SEARCH FOR REGULAR POLYPEPTIDES POSSESSING ESTERASE PROPERTIES. I. POLYPEPTIDES CONTAINING TYROSINE AND GLUTAMIC ACID RESIDUES

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An investigation has been made of the catalytic properties in relation to the hydrolysis of p-NPA of six polypeptides of regular structure: H-[Glu-Tyr]$_n$-OH (1), H-[Glu$_2$-Tyr]$_n$-OH (2), H-[Glu-Tyr$_3$]$_n$-OH (3), H-[Glu$_3$-Tyr]$_n$-OH (4), H-[Glu$_5$-Tyr]$_n$-OH (5), and H-[Glu-Tyr$_2$]$_n$-OH (6). It has been shown that polypeptides (I), (III), and (IV) catalyze the hydrolysis of p-NPA (p-nitrophenyl acetate). An enzyme-like type of catalysis has been found. Some catalytic characteristics have been calculated and the dependence of the rate of hydrolysis on the pH of the medium, the temperature, and the concentration of p-NPA have been discussed. The structures of the catalytically active and catalytically inactive polypeptides have been studied by the circular dichroism method. It has been shown that under conditions in which the catalytic properties of polypeptides are shown to the maximum degree there is a structure of the random coil type. The catalytic activity falls or disappears completely when ordered fragments of the α-helix and β-structure types appear in the structure. It has been found that polypeptide (I) possesses the maximum catalytic activity. It exceeds the activity of a copolymer of the same amino acids by an order of magnitude.

The hypothesis exists that the catalytic properties of enzymes may be partially retained even with some simplification of their structure. In other words, the possibility exists of the chemical synthesis of such a polypeptide as, in view of its primary structure and under certain conditions, will be capable of bringing about interaction of certain functional groups as a kind of "active center," similar in its main outlines to the active center of the enzyme being modeled.

We may note that, in spite of numerous investigations in this field, it is still not clear whether this idea can be realized at all in the practical sense [1-7]. Nevertheless, interest in synthetic oligopeptides and polypeptides with catalytic activity is high, since these compounds may open up new possibilities in a number of chemical technologies.

Recently, the greatest attention has been attracted by the idea of modeling the esterase properties of serine and acid proteases. This is connected with the fact that, in the first place, enzymes of this class have been studied in very great detail in relation both to their tertiary structure and to the mechanism of their catalytic action [8, 9]. In the second place, by their nature they are less specific and therefore may permit great structural deformation.

Since in the serine proteases the catalytic process is ensured by the cooperative interaction of a histidine, a serine, and a glutamic or aspartic acid residue, these are the amino acids that we used for the synthesis of copolymers and regular polypeptides in the hope that among them there might be found a polypeptide in which a successful structural combination of these amino acids permitting the manifestation of catalytic properties could arise. In actual fact, some of them have exhibited esterase activity in relation to the hydrolysis of p-nitrophenyl acetate (p-NPS) [10-15] or the corresponding ester of alanine [7]. However, the catalytic effect was low and even when histidine residues were present it was determined rather by the presence of the imidazole groups than by their cooperative interaction with the OH and COOH groups of the serine and dicarboxylic acid (glutamic or aspartic acid) residues [16].

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It has become obvious that the structure of a polypeptide chain based only on the amino acid residues participating in the active centers of esterases is inadequate. Consequently, in choosing the corresponding primary structure it is necessary also to take into account the tertiary structure that arises spontaneously under the appropriate conditions and in which it is possible to realize the corresponding interaction of amino acid residues. And since both serine proteases [17] and acid proteases such as pepsin have a globular structure [18], it was to be assumed that in a synthetic polypeptide, as well, a catalytic effect could be ensured only by some globular structure or one close to it. Unfortunately, it has not previously been possible to solve this problem since the laws underlying helix-coil transitions and depending on the primary structures of polypeptides have not yet been elucidated.

In these circumstances, it is necessary to make use of purely empirical approaches. In the present work we have started from the fact that poly-L-glutamic acid and poly-L-tyrosine are, depending on the conditions, capable of possessing either an α-helical structure, a β-structure, or a structure of the random coil type [19-21]. With some assumptions, the latter may be considered as an unstable globular structure which can be rearranged under the action of the corresponding substrate.

Our aim was to create regular polypeptides with esterase activity in relation to the hydrolysis of p-NPA and we therefore had to use primarily the functional groups that are present in the active center of such natural esterases as trypsin, chymotrypsin, elastase, etc.

