

## カルボキシペプチダーゼ C<sub>N</sub> の構造と機能

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### The Structure and Function of Carboxypeptidase C<sub>N</sub>

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**ABSTRACT** Carboxypeptidase C<sub>N</sub> from *Citrus natsudaidai* HAYATA is an acidic protease (*M*, 93000). The enzyme was inactivated by the incorporation of 1.0 mol of <sup>32</sup>P-labeled diisopropylphosphofluoridate, 1.3 mol of <sup>203</sup>HgCl<sub>2</sub>, or 2.2 mol of <sup>110m</sup>AgNO<sub>3</sub> per mol of enzyme at pH 5.5. Four or five radioactive peptides were isolated from a partial acid hydrolysate of the <sup>32</sup>P-labeled enzyme. These peptides provided evidence indicating the amino acid sequence Glx-Gly-Asx-Ser-Gly-Gly-Glu-Leu-Val around the reactive serine residue.

The enzyme reacted with 0.4 mol of <sup>203</sup>Hg-labeled *p*-chloromercuribenzoate without appreciable loss of enzymatic activity. On the other hand, 1 mol of <sup>203</sup>Hg-labeled *p*-chloromercuribenzoate was incorporated into the enzyme which had been denatured with 6 M guanidine hydrochloride and 8 M urea at pH 7.0 in the absence of dithiothreitol. Thus, the enzyme has one sulfhydryl group which possesses only poor reactivity.

Photooxidation using methylene blue as a sensitizer caused a loss of enzymatic activity and specific destruction of approximately 1 mol of histidine residue per mol of enzyme. The photooxidized, *p*-bromophenacyl bromide-treated and HgCl<sub>2</sub>-treated enzyme failed to react with <sup>32</sup>P-labeled diisopropylphosphofluoridate. From these findings, we inferred that one serine, one histidine, and the carboxyl groups are essential for catalytic activity.

抄録 ナツミカン外果皮から得たカルボキシペプチダーゼ C<sub>N</sub> は、分子量 93000の酸性プロテアーゼであって、 [<sup>32</sup>P]-iPr<sub>2</sub>PF, <sup>203</sup>HgCl<sub>2</sub> や <sup>110m</sup>AgNO<sub>3</sub> などが種々の程度に取り込まれ、阻害をうけた。活性セリン残基近傍のアミノ酸配列は、 Glx-Gly-Asx-Ser-Gly-Gly-Glu-

Leu-Val であった。0.4 mol の  $[^{203}\text{Hg}]$ -pCMB が酵素中に取り込まれても活性の減少は見られなかったが、塩酸グアニジンや尿素で変性させると、最終的に 1 mol の試薬が取り込まれ、SH 基が存在することが分った。メチレンブルーを用いる光酸化によって、1 mol のヒスチジン残基が破壊され、活性が消失した。光酸化、pBPB や  $\text{HgCl}_2$  などで処理した酵素は  $i\text{Pr}_2\text{PF}$  と反応しなかった。これらの結果から、本酵素の触媒部位には 1 個のセリン、ヒスチジンおよびカルボキシル基が必須であることが示唆された。

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