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Indium mediated allylation in peptide and protein functionalization†

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Indium-mediated allylation has been used in the site-selective functionalization of N-terminal aldehydes of peptides and proteins. This is the first demonstration of indium-mediated C–C bond formation in protein labelling studies under mild and environmentally friendly conditions.

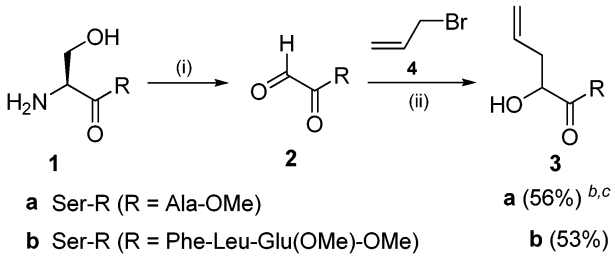
Protein bioconjugation has evolved as a powerful technique for the study of biological processes at a molecular level. Despite its well-known application the chemistry available for such transformation is rather narrow. One of the reasons is the limited use of metal catalyzed reactions which can play a significant role in expanding the tools available for protein modifications. The requirement of aqueous media, with a narrow pH, temperature window and low concentrations (below 100 μM) to preserve the structure and function of the biomolecule has made metal-mediated protein functionalization a challenging process. Yet several reports have successfully demonstrated the use of metals in this area.[1]

In their pioneering work, Bonmafous *et al.* have used Ni, Mn, Fe, Pd and Ru in the study of protein–protein interactions.[2] Francis and coworkers have used rhodium carbenoids to modify the indole functionality of tryptophan residues resulting in a mixture of N-alkylated and C-alkylated products. The same group also demonstrated the use of π-allylpalladium complexes in the alkylation of phenolate oxygens of surface-accessible tyrosines.[3] Tachibana and colleagues have used Heck coupling to modify genetically incorporated *p*-iodophenylalanine.[4] Different variations of Cu(I)-catalyzed [3 + 2] cycloaddition introduced by Sharpless and coworkers have been used to couple genetically introduced terminal alkynes with azides.[5] Elaborate genetic manipulation processes for the introduction of coupling partners, non-selective labeling leading to product mixtures, low conversion and the use of low pH limit the use of many of these strategies, thus the search for new metal mediated reactions is still important.

Metal-mediated allylation reactions are one of the most successful nucleophilic addition reactions to carbonyls leading to versatile homoallylic alcohols, which serve as building blocks in the synthesis of biologically important compounds.[6] Among them, the indium-mediated allylation discovered by Araki, Chan and Li is one of the most popular, because of its ability to be carried out in aqueous media under very mild reaction conditions.[7] In view of this, the reaction has been used by our group to functionalize water-soluble compounds such as carbohydrates.[8] In connection with our interest in the functionalization of proteins,[9] we envisaged that indium-mediated allylation reaction could be used in the chemical functionalization of biomolecules.

In this communication, we describe a novel application of indium-mediated allylation in aqueous media through the site-selective functionalization of peptides and proteins. This reaction furnishes a stable adduct and also expands the scope of metal-mediated protein functionalization by the addition of a new metal, indium (In). This was achieved through a dual approach where initially the reactive aldehyde site at the N-terminal was generated followed by indium-mediated allylation reaction to label peptides and proteins.

In our initial model studies periodate oxidation of N-terminal serine was used to furnish the N-terminal aldehyde in protected dipeptide **1a** and tetrapeptide **1b**.[10] We found that under optimum reaction conditions, when the dipeptide aldehyde **2a** was vigorously stirred with 2 equivalents of indium powder and 3 equivalents of allyl bromide **4** in 0.025 M sodium phosphate buffer (pH 7.0) for 18 h at room temperature, the allylation product **3a** was obtained in 56% isolated yield (over 2 steps). Under the same conditions the reaction with protected **2b** proceeded efficiently with comparable yield (53%) (Scheme 1).



Scheme 1 Indium mediated allylation of peptide aldehydes. *Reaction conditions*: (i) peptide **1a**, or **1b**, 2 equiv. $NaIO_4$, 10 mM sodium phosphate buffer (pH 7.0), dark, 10 min–2 h; (ii) aldehyde **2a**, or **2b**, 3 equiv. of allyl bromide **4**, 2 equiv. of indium powder, 25 mM sodium phosphate buffer (pH 7.0), rt, 18 h. [b]Yield refers to column-purified yield of the allylation product after two steps. [c]Isomeric ratios determined by ^{13}C NMR spectroscopy: **3a** (50 : 50), **3b** (60 : 40).