Although in these enzymes the catalytic process takes place as the result of a cooperative interaction of glutamic acid, serine, and histidine residues, we used only the —COOH group of glutamic acid and the OH group of tyrosine although the latter is only partially equivalent to the OH group of a serine residue. The choice of these two amino acid residues was also facilitated by the fact that some catalytic activity of copolymers of these amino acids in relation to the hydrolysis of p-NPA was already known [10, 11]. Since it was impossible previously to determine what ratio of the glutamic acid and tyrosine residues in the polypeptide was capable of ensuring a structure of the random coil type with a probable catalytic activity, it was necessary to perform the synthesis of a number of polypeptides with different ratios of the tyrosine and glutamic acid residues in the monomeric unit (Table 1) [22].

The catalytic activities of these polymers with respect to the hydrolysis of p-NPA were measured at pH 5-7.5 with temperatures of 25-60°C. Catalytic activity was found in compounds 1, 3, and 5. The dependence of the catalytic activity on the pH is shown in Fig. 1. It follows from the results given that with a rise in the pH of the medium from 5.6 to 7.5 the catalytic activity of the polypeptides also increases, reaching its maximum in the pH range from 6.9 to 7.2. On considering the curves obtained, attention is attracted by two facts: in the first place, the bell-shaped "enzyme-like" nature of the curves and, in the second place, the appearance of an additional weak maximum of activity in the pH range from 6.0 to 6.2. It is interesting that this maximum corresponds to the maximum activity of copolymers of these two amino acids obtained at ratios of tyrosine and glutamic acid of 1:1 and 1:5, respectively [10]. It is possible that the weak maximum is a consequence of the interaction of the functional groups with no appreciable contribution of structural factors, while the second activity maximum appears as the result of the production of some fairly stable structure that can ensure a better interaction of the polypeptide with the substrate.

It must be mentioned that the enzyme-like behavior of these polypeptides appears both in an investigation of the dependence of the catalytic activity on the temperature (Fig. 2) and in the comparatively close values of K_m for the polypeptides and for esterase enzymes such as chymotrypsin (Table 2).

The hydrolytic activities of the polypeptides investigated depended on the pH and the temperature and it could therefore be assumed that it was connected with some conformational rearrangements or other. It was interesting to detect that structure at which the maximum catalytic effect was observed. To evaluate the structural features of these polypeptides we made use of the method of circular dichroism (CD), which permits the identification of the main types of structures: α-helix, β-structure, and unordered structure, also known as the random coil.

The four polypeptides (see Table 1, compounds 2-5) were investigated in aqueous solutions both under the optimum conditions for the manifestation of the greatest catalytic activ-

TABLE 1. Regular Polypeptides Investigated for the Presence of Catalytic Activity in Relation to the Hydrolysis of p-NPA

Polypeptide [22]	Mean molecular weight, M_{av}	Degree of polymerization, $\bar{n}$	Catalytic activity*	Mean molecular weight of one residue	Concentration, %	pH at the following temperature, °C			
						20		45	
H-[Glu-Tyr]$_n$-OH	7 300	25	+	—					
H-[Glu$_2$-Tyr]$_n$-OH	27 000	64	—	—					
H-[Glu-Tyr$_3$]$_n$-OH	26 500	43	+	154,0	0,68	7,0	6,2	7,0	6,2
H-[Glu$_3$-Tyr]$_n$-OH	53 900	98	—	137,5	0,11	7,0	4,8	7,0	4,8
H-[Glu$_5$-Tyr]$_n$-OH	15 700	19,0	+	134,0	0,16	7,15	5,2	7,15	5,2
H-[Glu-Tyr$_2$]$_n$-OH	34 800	76,0	—	151,0	0,16	7,2	5,8	7,2	5,8

*A plus sign means that there was catalytic activity and a minus sign that there was none.

