

Aspergillus melleusより得たセミアルカリ プロテイナーゼの自己消化

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Chemical & Pharmaceutical Bulletin, **32** (8), 3105–3110 (1984)

Autolysis of Semi-alkaline Proteinase from *Aspergillus melleus*

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ABSTRACT The autolysis of semi-alkaline proteinase (SAP) purified from *Aspergillus melleus* was studied from the viewpoint of enzyme stability. We also prepared inactive phenylmethylsulfonyl semi-alkaline proteinase (PMS-SAP) in order to avoid the influence of autolysis and used it to study the denaturation profile or structural change in urea solution. Experiments with native SAP and PMS-SAP were performed in parallel under same conditions. It was found that the rate of inactivation of this enzyme, as determined from the decrease in enzyme activity, followed first order kinetics and that there was a good relationship between the degree of inactivation and the amount of autolyzed products during urea treatment. The rate of urea denaturation of PMS-SAP was followed by high-performance liquid chromatography (HPLC) and circular dichroism (CD) spectral measurement; the denatured enzyme could be completely separated from the intact PMS-SAP by HPLC. The results suggested that the inactivation of enzyme was a result of the denaturation, which was accompanied by conformational change. Thus, it seems likely that the cause of the inactivation of SAP is denaturation rather than autolysis, because during autolysis, SAP was proteolyzed through the denatured form produced in the process of inactivation.

抄録 抗炎症薬として用いられる *Aspergillus melleus* 由来のセミアルカリプロテイナーゼ (SAP) の自己消化について酵素製剤の安定性の観点から検討した。この酵素の失活は変性と自己消化との過程とで進行するので両者による失活機序を分離して解析することが困難である。そこでタンパク分解活性を有しないフェニルメチルスルホニルセミアルカリプロテイナーゼを製し、これとSAPとの失活速度を比較解析した。その結果、SAPの失活は1次反応速度に従うこと、変性過程が律速段階であること、変性した酵素がさらに自己消化により分解することなどを明らかにした。

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