POLYPEPTIDES POSSESSING CATALYTIC PROPERTIES

Sh. Kh. Khalikov, S. V. Alieva,
and R. V. Valiev

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The catalytic properties of synthetic polypeptides containing L-amino acids with the sequences H-(His-Glu)$_n$-OH, H-(Ser-Glu)$_n$-OH, H-(His-Tyr)$_n$-OH, and H-(Trp-Glu)$_n$-OH in the hydrolysis of p-NPA (para-nitrophenyl acetate) are considered. The dependences of the rates of the polypeptide-catalyzed hydrolysis of p-NPA on the pH of the medium, the temperature, and the concentration of p-NPA are discussed. V_{max}, K_m, and K" — the effective rate constants of the hydrolysis of p-NPA — and K_2 — the constant for the splitting out of p-nitrophenol from the substrate — have been found and calculated.

The idea of the active center of an enzyme has arisen as the result of a study of various functional groups of amino acids present in enzyme molecules and necessary for their biocatalytic activity.

There have been numerous publications describing attempts to model the active section of certain specific enzymes of the type of serine and acid proteases in various ways [1, 2]. These sections consisted of pure peptides, and also of peptide-like products containing mainly —OH, —COOH, and imidazole groups [3-6]. It must be mentioned that they all had comparatively low activities. This is apparently connected with a number of factors, one of which is the absence of a suitable chain length and a definite molecular weight and conformation. It appeared of interest to replace model oligopeptides by polypeptides of regular construction which could, under definite conditions, form structures similar in some respects to the active center of the enzyme being modeled. We have previously reported that polypeptides of regular construction containing tyrosine and glutamic acid residues possess catalytic properties in the hydrolysis of the ester bond [7].

In the present paper we give the results of an investigation of the catalytic properties of polypeptides of regular construction containing L-amino acids with the sequences: H-(His-Glu)$_n$-OH, H-(Ser-Glu)$_n$-OH, H-(His-Tyr)$_n$-OH, and H-(Trip-Glu)$_n$-OH which catalyze the hydrolysis of p-NPA (para-nitrophenyl acetate). The synthesis of these polypeptides has been described previously [8].

The hydrolytic activities of the polypeptides relative to p-NPA were measured at pH 5.4-8.2 and at temperatures of 25-60°C. The dependence of the catalytic activity on the pH is shown in Fig. 1. With a rise in the pH of the reaction medium (from 7.5 to 7.7) the rate of hydrolysis of p-NPA by the polypeptide H-(His-Glu)$_n$-OH (curve 1) rose but with a further increase in the pH it fell sharply; the optimum pH for the reaction was 7.7. The catalytic activity of H-(Ser-Glu)$_n$-OH (curve 2) rose from pH 7.4 to 7.8 and then fell gradually. In contrast to the two polypeptides mentioned above, H-(His-Tyr)$_n$-OH and H-(Trp-Glu)$_n$-OH showed comparatively low activities.

The dependence of the catalytic activities of the polypeptides on the temperature was measured in the interval 25-60°C at pH values of 7.7, 7.8, 7.5, and 8.0, respectively (Fig. 2). It is characteristic that all the polypeptides showed their greatest activity at 40°C, regardless of the pH of the reaction medium.

The dependence of the rate of the hydrolysis of p-NPA by the polypeptides on its concentration was measured at 40°C and the corresponding optimum pH values. As can be seen from Fig. 3, the rate of hydrolysis of p-NPA rose in all cases with an increase in the concentration of the substrate to about 3 mg/ml; at higher concentrations of p-NPA inhibition was observed, which was particularly pronounced in the case of H-(His-Tyr)$_n$-OH.

