

**Aspergillus melleus由来のセミアルカリプロ  
テイナーゼのトリプトファン及びヒスチ  
ジン残基の化学修飾**

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**Chemical Modification of Tryptophan and Histidine Residues  
in Semi-alkaline Proteinase from *Aspergillus melleus***

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**ABSTRACT** The amino acid residues responsible for the enzyme activity of semi-alkaline proteinase from *Aspergillus melleus* were identified by means of chemical modification studies. The modification of the enzyme with *N*-bromosuccinimide (NBS) resulted in the loss of the enzyme activity and a subtle alteration of conformation. NBS-modified enzyme still retained the antigenic structure, but became labile to heat and pH as the extent of modification of tryptophan increased. The relation between the extent of tryptophan oxidation and the enzyme stability suggested that 1 of the 3 tryptophan residues is important for the maintenance of structural integrity of the enzyme. The dye-sensitized photooxidation of the enzyme led to the loss of the enzyme activity with first-order kinetics. The rate of inactivation of this enzyme was pH-dependent and the rate constant-pH profile gave a sigmoidal curve with an inflection point at pH6.5. Amino acid analysis of photooxidized enzyme indicated that the inactivation of this enzyme was directly proportional to the loss of histidine residue. Thus, these result suggested that at least 1 histidine residue is involved in the active site of the enzyme.

抄録 *Aspergillus melleus* 由来のセミアルカリプロテイナーゼの活性中心について検討した。*N*-ブロモサクシニミドによる酸化と酵素の安定性の関係から3分子のトリプトファン残基の中の1残基がこの酵素の構造維持に重要であることが分かった。さらに色素増感光分解により酵素活性は1次反応速度に従って減少した。また光分解酵素のアミノ酸分析の結果から、6ヒスチジン残基の中1残基が失なわれており、少なくとも1分子のヒスチジン残基が活性に必須であることが判明した。

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