Anodic Modification of Proline Residues of Peptides for Protein Determination

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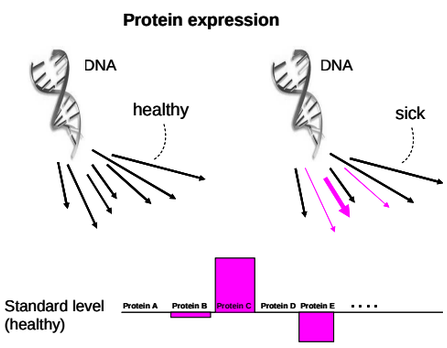
There has been considerable interest in the synthesis of unnatural and/or conformationally constrained amino acids, peptidomimetics, and small peptide fragments encompassing these residues. Of all the common α-amino acids, proline plays a particular role in peptide secondary structure formation. On the other hand, chemo-selective introduction of tags for some limited amino acid residues should be powerful weapons for isolating the fragments and identifying proteins or peptides. That is, in the course of recent proteomics researches, fine determination technologies of target peptides generated by enzymatic digestion of internal proteins became very important to identify constitutive pathogenic internal proteins and/or genes. In order to pick-up those target peptides, it is important to introduce marker tags on a defined kind of amino-acid residues in the mixture of peptides or modified peptides. This helps to purify and determine the peptides containing modified amino-acid. In this case, selective modification of proline residue is one of the most promising reactions for the identification and determination of peptides.



Electrochemical means are among the most useful methods for the modification of proline derivatives. It has been revealed that an amide or carbamate is oxidized to generate an iminium cation and that trapping of the iminium cation with the methoxy group leads to a product that has been functionalized on the carbon α to nitrogen.

It is, however, difficult to oxidize the starting material without affecting some nucleophiles because the oxidation potentials of nucleophiles are usually lower than those of the electrolytic substrates. For example, in anodic oxidation systems, a divided cell is often introduced to avoid cathodic re-reduction of electrogenerated products or their undesired reactions with cathodic products, but the application of higher electrolytic potentials is generally required, and there is an accumulation of electrogenerated acid accompanied by the generation of the products. This gave us the incentive to develop an extended, simple electrochemical method that would enable anodic generation and accumulation of unstable N-acyliminium cations of prolines in an undivided system for their diverse functionalization.

This paper describes novel method for the introduction of functional groups on the carbon α to nitrogen of proline moieties of peptides. A practical new pathway to the synthesis of substituted proline derivatives has been developed by using anodic reaction system. It was found that functionalized proline derivatives were efficiently generated[1] *via* the formation of corresponding iminium cation derivatives and trapping by nucleophiles in a lithium perchlorate / nitromethane solution[2] in mild conditions. In addition to the simple proline derivatives, model compounds of the proline-residue-containing peptides were also converted to the corresponding derivatives which enabled the identification of internal peptides and proteins.

Results and Discussion

In order to perform anodic introduce of tags for proline residues in various peptide mixture, anodically oxidizable amino-acid residues such as phenylalanine and methionine residues must be protected before anodic oxidation. It was found that phenylalanine and tyrosine were easily converted to corresponding cyclohexyl derivatives by a catalytic hydrogenation. These hydrogenated amino-acid residues were not oxidized during the anodic generation and tag-introduction for the iminium cation of proline moiety. Furthermore, sulfur-containing amino acid residues were beforehand oxidized to corresponding sulfones. There pretreatments were effective to avoid the generation of unexpected product through the anodic tag-introduction for proline moieties.

In conclusion, anodic introduction of pheylsulfanyl groups for proline-residue was accomplished in lithium perchlorate / nitromethane electrolyte solution. Even in the presence of benzene rings, sulfides, thiol groups, chemical pretreatment for anodically stable functional groups helped aimed selective activation of proline-residues. As the proline residues are distributed in most of all expressed proteins, the tag-introduced peptides should work as determination-marker of the expressed proteins

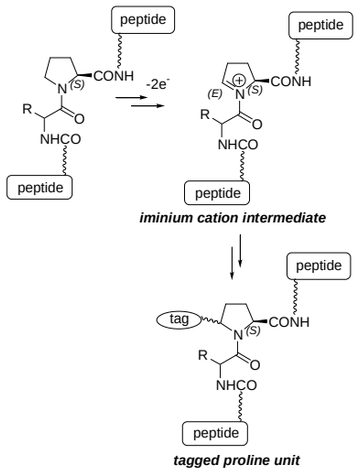


Fig. 1 Synthetic strategy for tagged peptide on C α to N of proline

References

1 Kim, S; Hayashi, K.; Kitano, Y.; Tada, M.; Chiba, K. *Org. Lett*., **2002**, 3735.
2 Chiba, K.; Miura, T.; Kim, S.; Kitano, Y.; Tada, M. *J. Am. Chem. Soc.* **2001,** *123*, 11314.