

Catalytic Activity of a Cysteine-containing Esterase Model

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THE pentapeptide L-threonyl-L-alanyl-L-cysteinyl-L-histidyl-L-aspartic acid (I) has been prepared and tested for activity as a catalyst for the hydrolysis of *p*-nitrophenyl acetate. This peptide can be considered as an analogue of the pentapeptide (II) which has the sequence present at the active site of phosphoglucomutase,¹ the L-serine residue of (II) being substituted by an L-cysteine residue in (I).

It has been shown² that peptide (II) exhibits an hydrolytic-catalytic activity³ towards *p*-nitrophenyl acetate as is true for some enzymes containing a serine residue at their active site *e.g.*, chymotrypsin, subtilisin, *etc.*,⁴ and for glyceraldehyde 3-phosphate dehydrogenase⁴ which bears a cysteine residue at its active site.

The cysteine-containing pentapeptide (I) has also been shown to catalyze the hydrolysis of *p*-nitrophenyl acetate under the conditions reported to be used² for the corresponding serine-containing pentapeptide (II). First-order rate plots for the liberation of *p*-nitrophenol in the presence of (I)

were linear from about 10% to about 70% of completion of the reaction. The concentration of *p*-nitrophenyl acetate was $3.09 \times 10^{-5}M$ and the peptide (I) concentration ranged from $1.55 \times 10^{-4}M$ to $4.65 \times 10^{-4}M$ [in phosphate buffer 0.2M, pH 7.7 containing 5% dioxan (v/v), at 23–24°].

The catalytic coefficient³ k_2 expressed in $l.mole^{-1} min.^{-1}$ was 30* for (I) compared with 31 for glutathione, 32 for cysteine hydrochloride, 3.4 for histidine hydrochloride (lit.,² gives 5.86), 23.3 for imidazole (lit.,² gives 20), and 1.1×10^4 for α -chymotrypsin (lit.,³ gives 10^4). Sheehan reports² a value of 92 for the pentapeptide (II).

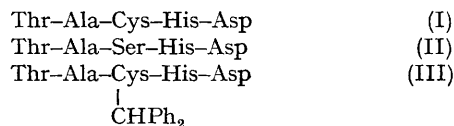
A free SH group in pentapeptide (I) is essential for the catalytic activity since the S-protected pentapeptide (III) has a catalytic coefficient of 6.7, measured under the conditions described above with the modification that the buffer system contained 10% dimethylformamide in place of dioxan. Under these conditions the k_2 for peptide (I) was 34.

It is obvious that substitution of the nucleophile

OH by SH reduces but does not abolish completely the hydrolytic-catalytic effect of the pentapeptide sequence (II).⁶ On the other hand the fact that the value of k_2 for (I) is close to that found for cysteine or glutathione, does not permit conclusions concerning the effect of the neighbouring amino-acids and the conformation of the molecule, on its catalytic activity.

A report on the synthesis of pentapeptide (I) with

further details concerning its catalytic activity will be presented elsewhere.



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* This value could be somewhat higher because the cysteinyl pentapeptide (I) showed a free SH-group 85% of the theory as measured by the Ellman method (ref. 5).

¹ C. Milstein and F. Sanger, *Biochem. J.*, 1961, **79**, 456; C. Milstein, *ibid.*, 1966, **100**, 40 C.

² P. Cruickshank and J. Sheehan, *J. Amer. Chem. Soc.*, 1964, **86**, 2070; J. Sheehan, G. Bennett, and J. Schneider, *ibid.*, 1966, **88**, 3455.

³ E. Katschalski, G. D. Fasman, E. Simons, E. R. Blout, F. R. N. Gurd, and W. L. Koltun, *Arch. Biochem. Biophys.*, 1960, **88**, 361.

⁴ R. A. Oosterban and J. A. Cohen in: "Structure and activity of enzymes", Federation of European Biochemical Societies Symposium No. 1, ed. T. W. Goodwin, J. Harris, and B. S. Hartley, Academic Press, London and New York, 1964, p. 89.

⁵ G. Ellman, *Arch. Biochem. Biophys.*, 1959, **82**, 70.

⁶ The same substitution when made at the active site of the enzyme subtilisin leads to the formation of "thiol-subtilisin" which still acts as a catalyst *e.g.*, in the hydrolysis of *p*-nitrophenyl acetate: L. Polgar and M. L. Bender, *J. Amer. Chem. Soc.*, 1966, **88**, 3153.