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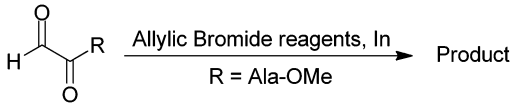
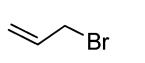
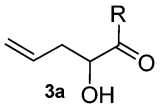
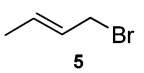
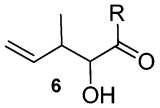
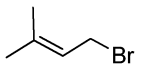
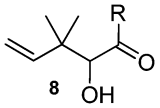
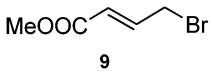
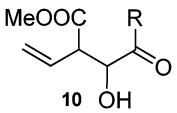
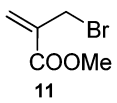
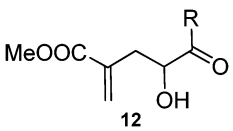
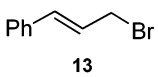
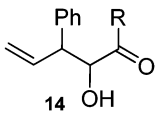
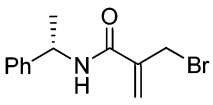
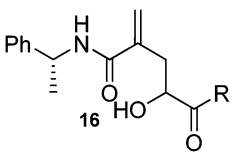
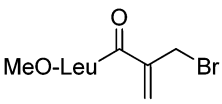
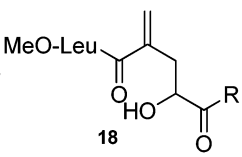
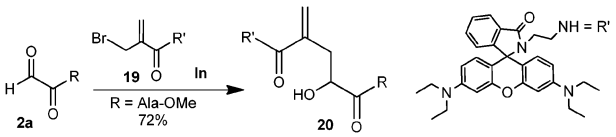
Table 1 Allylation of peptide aldehyde **2a** with various allylic bromides[a]



Entry	Allylic bromide	Product	Yield[b,c]
1	 **4**	 **3a**	56 50 : 50
2	 **5**	 **6**	50 21 : 24 : 23 : 22
3	 **7**	 **8**	45 51 : 49
4	 **9**	 **10**	34 50 : 35 : 1 : 4
5	 **11**	 **12**	19 65 : 35
6	 **13**	 **14**	25 45 : 44 : 6 : 5
7	 **15**	 **16**	42 63 : 37
8	 **17**	 **18**	46 60 : 40

[a] Reaction conditions: Scheme 1 step (ii). [b] Yield refers to column-purified yield of the allylation product. [c] Isomeric ratios determined by ^{13}C NMR spectroscopy.

Further screening with a variety of allylic bromide reagents with peptide aldehyde **2a** provided allylation products with yields ranging from 19 to 56% (Table 1). Yields comparable to compound **4** were obtained with alkyl substituted allylic bromides (Table 1, entries 1–3). Important functionalities (amines, amino acids) were successfully introduced into the peptides in the form of acrylate derivatives through this method (Table 1, entries 5, 7 and 8). Furthermore, entry 8 in Table 1 indicates that peptide conjugated proteins could be synthesized using this method. These results were very encouraging for the metal mediated functionalization of proteins.



Scheme 2 Fluorescent tagging of peptide.

The utility of this reaction was demonstrated by the successful coupling of rhodamine labeled fluorescent tag **19** to peptide **2a** in 72% yield (Scheme 2).

Pyridoxal-5′-phosphate (PLP, **21**) mediated biomimetic transamination reaction developed by Francis group was used to generate the N-terminal aldehyde in proteins.[11] This method is versatile and can be used for more classes of proteins irrespective of their N-terminus amino acid.

Application of water-based indium-mediated allylation to a protein molecule was illustrated with horse heart myoglobin. The 153-amino acid protein has an N-terminal glycine residue. To generate the N-terminal aldehyde, the procedure reported by Francis group was adopted. Protein in 75 μM concentration was reacted with 10 mM PLP for 20 h at 37 °C in 25 mM sodium phosphate buffer (pH 6.5). Following removal of excess PLP with NanoSep 10 filtration devices, the product solution was divided into two portions. One portion was incubated with 25 mM biotin amidohexanoicacid hydrazide[12] (**22**) overnight to trap the aldehyde and confirm the success of transamination,[12] while the other portion was incubated at room temperature with indium (2 equiv.) and 2.85 mM allyl bromide **4** (a solution in *t*-BuOH) (200 equiv.) in 25 mM phosphate buffer (pH 7.0) after proper pipette mixing. 60% conversion to the biotinylated product **23** was determined by liquid chromatography–electrospray ionization mass spectrometry (LC-ESI-MS) after overnight reaction (Fig. 1a and b). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis and Western blotting of the SDS-PAGE gel were used to further confirm biotinylation.[12] However to our surprise there was no evidence of allylation product **24** (Scheme 3).

