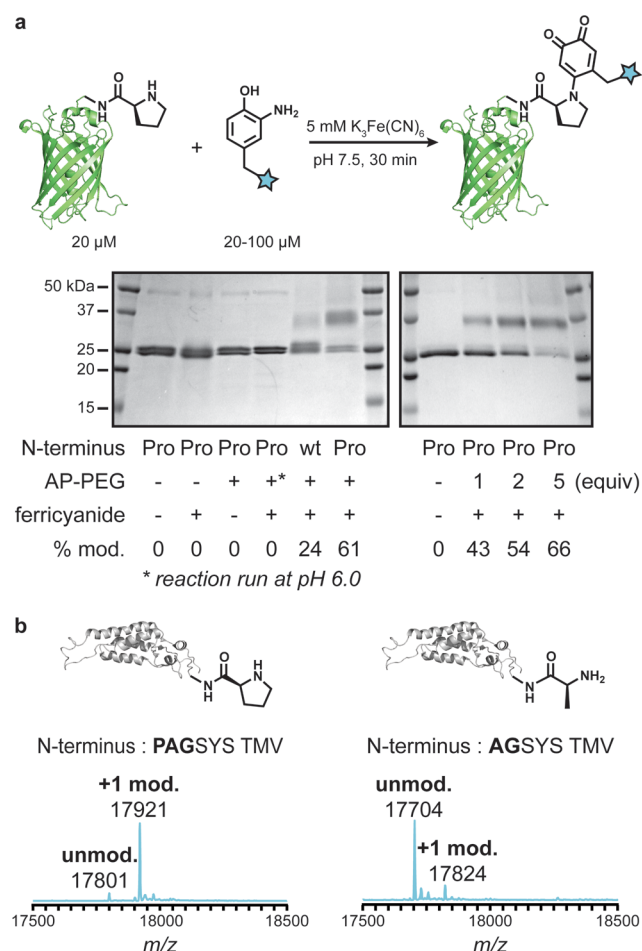
Figure 4. Effect of N-terminal proline on protein modification. (a) A proline was introduced to the N-terminus of GFP. Reactivity with o-aminophenol-PEG was monitored by SDS-PAGE. The proline terminal variant showed much higher levels of modification than the wild-type protein. No modification of N-terminal proline-GFP occurred at pH 6. The band doubling is due to a gel artifact, and appears in all lanes. (b) Mutants of TMV were reacted with 5 equiv of 2-amino-p-cresol and 1 mM $K_3Fe(CN)_6$ for 30 min and analyzed by LC−MS.

PEG was used. The site of modification was confirmed to be the N-terminal proline by LC−MS/MS analysis of a tryptic digest of proline-GFP modified with 2-amino-p-cresol (Supporting Information Figure S16).

Reaction conditions for both native and proline terminal proteins were optimized by evaluating reactivity with myoglobin and proline-GFP. The reaction time, buffer, and pH were screened (Supporting Information Figures S17 and S18). Similar to the results obtained with peptide substrates, the reaction reached its highest level of conversion after about 15−30 min. In addition, most buffer salts tested were compatible with the reaction with the exception of buffers containing morpholine (MOPS) or piperazine moieties (HEPES), as was observed with peptide substrates. These buffers decreased the level of modification slightly, but did not completely inhibit reactivity. The effect of reaction pH was tested using both $K_3Fe(CN)_6$ and $NaIO_4$ as the oxidants. Little reactivity was observed at acidic pH, and the level of conversion increased between pH 7.0 and 8.0. At higher reaction pH (≥8.0) a second modification was observed, indicating that lysines may also participate in the reaction. However, it is also

possible that under more forcing conditions, such as higher reaction pH or increased concentration of aminophenol substrate, the aminophenol reacts with both itself and the N-terminal amino group resulting in double modification of the N-terminus.

N-terminal mutants of TMV were also evaluated for their reactivity with o-aminophenols. The TMV monomers assemble into well-known double disk structures, displaying 34 copies of the N-terminal groups on their peripheries.[60] Two N-terminal mutants (PAG and AG) were reacted with 5 equiv of 2-amino-p-cresol (100 μM) and 1 mM $K_3Fe(CN)_6$ for 30 min. Analysis by LC−MS demonstrated that the proline terminal mutant reached nearly complete conversion, while the alanine terminal mutant showed low levels of modification under these coupling conditions (Figure 4b, see Supporting Information Figure S19 for wider mass range and ion series).