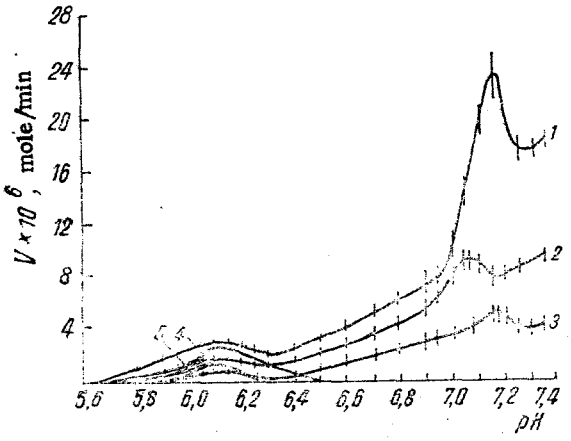

Fig. 1

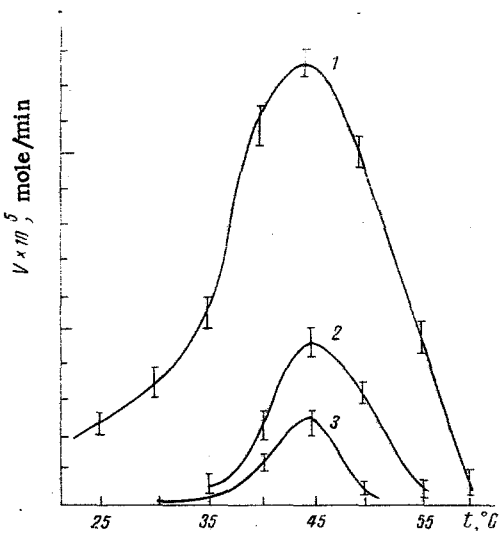

Fig. 2

Fig. 1. Dependence of the hydrolysis of p-NPA by polypeptides on the pH: 1) H-[Glu-Tyr]$_n$-OH; 2) H-[Glu$_5$-Tyr]$_n$-OH; 3) H-[Glu-Tyr$_3$]$_n$-OH (concentration of the polypeptides C = 0.2 mg/ml; concentration of p-NPA, C = 3 mg/ml, reaction temperatures 45°C); 4) copolymer of tyrosine and glutamic acid (1:1) [10]; 5) copolymer of tyrosine and glutamic acid (1:5) [10]. (Concentration of copolymer 1 mg/ml; concentration of p-NPA 2 mg/ml; temperature 40°C).

Fig. 2. Dependence of the rate of hydrolysis of p-NPA catalyzed by polypeptides on the temperature at the optimum pH values: 1) H-[Glu-Tyr]$_n$-OH (pH 7.17); 2) H-[Glu$_5$-Tyr]$_n$-OH (pH 7.07); 3) H-[Glu-Tyr$_3$]-OH (pH 7.17).

ity in relation to the hydrolysis of p-NPA and under nonoptimum conditions (acid pH values).

The interpretation of the CD spectra of the polypeptides containing tyrosine residues presents some difficulty, since they are complicated by the additional bands due to the influence of the lateral tyrosine chromophores [23]. In view of this, the spectra of the model structures poly-L-lysine and poly-L-glutamic acid, which are usually used for the analysis of the CD spectra, proved unsuitable in our case. Nevertheless, we attempted to characterize the structures of the polypeptides under investigation, even if only qualitatively, on the basis of literature information [20, 21, 23]. In addition to this, we also attempted to take into account the fact that the predominance of a particular amino acid residue should have an influence on the type of structure formed. Thus, α-helical sections may appear in the secondary structures of compounds 4 and 5 (Table 1) at acid pH values because at pH 4.4 poly-L-glutamic acid passes from the state of a random coil into that of an α-helix [19]. Similar considerations can be taken into account when tyrosine residues predominate in a polypeptide, since at low alkaline pH values poly-L-tyrosine has either the β-structure or an unordered

TABLE 2. Catalytic Activities of Polypeptides, Copolymers, Amino Acids, Histidine, Imidazole, and α-Chymotrypsin in Relation to the Hydrolysis of p-NPA

Catalyst*	Polymerization, $\bar{n}$	$\frac{V_{max}\ mole/min}{C_{catalyst},\ mg/ml}$	K_m, M	K_2, min^{-1}	$K'' = K_2/K_m$, liter/mole·min	pH	Temperature, °C
1. Poly(Tyr-Glu)	25	$6,3\cdot10^{-5}/0,2$	$2,32\cdot10^{-3}$	$9\cdot10^{-2}$	38,8	7,17	45
2. Poly(Glu-Tyr_3)	43	$1,4\cdot10^{-5}/0,2$	$4,0\cdot10^{-2}$	$4,4\cdot10^{-2}$	1,1	7,17	45
3. Poly(Tyr-Glu_5)	19	$2,8\cdot10^{-5}/0,2$	$8,3\cdot10^{-2}$	$1,1\cdot10^{-1}$	13,2	7,07	45
4. Poly(Tyr-Glu) [11]	9	$8,0\cdot10^{-7}/1$	$2,2\cdot10^{-2}$	$2,3\cdot10^{-4}$ †	$1,05\cdot10^{-2}$ †	6,13	45
5. Poly(Tyr,Glu, 1:1) [10]	188	$9,4\cdot10^{-6}/1$	$3,0\cdot10^{-2}$	$2,76\cdot10^{-3}$ †	$9,2\cdot10^{-2}$ †	6,13	40
6. Poly(Tyr,Glu, 1:5) [10]	178	$5,1\cdot10^{-6}/1$	$2,6\cdot10^{-2}$	$4,14\cdot10^{-3}$ †	$1,6\cdot10^{-1}$ †	6,07	42,5
7. Histidine [3]	—				3,4	7,7	23–24
8. Imidazole [3]	—				23,3	7,7	23–24
9. α-Chymotrypsin [3]	—				$1,0\cdot11^{-4}$	7,7	23–24