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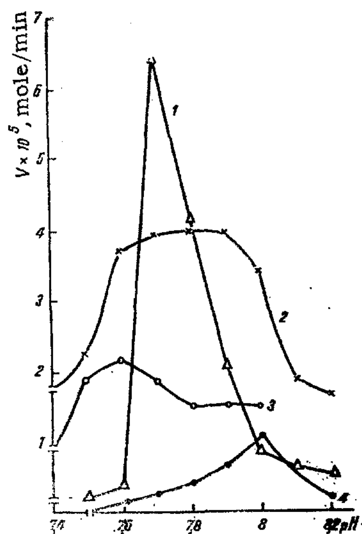


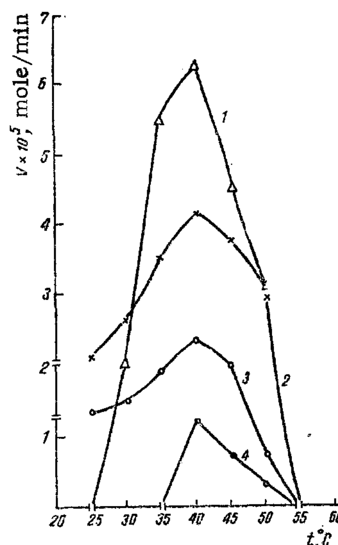


Fig. 1 Fig. 2

Fig. 1. Dependence of the hydrolysis of p-NPA by polypeptides on the pH: 1) H-(His-Glu)$_n$-OH; 2) H-(Ser-Glu)$_n$-OH; 3) H-(His-Tyr)$_n$-OH; 4) H-(Trp-Glu)$_n$-OH (concentration of polypeptides C = 0.2 mg/ml; concentration of p-NPA C = 2 mg/ml; reaction temperature 40°C).

Fig. 2. Dependence of the rate of hydrolysis of p-NPA by polypeptides on the temperature: 1) H-(His-Glu)$_n$-OH; pH 7.7; 2) H-(Ser-Glu)$_n$-OH, pH 7.8; 3) H-(His-Tyr)$_n$-OH, pH 7.5; 4) H-(Trp-Glu)$_n$-OH, pH 8.0.

The values of the maximum rates (V_{max}) of the hydrolysis of p-NPA under the action of the polypeptides were determined by the Lineweaver–Burk graphical method (Fig. 4). Table 1 gives the values of these constants, and also the calculated values of the effective rate constant of the hydrolysis of p-NPA (K") and of the rate constant of the splitting out of p-nitrophenol (K_2).

It is interesting to compare the values of the kinetic constants V_{max} and K_m which we measured for the hydrolysis of p-NPA by the synthetic polypeptides with the analogous constants for the hydrolysis of this ester by the enzyme α-chymotrypsin and some catalysts. In the case of α-chymotrypsin, V_{max} for the hydrolysis of p-NPA was $1.0 \cdot 10^4$ liter/mole·min, and in the cases of histidine and imidazole it was 3.4 and 23.3 liter/mole·min, respectively [9]. The synthetic polypeptides that we have investigated occupy an intermediate position with respect to the efficiency of catalysis: the values of K" for them lie in the range of $0.13\text{-}0.87 \cdot 10^2$ liter/mole·min.

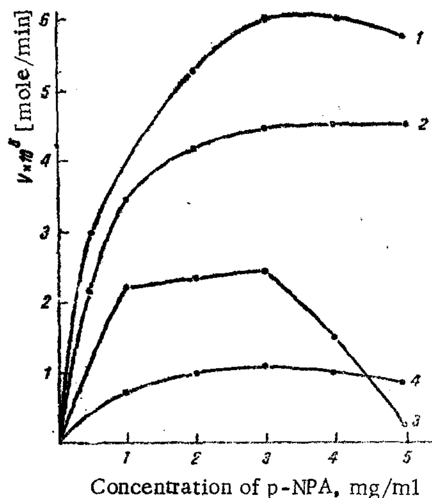
EXPERIMENTAL

Preparation of the Solutions. With stirring, 3 ml of 0.1 N HCl was carefully added to a solution of 25 ml of the free polypeptide H-(Ser-Glu)$_n$-OH in 2 ml of 0.2 N NaOH. Then the solution was titrated to pH 7 on a pH-meter with the aid of 0.1 N HCl.