The compatibility of the reaction with cysteine residues was also tested using TMV. A single cysteine residue (S123C) was introduced into the TMV coat protein with a proline N-terminus (PAG S123C TMV). This mutant was reacted with 2-amino-p-cresol and analyzed by LC−MS (Supporting Information Figure S20). The cysteine residue also reacted with the o-aminophenol, resulting in two modifications.[61,62] However, it was found that the N-terminal proline could be modified selectively if the cysteine was first capped (Figure 5a,b, see Supporting Information Figures S21−22 for wider mass range and ion series). To do this, the cysteine residue was protected as a disulfide bond by reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent). After the oxidative coupling step the disulfide bond was readily reduced by TCEP, leaving the free cysteine and the modified N-terminus. Alternatively, the cysteine residue was modified with a maleimide, followed by modification at the N-terminus with an o-aminophenol reagent.

This strategy allowed for the direct, dual modification of the protein at both the cysteine residue and the N-terminus. Two fluorophores paired for Förster resonance energy transfer (FRET, Alexa Fluor 488 C_5-maleimide and o-aminophenol functionalized rhodamine B) were thus conjugated to TMV using this strategy to create a templated array of chromophores for light harvesting applications.[63−65] The free cysteine was first quantitatively labeled with an Alexa Fluor maleimide (Supporting Information Figure S22). The N-terminal proline was then coupled to a fluorescent o-aminophenol resulting in ~50% modification of the TMV monomers with both fluorophores (Figure 5b). Complete modification of the N-terminus was not observed as TMV precipitated from solution with increasing levels of modification with the rhodamine dye. In current experiments, we are using this dual-labeling strategy to introduce more soluble chromophores. We are also evaluating the energy transfer capabilities of the resulting systems.[65]

The oxidative coupling reaction was also compared to the reaction of protein amines with activated esters. This acylation methodology is commonly employed, and can be targeted to the N-terminus by controlling the reaction pH in some cases.[66] The reactions were compared on creatine kinase, a protein with a native proline N-terminus. Reaction with 1−5 equiv of o-aminophenol PEG resulted in good levels of modification of creatine kinase (~50−60%), while reaction with 1−5 equiv of N-hydroxysuccinimide (NHS) PEG resulted in low levels of modification (5−25%, Figure 6). Only when a vast excess of the NHS PEG was used were moderate levels of modification
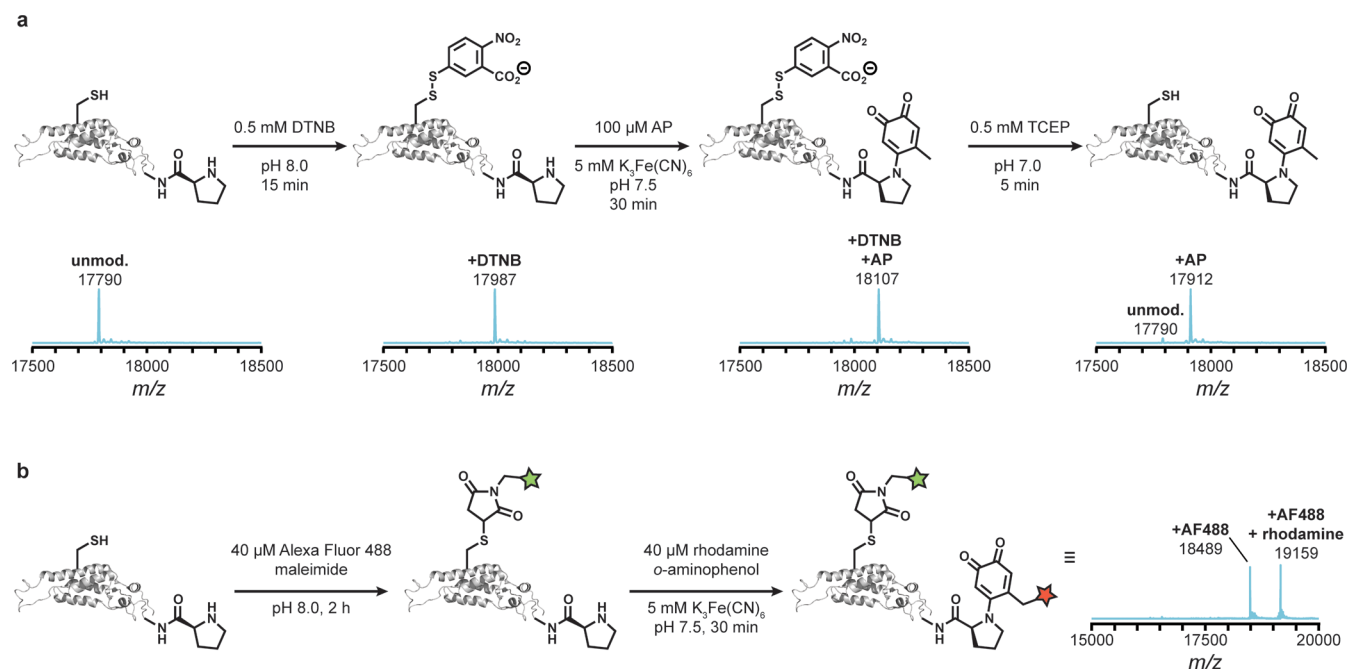
Figure 5. N-terminal oxidative coupling for proteins with free cysteines. (a) PAG S123C TMV was reacted with small molecule substrates and analyzed by LC−MS. Cysteine residues were protected as a disulfide using Ellman's reagent (DTNB) before oxidative coupling. Subsequent reduction of the disulfide resulted in selective modification of the N-terminus. (b) PAG S123C TMV was labeled with two fluorophores. The cysteine was first alkylated with an Alexa Fluor maleimide. The N-terminal proline was then modified with a rhodamine-functionalized o-aminophenol.
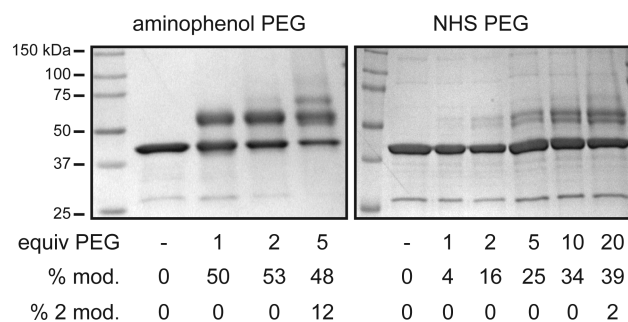
Figure 6. The modification of the N-terminus of creatine kinase with aminophenol PEG was compared to the reaction of creatine kinase with NHS PEG. The reactions were analyzed by SDS-PAGE.

