カルボキシペプチダーゼYによるヒドラジノリシスを利用した配列非依存的ペプチド・タンパク質チオエステル調製法の確立

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Sequence-independent traceless method for preparation of peptide/protein thioesters using CPaseY-mediated hydrazinolysis

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ABSTRACT　Proteins incorporating artificial moieties such as fluorophores and drugs have enjoyed increasing use in chemical biology and drug development research. Preparation of such artificial protein derivatives has relied mainly on native chemical ligation in which peptide/protein thioesters chemoselectively react with Nterminal cysteine (Cys) peptides to afford protein molecules. The protein thioesters derived from expressed proteins represent thioesters that are very useful for the preparation of artificial proteins by native chemical ligation with synthetic peptides with N-terminal Cys. We recently have developed a traceless thioesterproducing protocol using carboxypeptidase Y (CPaseY) which is compatible with an expressed protein. The traceless character is ensured by CPaseY-mediated hydrazinolysis of C-terminal Xaa (X)-Cys-proline (Pro)-leucine (Leu)-OH sequence followed by an auto-processing of the Cys-Pro (CP) dipeptide unit, affording the corresponding X-thioester (X-SR). However, hydrazinolysis of the amide bond in the prolyl leucine junction depends significantly on the nature of X. In the case of hydrophobic X residues, the hydrazinolysis overreacts to give several hydrazides while the reaction of hydrophilic X residues proceeds slowly. In this research, we attempted to develop an X-independent CPaseY-mediated protocol and found that the incorporation of a triple CP sequence into the C-terminal end (X-(CP)3-Leu-OH) allows for efficient X-SR formation in a manner that is independent of X.

抄録　ケミカルバイオロジー分野において、非天然部位を導入したタンパク質の需要が高まっている。これらタンパク質の調製法の一例として、発現タンパク質のC末端をチオエステル化したものと、非天然部位およびN末端システインを有する化学合成ペプチドの Native Chemical Ligation (NCL) が挙げられる。著者らは以前、カルボキシペプチダーゼ Y によるヒドラジノリシスを利用した発現タンパク質C末端チオエステル化法を報告した。本論文ではこれを改良し、反応速度の配列依存性を下げることで汎用性を向上させることに成功した。

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