*Conditions of hydrolysis of p-NPA in the presence of the catalysts 1-3: 0.067 M phosphate buffer containing 20% of dioxane; C of p-NPA = $1.1\cdot10^{-2}$ M; concentration of polypeptide in moles of polypeptide unit $2.47\cdot10^{-4}$-$6.84\cdot10^{-4}$ M. For compound 4 the conditions were similar but the buffer contained 20% of acetonitrile. For compounds 5 and 6 the conditions were also similar to those for 1-3, but the buffer contained 20% of methanol. For 7 and 9: 0.2 M phosphate buffer containing 5% of dioxane; C of p-NPA = $3.09\cdot10^{-5}$ M; concentration of the catalyst $5\cdot10^{-5}$ M. For 8: imidazole buffer containing 5% of dioxane C of p-NPA = $3.09\cdot10^{-5}$ M.

†We obtained these values on the basis of values of V_{max} and K_m given in the literature and the concentration of the copolymer unit [10, 11].

structure, depending on the method of preparing the polypeptide solution [20]. It may be assumed that these structural forms will be retained both under normal conditions and at acid pH values. The structural features of a polypeptide in which the ratio of tyrosine and glutamine residues is 1:1 (compound 1, Table 1) will be considered in a special communication.

Thus, the structures of compounds 4 and 5 must be considered as the most sensitive to a change in the pH conditions in the range studied, since the lateral carboxy groups of the glutamic acid residues that are present in predominating amount in these polypeptides are ionized completely or partially precisely in the range of pH values studied.

Analysis of the CD curves and values of [θ] (Table 3) shows that under the optimum conditions of hydrolysis when the polypeptides H-[Glu_5-Tyr]$_n$-OH and H-[Glu_3-Tyr]$_n$-OH exhibit their maximum catalytic activity, they actually have an unordered structure (Figs. 3 and 4), as is shown by the negative band in the 197-nm region. The $[\theta]_{197}$ value of the former is almost twice that of the latter, apparently because of the relatively high tyrosine content in the polypeptide molecule.

On passing to the region of the acid pH values, α-helical sections arise in the structures of these polypeptides (Figs. 3 and 4), since the broad negative band in the CD spectra with maxima at 208 and 222 nm that is characteristic of poly-L-glutamic acid is observed in the CD spectra of both components. Some difference in the shapes of the bands and [θ] values can probably be explained by different quantitative amounts of α-helical residues, and also by the quality of the secondary structure formed. It is just at this moment when the polypeptide molecule acquires some additional structural organization that its catalytic properties disappear. The fact that with the presence of an unordered structure in the polypeptides H-[Glu_5-Tyr]$_n$-OH and H-[Glu_3-Tyr]$_n$-OH, existing under conditions when the catalytic properties with respect to the hydrolysis of p-NPA exist to the maximum extent, the former catalyzes it and the latter does not, may indicate that the presence of an unordered structure is insufficient for the realization of catalytic properties in a polypeptide and that in this case the actual primary structure is of equal value, which must simultaneously ensure the necessary number of functional groups present in the "catalytic center" and their appropriate arrangement in space.

The CD spectra of compounds 3 and 6 (Table 1) differ by the position of the negative bands and the value of [θ] and, consequently, their secondary structures are different. This observation is important in itself, since H-[Glu-Tyr_3]$_n$-OH has catalytic activity while H-[Glu-Tyr_2]$_n$-OH has not. Furthermore, the polypeptides react differently from the structural point of view to a change in conditions. For example, the secondary structure of the latter is less sensitive to a change in the pH of the medium and of the temperature (Fig. 5) and is less mobile than the secondary structure of the first polypeptide (Fig. 6). However, it is difficult to make a concrete statement as to what types of secondary structures are present in the two polypeptides. It can only be assumed that it may be an unordered structure with elements of β-structure.

We have been unable to perform a more detailed analysis, since we did not possess the CD spectra of model structures of poly-L-tyrosine. Unfortunately, we also have no ideas concerning the tertiary structure of our polypeptides, since the very term "unordered structure" may represent a structure to some extent resembling the globular structure but fairly flexible and capable of rearranging under the structure of the substrate with the formation of a polypeptide-substrate complex.