The solution was transferred to a measuring flask (V = 25 ml) and diluted to the mark with water (C = 1 mg/ml). The solution of the substrate was prepared by dissolving 0.5 g of p-NPA in absolute dioxane and bringing the volume to 50 ml (C = 10 mg/ml). Solutions of H-(His-Glu)$_n$-OH and H-(Trp-Glu)$_n$-OH were prepared similarly.

A solution of H-(His-Tyr)$_n$-OH was prepared by dissolving 25 mg of the polypeptide in 25 ml of water (C = 1 mg/ml).

Activity Measurement. The thermostated cell of a Hitachi spectrophotometer (optical path length 1 cm) was charged with 0.6 ml of polypeptide solution and 1.8 ml of 1/15 M phosphate buffer with the predetermined pH value, and the cell was heated to the appropriate temperature. A similar cell but without the polypeptide served as control. To each cell was



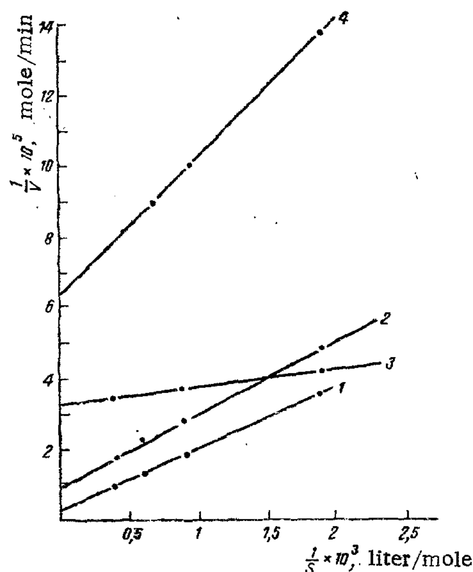


Fig. 3 Fig. 4

Fig. 3. Dependence of the rate of hydrolysis of p-NPA by polypeptides on its concentration 1) N-(His-Glu)$_n$OH, pH 7.7; 2) H-(Ser-Glu)$_n$-OH, pH 7.8; 3) H-(His-Tyr)$_n$-OH, pH 7.5; 4) H-(Trp-Glu)$_n$-OH, pH 8.0.

Fig. 4. Dependence of the rate of hydrolysis of p-NPA by polypeptides on the concentration of p-NPA (curves in the form of Lineweaver–Burk plots): 1) H-(His-Glu)$_n$-OH; 2) H-(Ser-Glu)$_n$-OH; 3) H-(His-Tyr)$_n$-OH; 4) H-(Trp-Glu)$_n$-OH.

TABLE 1. Catalytic Activities of the Polypeptides in the Hydrolysis of p-NPA

Synthetic polypeptide	Mol. wt.	Degree of polymerization	V_{max}, mole/min	K_m, mole/liter	K_2, min^{-1}	K", liter/mole·min	pH	T, C
1. H-[His-Glu]$_n$-OH	14000	48	$3{,}3\cdot10^{-5}$	$4{,}0\cdot10^{-4}$	$3.5\cdot10^{-2}$	$0\,87\cdot10^{2}$	7,7	40
2. H-[Ser-Glu]$_n$-OH	11000	46	$1\,1\cdot10^{-5}$	$2{,}5\cdot10^{-4}$	$7{,}9\cdot10^{\ 3}$	$0{,}31\cdot10^{2}$	7,8	40
3. H-[His-Tyr]$_n$-OH	13000	38	$3{,}1\cdot10^{-6}$	$2{,}0\cdot10^{-4}$	$3{,}5\cdot10^{-3}$	$0{,}17\cdot10^{2}$	7 5	40
4. H-[Trp-Glu]$_n$-OH	14000	41	$1{,}5\cdot10^{-6}$	$1{,}33\cdot10^{-4}$	$1{,}8\cdot10^{-3}$	$0{,}13\cdot10^{2}$	8,0	40
5 Histidine [9]						3,4	7,7	23–24
6. Imidazole [9]						23,3	7,7	23–24
7. α-Chymotrypsin [9]						$1{,}0\cdot11^{4}$	7 7	23–24

