

Kinetic Analysis of the Catalytic Properties of Peptides in Ester Hydrolysis

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(Z. Naturforsch. 31 c, 675—678 [1976]; received September 20, 1976)

Kinetic Properties, Peptides, Ester Hydrolysis

The catalytic properties of peptides containing histidine, cysteine and aspartic acid in ester hydrolysis were studied. Saturation kinetics were found for the reaction of *p*-nitrophenyl acetate (NPA) with Z-His-Ala-Asp-Gly-Cys-NH₂ and Z-His-Ala-Gly-Gly-Cys-NH₂. The Brönsted equation for the hydrolysis of *tert*-butyloxycarbonyl-L-alanine-*p*-nitrophenylester (Boc-Ala-ONp) catalyzed by simple imidazole and SH-compounds was determined. The catalytic behaviour of the peptides in ester hydrolysis could not be described by the Brönsted equations for imidazole or thiole catalyzed hydrolysis of NPA and Boc-Ala-ONp. The pH dependence of the rate constants of the catalyzed ester hydrolysis gave no linear plots in $1/k$ versus H^+ diagrams.

In a previous communication we have described the synthesis of a number of peptides containing histidine, cysteine and aspartic acid¹ and have further reported the first experiments on their catalytic properties in ester hydrolysis^{1,2}. These investigations have shown, that the catalytic efficiency of the peptides changes considerably with their structure, but it became also evident, that structural differences alone cannot account for their different catalytic activities. It is rather to be assumed that cooperative effects of their functional groups are responsible for the enhanced hydrolytic activity of some of the peptides studied. We therefore have performed an extensive kinetic analysis of their catalytic behaviour. We suggest that this is a reasonable approach to look for cooperative effects possible in peptides with different catalytically active functional groups, though studies on the conformation of the peptides in solution would also be valuable.

In the present paper we describe the results of our experiments on 1. the relation between v and S in the catalysis of the hydrolysis of *p*-nitrophenyl acetate as substrate, 2. the evaluation of the Brönsted equation for the hydrolysis of Boc-Ala-ONp catalysed by imidazole and SH compounds, 3. the validity of the Brönsted law for the reaction of the peptides with *p*-nitrophenyl acetate and Boc-Ala-

ONp and 4. the determination of the kinetic pK -values from the pH dependence of the rate constants.

Materials and Methods

The syntheses of the peptides studied are described¹⁻³; their SH content was estimated with Ellman reagent⁴. *p*-nitrophenyl acetate (NPA), ethylmercaptane, cysteine, imidazole and benzimidazole were from Merck, Darmstadt. Boc-Ala-ONp⁵, 4-phenylimidazole⁶, 4-hydroxyethylimidazole⁷ were synthesized according to the references given. The measurement of the rate constants of ester hydrolysis and the determination of the pK values of the SH and imidazole groups of the peptides are described^{2,3}. The rate constants for the evaluation of the Brönsted equation were determined in a 0.15 M phosphate buffer pH 7.5 at 25 °C. For the calculation of the second order rate constants see l. c. 2.

Results and Discussion

The v/S -diagram of the hydrolysis of NPA

The relation between substrate concentration and rate of ester hydrolysis at fixed concentration of the catalyst was measured only with NPA as substrate because the poor solubility of Boc-Ala-ONp did not allow measurements at higher concentrations of this ester. The results of the experiments with NPA are shown in Fig. 1. From the v/S diagram (Fig. 1 a and b) for the pentapeptides Z-His-Ala-Asp-Gly-Cys-NH₂ (IV) and Z-His-Ala-Gly-Gly-Cys-NH₂ (VI) apparent saturation kinetics are observed, while with the other peptides a linear increase of the

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Abbreviations: NPA, *p*-Nitrophenylacetate; Boc-Ala-ONp, *tert*-butyloxycarbonyl-L-alanine-*p*-nitrophenylester.

