

CATALYTIC PROPERTIES OF PEPTIDES WITH HYDROLYTIC ACTIVITY

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1. Introduction

The functional groups of the amino acids are the tools of catalysis in those enzymes which operate without coenzymes and cofactors [1,2]. Among the functional groups of proteins the imidazole, thiol and carboxyl groups are the most interesting ones with respect to their possible role in enzyme catalysis. While the mechanism of action of these groups in monofunctional catalysis has been thoroughly investigated [3], there exist only few studies on multifunctional cooperative catalysis including imidazole, thiol and carboxyl groups [4–7]. We therefore have synthesized a number of peptides containing histidine, cystein and aspartic acid and have looked for possible cooperative effects of the functional groups by an extensive kinetic analysis of their catalytic properties in ester hydrolysis. In the present communication we report the first results of our studies on the catalytic properties of the synthetic peptides.

2. Material and methods

p-Nitrophenylacetate (NPA) and Boc-L-Ala-ONp were synthesized according to [8] and [9] respectively. The synthesis of the peptides studied is described in [10]. Peptide disulfides were reduced with mercaptoethanol. 5,5'-Dithio-bis(2-nitro-benzoic acid) Ellman reagent was from Serva, Heidelberg. Buffer substances were p.a. grade from Merck, Darmstadt. Argon was purchased from Messer Griessheim.

2.1. *pK'*-values of the SH and imidazole groups of the peptides

The *pK'*-values of the SH groups of the peptides were determined by photometric titration according to Benesch and Benesch [11]. A phosphate borate buffer was used for the measurement of the extinction of the thiolate anion between pH 6 and 12. All buffer solutions were freed from oxygen by argon. The *pK'*-values of the imidazole residues were determined by potentiometric titration in 0.1 M NaCl in the pH range from 4–9. A pH-stat arrangement from Radiometer Copenhagen was used. All measurements were performed under argon.

2.2. Measurement of ester hydrolysis

Hydrolysis of the esters NPA and Boc-L-Ala-ONp was followed spectrophotometrically at 405 nm with a Beckman Acta III photometer with automatic registration. All measurements were performed under first order reaction conditions. The ionic strength of the solutions was brought to 0.2 M by addition of NaCl. The reactions were started in a cuvette containing 3 ml buffer and 10^{-2} M of catalyst by addition of 30 μ l 10^{-2} M ester. For the measurement of the pH dependence of the rate of hydrolysis a Sørensen phosphate buffer pH 6–8 and a borate buffer pH 8–9 was used. The buffers were freed from oxygen by argon. Ester substrates and catalysts were dissolved in methanol, except Z-His-Ala-Asp-Gly-Cys-NH₂ and Z-His-Ala-Asp-Gly-OCH₃ which were soluble in buffer. The observed first order rate constants k_b were calculated

from $k_b = \frac{2,303}{t} \log \frac{E_{\infty} - E_o}{E_{\infty} - E_t}$; in those cases where

Table 1
Second-order rate constants k_2 of ester hydrolysis catalysed by synthetic peptides and pK values of the imidazole and SH groups at 25°C

Peptides	pK_{SH}	pK_{im}	$k_2 \times 10^{-2}$ at pH 7.5 ($l \times mol^{-1} \times min^{-1}$)	
			NPA	Boc-Ala-ONp
Z-Asp-Cys-NH ₂	8.78 \pm 0.04	—	7.186	65.40
Z-Asp-Gly-Cys-NH ₂	9.02 \pm 0.02	—	8.592 ^b	105.66
Z-Glu-Gly-Cys-NH ₂	9.03 \pm 0.03	—	12.210 ^b	131.04 ^a
Z-His-Ala-Gly-Gly-Cys-NH ₂	8.77 \pm 0.03	6.42 \pm 0.04	15.312 ^b	158.64 ^a
Z-His-Ala-Asp-Gly-OCH ₃	—	6.83 \pm 0.02	0.198	0.60
Z-His-Ala-Asp-Gly-Cys-NH ₂	9.28 \pm 0.04	6.82 \pm 0.03	25.344 ^a	246.12 ^a

^a k_2 pH dependent.

^b k_2 pH dependent above pH 8.0.

E_∞ could not be determined exactly, the method of Guggenheim [12] was used for the calculation of the rate constants. The relation between the observed rate constant k_b , the first order rate constant k_1 , the apparent second order rate constant k_2 and the true second order rate constant k_2 is given by the following equations: $k_1 = k_b - k_w$ (k_w = rate constant of the

spontaneous hydrolysis). $k'_2 = \frac{k_1}{c}$ (c = concentration of catalyst). $k_2 = \frac{k'_2}{\alpha} = \frac{k_1}{\alpha \cdot c}$ (α = degree of dissociation

of the catalytically active group, which is calculated from the Henderson-Hasselbalch equation $pH - pK =$

$$\log \frac{\alpha}{1-\alpha}.$$

3. Results and discussion

3.1. The peptides and their pK' -values

The peptides studied together with the pK' -values of the SH and imidazole groups are shown in table 1. A characteristic structural feature of the peptides is the presence of carboxyl, thiol and imidazole groups in different combinations and arrangements, thereby varying the possibilities of approximation and cooperation of the potential catalytically active groups. The amino groups and C-terminal carboxyl groups of the peptides are protected to avoid any effects of these groups on the catalytic behaviour.