Conditions for the hydrolysis of p-NPA in the presence of catalysts 1-4: 0.067 M phosphate buffer containing 20% of dioxane; $C_{p\text{-}NPA} = 1.1\cdot10^{-2}$ M; concentration of polypeptides in moles of polypeptide unit, $1.0\text{-}1.5\cdot10^{-3}$ M. For 5-7: 0.2 M phosphate buffer containing 5% of dioxane; $C_{p\text{-}NPA} = 3.09\cdot10^{-5}$ M. Concentration of catalyst $5\cdot10^{-5}$ M. For 6: imidazole buffer containing 5% of dioxane, $C_{p\text{-}NPA} = 3.09\cdot10^{-5}$ M.

added 0.6 ml of a freshly prepared solution of the substrate that had been heated to the appropriate temperature; then the mixture was stirred for a few seconds and the optical density (D) was measured at a wavelength of 400 nm. To measure the rate of hydrolysis of p-NPA we used the linear section of the curve of the dependence of D for the p-nitrophenol liberated during hydrolysis on the time.

To determine V_{max} and K_m we used the Lineweaver–Burk graphical method.

The effective rate constant of the reaction (K") was determined from the formula $K'' = K/K_2$, where $K = V/C$ and K_2 is the rate constant of the splitting out of p-nitrophenol and C is the molar concentration of a monomeric unit of the polypeptide in the reaction medium, mole/liter.

SUMMARY

The hydrolytic properties of polypeptides of regular structure and containing polyfunctional amino acids with respect to the hydrolysis of p-NPA have been determined as functions of the pH, the temperature, and the concentration of the substrate.

Calculations have been made of V_{max}, K_m, and K" — the effective rate constant of the hydrolysis of p-NPA — and of K_2 — the rate constant of the splitting out of p-nitrophenol.

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ISOLATION OF PROTEASE B FROM COTTON SEEDS

T. D. Kasymova and P. Kh. Yuldashev

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Protease B has been isolated from dormant cotton seeds by fractionation with ammonium sulfate, ion-exchange chromatography on CM-cellulose, and gel filtration through Acrilex P-10 and Sephadex G-75, with 128-fold purification. The enzyme exists in dimeric and monomeric forms. According to the results of gel filtration, their molecular weights are 72,000 and 36,000, respectively. The enzyme consists of a single polypeptide chain including sugars. The N-terminal amino acid of protease B is alanine. The enzyme possesses proteolytic activity in the pH range from 4 to 6.

The recently increasing interest in plant enzymes is due to their unique properties, the study of which is opening up broad possibilities for the fine regulation of the processes involved in the preparation and storage of plant foodstuffs for animal husbandry. In addition, the proteolytic enzymes of plant seeds fulfill an important function in the growth process. They cleave the globulin components and provide the developing germ with low-molecular-weight nitrogen compounds [1, 2].

Dormant seeds of the cotton plant of the Tashkent 1 variety contain proteolytic enzymes acting over a wide pH range with three maxima: 2.8-3, 4.5-5, and 9-9.3 [3, 4]. The aim of the present investigation was to determine their role in the metabolism of the cell and to study the catalytic action of the cotton proteases.

It is impossible to elucidate the chemical essence of the action of proteolytic enzymes without a detailed study of their primary and spatial structures. We have developed a method for isolating a proteolytic enzyme, which we have called protease B, from dormant

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Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.

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