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 shown[2] that peptide (II) exhibits an hydrolytic-catalytic activity[3] 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 dehydrogenase[4] 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 used[2] 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 $3{\cdot}09 \times 10^{-5}$M and the peptide (I) concentration ranged from $1{\cdot}55 \times 10^{-4}$M to $4{\cdot}65 \times 10^{-4}$M [in phosphate buffer 0·2M, pH 7·7 containing 5% dioxan (v/v), at 23—24°].

The catalytic coefficient[3] 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 $1{\cdot}1 \times 10^4$ for α-chymotrypsin (lit.,[3] gives 10^4). Sheehan reports[2] 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.

Thr–Ala–Cys–His–Asp (I)
Thr–Ala–Ser–His–Asp (II)
Thr–Ala–Cys–His–Asp (III)
|
$CHPh_2$

(*Received, February 13th*, 1967; *Com.* 139.)

* 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).

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[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.