A somewhat indirect idea of the relative changes in it can nevertheless be obtained from measurements of the CD spectra taken in the region of the absorption of the phenol chromophore at 240-300 nm under conditions ensuring the appearance of the maximum catalytic activity and under conditions not ensuring this. Thus, attention is attracted by the fairly considerable change in the CD spectra with a change in the conditions which, even in itself, may show some conformational rearrangement in the structure of the polypeptides. It is particularly appreciable for the polypeptides H-[Glu_5-Tyr]$_n$-OH and H-[Glu-Tyr_3]$_n$-OH at the moment of passage from conditions not the optimum for hydrolysis to the optimum conditions (Figs. 7 and 8). At the same time, in the polypeptides H-[Glu-Tyr_2]$_n$-OH and H-[Glu_3-Tyr]$_n$-OH these changes are only slight (Figs. 9 and 10). One more feature of the CD spectra has been found which is characteristic of the polypeptides not possessing catalytic properties: they have a negative band with two maxima at 276 and 284 nm. However, we have been unable to explain this fact on the basis of the results of the investigations performed. We have also been unable to estimate the percentages of α-helical and β-structural sections in the polypeptide chains,

TABLE 3. Molar Ellipticities

Polypeptide	Temperature, °C	pH	λ, nm [θ]×10⁻⁴ (deg · cm²/dmole)					
1. H-(Glu-Tyr$_2$)$_n$ -OH	20	7,2	195 (−0,8)	215 (−0,06)			230 (+0,24)	276 (−0,004)
		5,8	195 (−0,74)	215 (−0,06)			230 (+0,24)	276 (−0,002)
	45	7,2		215 (−0,078)			230 (+0,20)	276 (−0,009)
		5,8	195 (−0,61)	215 (−0,045)			230 (+0,19)	276 (−0,001)
2. H-(Tyr-Glu$_3$)$_n$ -OH	20	7,0	196 (−0,94)	215 (−0,04)			230 (+0,18)	276 (−0,004)
		4,8		215 (+0,066)		277 (+0,24)	230 (+0,21)	274 (−0,003)
	45	7,0	196 (−0,60)	208 (+0,22)	217 (−0,13)			274 (−0,003)
		4,8		208 (−0,165)	217 (−0,11)			
3. H-(Glu-Tyr$_3$)$_n$ -OH	20	7,0		212 (−3,3)			230 (0)	276 (+ 0,012)
		6,2					230 (+0,26)	276 (0,015)
	45	7,0		215 (−2,5)			230 (+0,04)	
		6,2		215 (−2,08)			230 (−0,07)	
4. H-(Tyr-Glu$_5$)$_n$ -OH	20	7,15	197 (−2,08)		215 (+ 0,14)		230 (+0,08)	277 (−0,0013)
		5,2		205 (−0,73)	215 (−0,48)		230 (−0,03)	
	45	7,15	197 (−1,52)		215 (+0,19)		230 (−0,09)	277 (−0,001)
		5,2			215 (+ 0,06)		230 (0)	

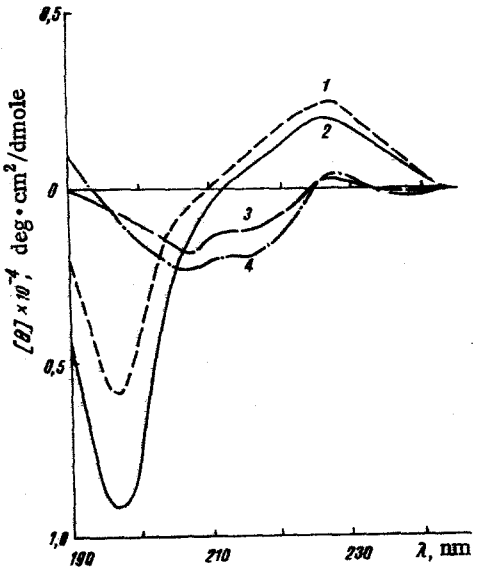

Fig. 3

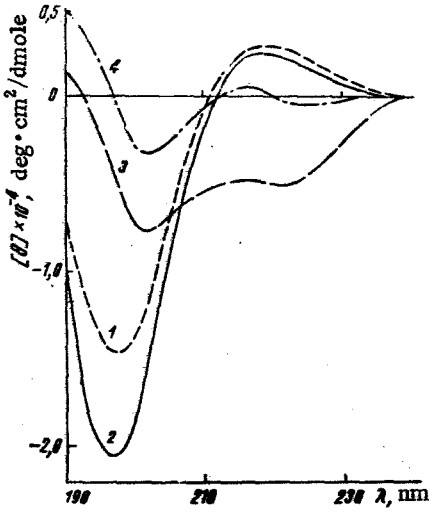

Fig. 4

Fig. 3. CD spectra of H-[Glu$_3$-Tyr]$_n$-OH in aqueous solution: 1) pH 7.0, t = 45°; 2) pH 7.0, t = 20°; 3) pH 4.8, t = 45°; 4) pH 4.8, t = 20°.