The unsatisfactory result could be ascribed to the poor solubility of allyl bromide in water, together with a lack of vigorous stirring. To address this problem in the first instance the reaction was subjected to shaking on a laboratory rotisserie. It is known that proteins will lose their stability upon stirring,[13] hence the use of a cosolvent was explored. Thus the reaction was repeated in a 50% *tert*-butyl alcohol/buffer system with gentle shaking as this solvent system gave us positive results in our Mukaiyama aldol methodology.[9a] This time trace amounts of the product were obtained. After a number of experiments we identified *tert*-butyl alcohol/water (40 : 60) as the best solvent, with 54% conversion to the desired product (Scheme 3 and Fig. 1c). The partial dissociation of the heme[14] in the presence of high *tert*-butyl alcohol concentration was addressed through reconstitution experiments.[12] The circular dichroism (CD) and UV spectroscopy traces of the modified reconstituted protein and unmodified protein were identical showing the success of reconstitution experiments (Fig. 1d and e).

To confirm the site specificity of the modification, products **23** and **24** were subjected to tryptic digestion. The resulting peptide fragments were then analyzed by LC-ESI-MS which

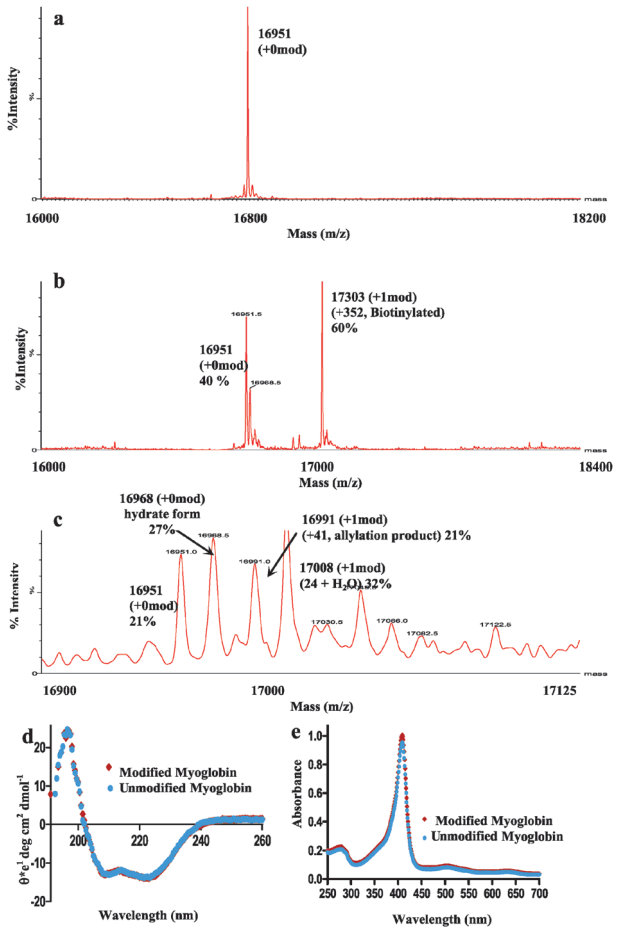
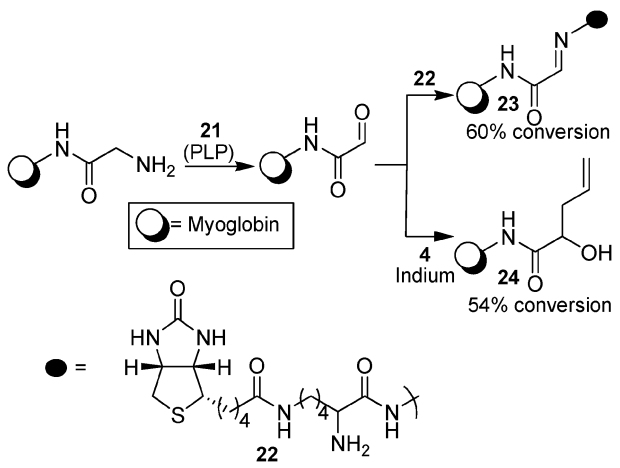


Fig. 1 Allylation of myoglobin. (a–c) LC-ESI-MS data for (a) unmodified myoglobin and the (b) biotinylated 60% and (c) allylation modified (54%) protein products. (d) CD and (e) UV traces of modified reconstituted and unmodified myoglobin.



Scheme 3 Indium mediated allylation of myoglobin.

showed that the reactions had successfully modified the protein N-terminus.

In summary, we have described here the first demonstration of indium-mediated allylation in the functionalization of N-terminal aldehydes of peptides and proteins through the formation of a stable carbon–carbon bond allowing the protein to be refolded without loss of structural stability. It equips the protein with a terminal olefin handle that opens up a wide avenue for their orthogonal modifications through ruthenium-catalyzed metathesis.[15] It complements existing methods of introducing olefins into proteins which face the challenges of non-selective reactions and undesired side product formation.[16] The use of indium as the metal-mediator adds to the growing repertoire of metal-mediated reactions in protein functionalization encouraging protein chemists to further explore into the untapped elements of the periodic table. We believe that this method has the potential to be used for the efficient labeling of proteins, with an array of functionalities thus aiding in the study and manipulation of biological functions.

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