With regard to the pK' -values of the SH groups, which are most important for the catalytic properties, the peptides may be divided into three groups with pK_{SH} values of 8.8, 9.0 and 9.3. Concerning the pK_{im} values peptides with pK of 6.4 and 6.8 may be distinguished. We suggest, that the significant differences in the pK' -values are not only a consequence of the structural differences of the peptides but also of different interactions of the functional groups produced by different conformations of the peptides in solution.

3.2. Catalytic properties

The catalytic properties of the peptides were tested with NPA and Boc-L-Ala-ONp as ester substrates. Since these esters were also studied as substrates for several proteinases, a comparison of the activity of the peptides and hydrolytic enzymes is possible. The pH and temperature dependence of the apparent second order rate constants of the hydrolysis of NPA and Boc-L-Ala-ONp catalyzed by Z-His-Ala-Asp-Gly-Cys-NH₂ is demonstrated in fig.1a and 1b. The rate constants increase with increasing pH because the catalytically active SH and imidazole groups only operate in the deprotonated state [3]. The rate constants of the hydrolysis of the two esters differ by one order of magnitude, NPA being the more stable compound. The activation energies for spontaneous hydrolysis of NPA and Boc-L-Ala-ONp are 85.4 kJ/mol and 63.8 kJ/mol respectively

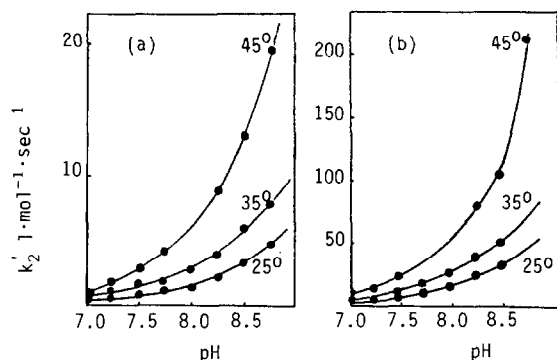


Fig. 1. pH and temperature dependence of k'_2 of the hydrolysis of a) NPA and b) Boc-Ala-ONp catalysed by Z-His-Ala-Asp-Gly-Cys-Cys-NH₂.

[10]. The real catalytic potency of the peptides manifests itself in the second order rate constants k_2 which should be pH independent. Investigation of the pH dependence of k_2 revealed however, that for some peptides the second order rate constants are not constant in the pH range tested as is demonstrated in fig. 2a and 2b for both esters. One recognizes that the strongest pH dependence of k_2 is found for the peptides Z-His-Ala-Asp-Gly-Cys-NH₂ and Z-His-Ala-Gly-Gly-Cys-NH₂ and further that the k_2 -values increase with decreasing pH. It looks as if k_2 is apparently too high at low pH because α , which is calculated from the pK' -values of the strongest nucleophilic SH groups is too low. In other words the concentration of the catalytically active species at low pH is higher than is expressed by α -c. With increasing pH this effect becomes smaller because α approaches one if all SH groups are present in the deprotonated state.

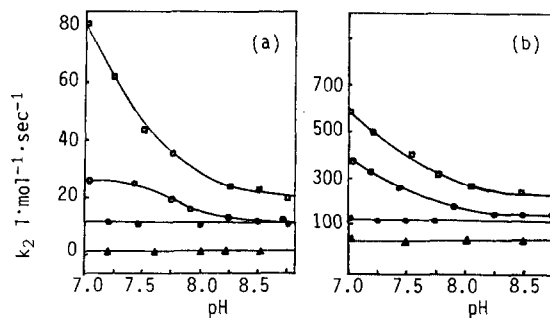


Fig. 2. pH dependence of k_2 at 35°C of the reaction of Z-His-Ala-Asp-Gly-Cys-NH₂ (□—□) Z-His-Ala-Gly-Gly-Cys-NH₂ (○—○) Z-His-Ala-Asp-Gly-OCH₃ (▲—▲) Z-Asp-Cys-NH₂ (●—●) with (a) NPA and (b) Boc-L-Ala-ONp.

From these results we conclude that the nucleophilic reactivity of the SH groups of some of the peptides is higher than is expressed by their pK' -values. We suggest that this is a consequence of cooperative effects of the catalytically active groups especially between SH and imidazole as was shown by us previously with low molecular weight imidazole-SH compounds such as 4(β -mercaptoethyl)-imidazole and 4-mercapto-methyl-imidazole [4,5,6].

An immediate comparison between the catalytic efficiency of the peptides and of SH-proteinases is possible on the basis of the second order rate constants of the peptides and the k_{cat}/K_M values of the enzymes. This comparison shows (table 2) that under nearly identical conditions the catalytic efficiency of streptococcal proteinase is 10 times and that of papain is three orders of magnitude greater than the activity of the most active peptides with Boc-L-Ala-ONp as

Table 2
Comparison of the catalytic efficiency of the most active peptides and of SH proteinases with Boc-L-Ala-ONp as substrate

Peptides	k_2 at 25°C ($1 \times \text{mol}^{-1} \times \text{sec}^{-1}$)	Enzymes	k_{cat}/K_M 25°C ($1 \times \text{mol}^{-1} \times \text{sec}^{-1}$)
Z-His-Ala-Gly-Gly-Cys-NH ₂	388 ^a	Streptococcal Proteinase	7.0×10^3 ^b
Z-His-Ala-Asp-Gly-Cys-NH ₂	600 ^a	Papain	10.5×10^5 ^b

^a At pH 6.5.

^b At pH 5.5 [11].

substrate. These data can however serve only for orientation since with other substrates [3,4] other relations are observed. Further kinetic studies are underway to confirm the interpretation of our experiments.

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