Fig. 4. CD spectra of H-[Glu$_3$-Tyr]$_n$-OH in aqueous solution: 1) pH 7.15, t = 20°; 2) pH 7.15, t = 45°; 3) pH 5.20, t = 20°; 4) pH 5.20, t = 45°.

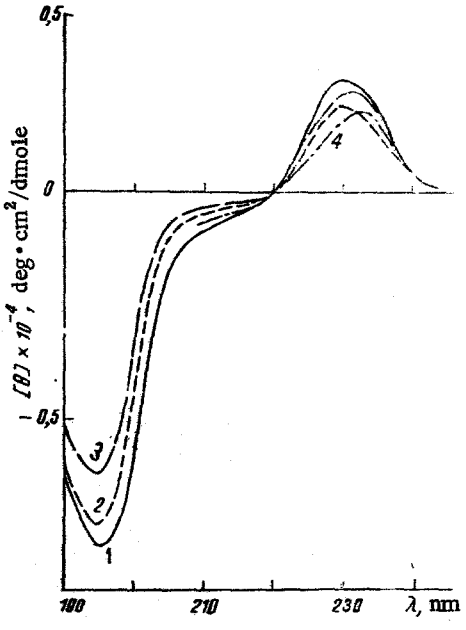

Fig. 5

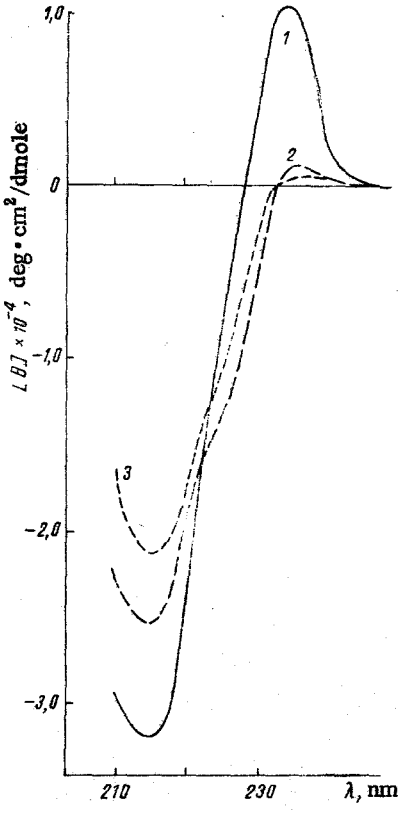

Fig. 6

Fig. 5. CD spectra of H-[Glu-Tyr$_2$]-OH in aqueous solution: 1) pH 7.20, t = 20°; 2) pH 7.20, t = 45°; 3) pH 5.80, t = 0°; 4) pH 5.80, t = 45°.

Fig. 6. CD spectra of H-[Glu-Tyr$_3$]$_n$-OH in aqueous solution: 1) pH 7.0, t = 20°; 2) pH 6.2, t = 20°; 3) pH 6.2, t = 45°.

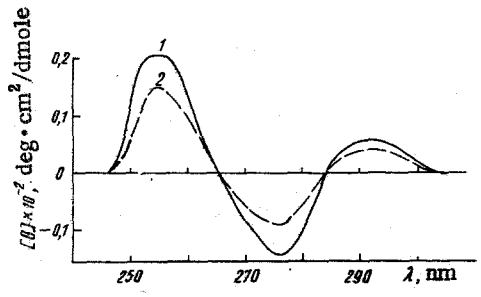

Fig. 7

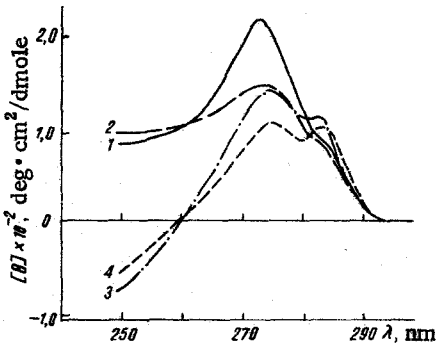

Fig. 8

Fig. 7. CD spectrum of H-[Glu$_3$-Tyr]$_n$-OH in aqueous solution: 1) pH 7.15, t = 20°C; 2) pH 7.15, t = 45°C.

Fig. 8. CD spectra of H-[Glu-Tyr$_3$]$_n$-OH in aqueous solution: 1) pH 7.0, t = 20°; 2) pH 6.2, t = 20°; 3) pH 7.0, t = 45°; 4) pH 6.2, t = 45°.