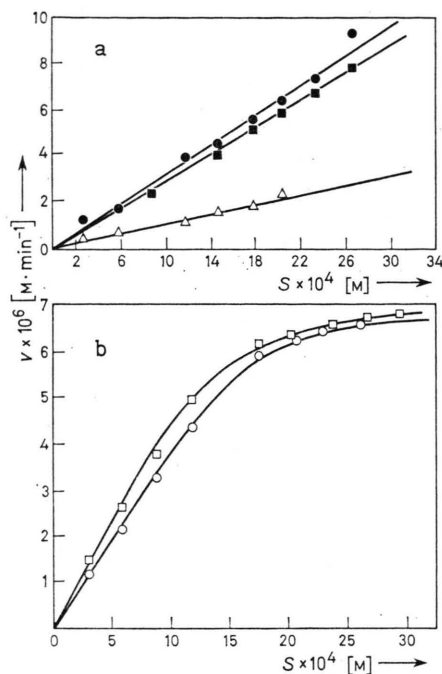


Fig. 1. v/S diagram of the hydrolysis of NPA catalysed by: a) Z-His-Ala-Asp-Gly-OCH₃ $\triangle$ — $\triangle$, Z-Asp-Gly-Cys-NH₂ $\blacksquare$ — $\blacksquare$, Z-Asp-Cys-NH₂ $\bullet$ — $\bullet$. b) Z-His-Ala-Asp-Gly-Cys-NH₂ $\square$ — $\square$ and Z-His-Ala-Gly-Gly-Cys-NH₂ $\circ$ — $\circ$. Conditions 0.2 M phosphat buffer pH 7.59, $t=35^\circ\text{C}$, concentration of catalyst 50 μM .

initial rate of hydrolysis with increasing ester concentration was observed. A Lineweaver Burk diagram for the hydrolysis of NPA catalysed by the pentapeptides reveals $K_{m\text{app}}$ values in the range of 1 mM. It seems that the hydrolysis of NPA catalysed by the pentapeptides involves either bind-

ing of the substrate, followed by acylation and deacylation as described for several proteinases⁸, or a mechanism involving a change in rate determining step with increasing substrate concentration. Formation of S-acetyl derivatives on reaction of thiols with NPA is well established⁹ as well as the catalysis of the deacylation by imidazole⁹.

Validity of the Brönsted law

Further information on the catalytic processes results from the examination of the validity of the Brönsted law. The Brönsted equation for the reaction of imidazole and thiol compounds with NPA are found to be $\log k_2 = 0.8 \text{ pK} - 4.3$ ¹⁰ and $\log k_2 = 0.38 \text{ pK} - 0.75$ ¹¹ respectively. We have determined the Brönsted equations for Boc-Ala-ONp; the results of these experiments are shown in Fig. 2 a and b. The equations describing the plots for the thiolate and imidazole nucleophiles are $\log k_{2\text{SH}} = 0.41 \text{ pK} + 0.075$ and $\log k_{2\text{im}} = 0.8 \text{ pK} - 3.5$.

With these equations it is possible to calculate the second order rate constants for the imidazole and thiol catalysed hydrolysis of NPA and Boc-Ala-ONp only from the pK-values. Applying this procedure to the peptides studied one may calculate theoretical rate constants from the total of the imidazole and thiol effects without consideration of cooperative actions. The calculated and measured rate constants are compared in Table I. From the table it becomes obvious that the catalytic behaviour of the peptides cannot be described by the Brönsted equations which are valid for simple imidazole- and SH-compounds. Since the observed differences be-

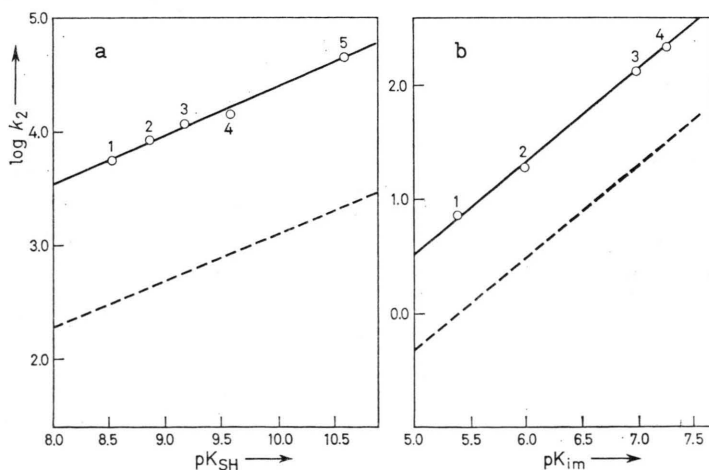


Fig. 2. Brönsted diagram of the reaction of a. thiol nucleophiles with Boc-Ala-ONp. 1. Cysteine, 2. acetyl-cysteine-amide, 3. glutathione, 4. mercaptoethanol, 5. ethylmercaptane. b. Imidazole nucleophiles with Boc-Ala-ONp. 1. Benzimidazole, 2. 4-phenylimidazole, 3. imidazole, 4. 4-hydroxyethylimidazole. The dotted lines are the Brönsted plots for the thiol and imidazole catalysed hydrolysis of NPA^{10, 11}.