	aminophenol PEG				NHS PEG					
equiv PEG	-	1	2	5	-	1	2	5	10	20
% mod.	0	50	53	48	0	4	16	25	34	39
% 2 mod.	0	0	0	12	0	0	0	0	0	2

achieved. As was the case with proline-GFP, some over modification was observed under the oxidative coupling conditions when using five or more equivalents of aminophenol. This could result from dimerization of the oxidized species before protein coupling, but has yet to be characterized due to the low abundance of this product. In any case, lowering the reaction pH slightly or using fewer equivalents of the o-aminophenol substrate prevented the over modification from occurring.

■ CONCLUSION

In this study, we have identified conditions for the oxidative coupling of o-aminophenols to N-terminal amino acids. Proline residues work particularly well with this strategy, and are therefore strongly recommended when using it. These groups can be introduced readily in N-terminal positions using site-directed mutagenesis and *Escherichia coli* expression, especially since the methionine residue resulting from the start codon is cleaved when proline is in the second position.[67] The fast kinetics of the reaction allow it to be successful even at low reagent and substrate concentrations, and suggest that it can be used for sterically demanding bioconjugations.

The oxidative coupling strategy reported here offers two distinct advantages over other N-terminal labeling methods. First, the modification occurs in a single step and does not require initial oxidation of the N-terminus. Second, the fast second-order kinetics allow for low concentrations of the coupling partners to be used. However, to achieve high levels of modification on protein substrates, proline was required as the N-terminal residue. Other methods may show a broader scope for different N-terminal residues.[42]

This new protein modification strategy is currently being explored in our lab for the generation of protein-based materials. In the larger context, new techniques for the introduction of a single functional group in a specific position on a protein surface are always in demand. The ability of the N-terminal oxidative coupling method to achieve this in a single, brief reaction step is highly advantageous, and the fact that it can be combined with cysteine modification chemistry provides new opportunities for complex bioconjugate synthesis.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Full experimental procedures and additional characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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