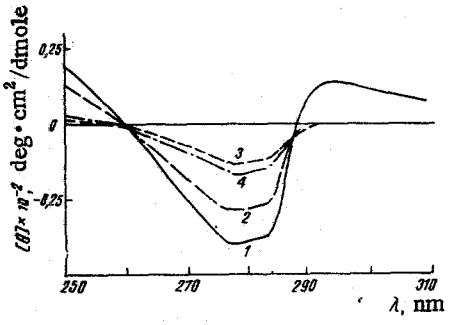

Fig. 9

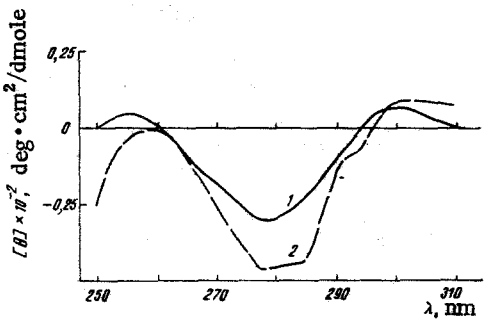

Fig. 10

Fig. 9. CD spectra of H-[Glu-Tyr$_2$]$_n$-OH in aqueous solution: 1) pH 7.2, t = 20°C; 2) pH 7.2, t = 45°C; 3) pH 5.8, t = 45°C; 4) pH 5.8, t = 20°C.

Fig. 10. CD spectra of H-[Glu$_3$-Tyr]$_n$-OH in aqueous solution: 1) pH 7.0, t = 20°C; 2) pH 4.8, t = 20°C.

since the reference spectra obtained with the aid of poly-L-lysine proved to be inadequate because of the high tyrosine content in the polypeptides.

Thus, regular polypeptides containing tyrosine and glutamic acid residues can catalyze the hydrolysis of the ester bond in p-NPA while with the appearance of additional elements of structuration in the polypeptide chain the catalytic properties decrease or disappear completely.

EXPERIMENTAL

Measurement of the Hydrolytic Activity of the Polypeptides. Carefully, with stirring, 3 ml of 0.1 N HCl was added to a solution of 25 mg of a polypeptide in 2 ml of 0.2 N NaOH. Then, using a pH-meter, the solution was back-titrated with 0.1 N HCl to pH 7. The solution was transferred to a 25-ml measuring flask and was made up to the mark with water (concentration 1 mg/ml). Solutions of p-NPA as substrate were obtained by dissolving 500 and 750 mg of p-NPA in absolute dioxane. The volume of each solution was made up to 50 ml with dioxane (concentrations 10 and 15 mg/ml). The thermostated cell of a Hitachi EPS-3T spectrophotometer (optical path length 1 cm) was charged with 0.6 ml of the polypeptide solution and 1.8 ml of 1/15 M phosphate buffer with a predetermined pH, and the cell was heated to the appropriate temperature. An identical cell but without the polypeptide was prepared in parallel. To each cell was added 0.6 ml of a solution of the substrate in dioxane that had been freshly prepared and heated to the appropriate temperature and then the cell contents were mixed 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 rectilinear section of the curve of the dependence of D for the p-nitrophenol (p-NP) liberated in the hydrolysis on the time t from the beginning of the reaction. The tangent of the angle of slope of this section was taken as the initial rate of hydrolysis of the substrate, this being a measure of the catalytic activity of the polypeptide. The rate of catalytic hydrolysis of the substrate by the polypeptides was determined from the formula

$$V_{obs} = V_{meas} - V_{contr},$$

where V_{meas} and V_{contr} are the rates of hydrolysis of p-NPA in the presence and in the absence of the polypeptide, respectively. Then

$$V_{meas} = \frac{\Delta D_1}{\Delta t_1} \cdot K, \quad V_{contr} = \frac{\Delta D_2}{\Delta t_2} \cdot K,$$

$$V_{obs} = \left| \frac{\Delta D_1}{\Delta t_1} - \frac{\Delta D_2}{\Delta t_2} \right| \cdot K;$$

where K is a factor for calculating the initial rate of the reaction V, which is derived from the relation

$$K = a\,d/\xi,$$

where ξ is the molar extinction coefficient of p-NPA under the given conditions (pH and temperature); a, rate of movement of the paper strip of the recorder of the instrument, 1 mm/min; and d, change in optical density corresponding to 1 mm on the paper strip of the recorder.

Since the instrument is a double-beam instrument, we obtain the difference between $\Delta D_1/\Delta t_1$ and $\Delta D_2/\Delta t_2$ as $\Delta D/\Delta t$ or tan α, and

$$V_{obs} = \frac{\Delta D}{\Delta t} \cdot K = \frac{\Delta D}{\Delta t} \cdot \frac{ad}{\xi}.$$

Determination of V and K_m. Since these magnitudes cannot be determined from graphs of the dependence of V on C for p-NPA, because the latter is not rectilinear (Fig. 11), we used the Lineweaver-Burk method for transforming them into linear functions (Fig. 12). The effective rate constant of the reaction K" was determined from the formula

$$K'' = \frac{K_2}{K},$$

where $K_2 = V/C$, the rate constant of the splitting off of p-nitrophenol, min^{-1}; C being the molar concentration of the monomeric unit of the polypeptide in the reaction mixture, M.