Table I. Comparison of the calculated and experimental rate constants of the hydrolysis of NPA and Boc-Ala-ONp catalysed by different peptides.

Substrate	Peptides	I	II	III	IV	V	VI
NPA	$k_2 \times 10^{-3}$ [$l \times mol^{-1} \times min^{-1}$] calculated	0.386	0.476	0.480	0.390	0.015	0.616
	$k_2 \times 10^{-3}$ [$l \times mol^{-1} \times min^{-1}$] found	0.719	0.859	1.221	1.530 *	0.020	2.534 *
Boc-Ala-ONp	$k_2 \times 10^{-3}$ [$l \times mol^{-1} \times min^{-1}$] calculated	7.763	9.763	9.863	7.655	0.103	12.768
	$k_2 \times 10^{-3}$ [$l \times mol^{-1} \times min^{-1}$] found	6.540	10.560	13.080	15.864	0.060	24.612

I, Z-Asp-Cys-NH₂; II, Z-Asp-Gly-Cys-NH₂; III, Z-Glu-Gly-Cys-NH₂; IV, Z-His-Ala-Gly-Gly-Cys-NH₂; V, Z-His-Ala-Asp-Gly-OCH₃; VI, Z-His-Ala-Asp-Gly-Cys-NH₂.

* k_2 — values are pH dependent; given is the value at pH 7.0.

tween the theoretical and experimental rate constants cannot be explained by steric factors, we assume that they are manifestations of cooperative catalytic actions especially of imidazole and SH-groups. From the table it is also evident that the catalytical efficiency of the peptides depends on the nature of the substrate, NPA being the better substrate than Boc-Ala-ONp: the quotient k_2 found/ k_2 calc. for peptide VI is 4.1 with NPA and 1.9 with Boc-Ala-ONp for instance.

The kinetic pK values of the peptides

The most convenient method for the determination of pK values from the pH dependence of the thiol and imidazole catalysed ester hydrolysis is the Lindley diagram ($1/k$ versus $[H^+]$)¹². In normal cases the Lindley diagram gives linear plots, not so with most of the peptides studied. Especially with the most active pentapeptides we obtained non linear plots, which did not permit the estimation of k_2 and pK values. The Lindley diagram for the reaction of Z-His-Ala-Asp-Gly-Cys-NH₂ with NPA at three temperatures is given in Fig. 3. One can predict, that the deviations from a straight line are the stronger the greater the differences between the calculated and measured rate constants. In those cases where only small deviations from a linear plot are observed, it is possible to interpolate pK-values,

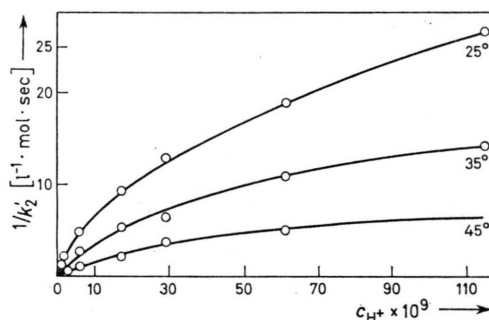


Fig. 3. Lindley diagram of the reaction of Z-His-Ala-Asp-Gly-Cys-NH₂ with NPA at different temperatures.

but they do not agree with the values found from photometric titrations. These results demonstrate that the pH dependence of the rate constants of these reactions cannot be described by a regular dissociation curve.

Concerning the molecular mechanism of the catalytic processes, we assume that the interaction of SH and imidazole groups is an important factor¹³. At the moment it is not possible to decide whether a concerted reaction or a catalysis by subsequent neighbouring group effects is operating. It is now well established that even small peptides may have a specific conformation in solution¹⁴ which should make possible cooperative action of catalytically active groups.

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