Measurement of the Circular Dichroism Spectra. The CD spectra of the polypeptides were taken on a Jobin-Yvon Mark III dichrograph (France). The molar ellipticity was evaluated from the relation

$$[\Theta] = 3300 \cdot \frac{\Delta D}{C \cdot l},$$

where ΔD is the recorded dichroic optical density; C, molar concentration of the polypeptide solution, M; and l, the length of the sample-containing cell.

Quartz cells with optical path lengths of 0.01, 0.05, and 0.1 cm were used in the 190-250-nm region, and 0.1-, 0.5-, and 1-cm cells in the 250-320-nm region. The mean molecular weights of one residue, the working concentrations of the polypeptides, and the conditions for performing the measurements (pH and temperature) are given in Table 2.

H-$[Glu\text{-}Tyr_2]_n$-OH does not possess catalytic activity. The CD spectrum taken at 20°C and pH 7.2-7.4 has a negative band in the 190-210-nm region with a maximum at 197 ($[\Theta]_{197} \sim 0.8 \cdot 10^{-4}$) and a positive band in the 220-240-nm region with a maximum at 230 nm ($[\Theta]_{230} \sim 0.24 \cdot 10^{-4}$). A rise in the temperature to 45°C led to a slight uniform increase in the intensity of the whole negative band from 190 to 210 nm while the positive band with a maximum at 230 nm remained unchanged.

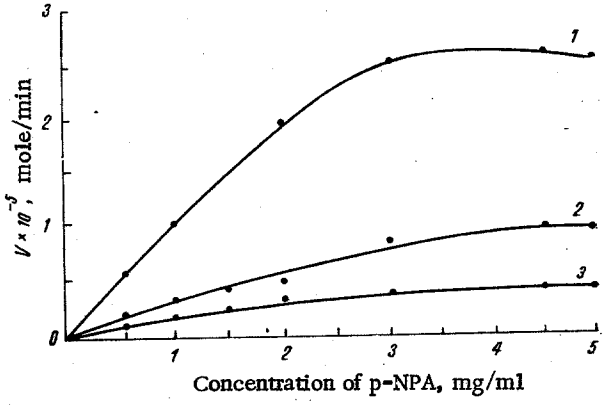

Fig. 11. Dependence of the rate of hydrolysis of p-NPA by polypeptides on the concentration of p-NPA: 1) H-[Tyr-Glu]$_n$-OH; 2) H-[Glu$_5$-Tyr]$_n$-OH; 3) H-[Glu-Tyr$_3$]$_n$-OH.

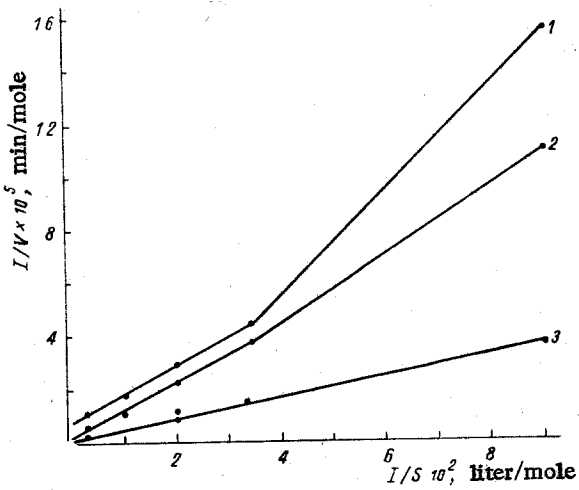

Fig. 12. Dependence of the rate of hydrolysis of p-NPA on the concentration of p-NPA (curves in the form of Lineweaver-Burk diagrams): 1) H-[Glu-Tyr$_3$]-OH; 2) H-[Tyr-Glu$_3$]$_n$-OH; 3) H-[Tyr-Glu]$_n$-OH.

A change in the pH of the medium from 7.2 to 5.8 was accompanied by appreciable decreases in the ellipticities of the negative and positive bands with a shift in the maximum of the latter from 230 to 232 nm (Fig. 5). The form of the spectrum under the optimum conditions of hydrolysis is characteristic for a coil containing β-structure, as may be indicated by a shoulder with an inflection in the 207-219-nm region.

H-[Glu-Tyr$_3$]$_n$-OH possesses catalytic properties. The CD spectrum taken at 20°C and pH 7 has a strong negative band at 207-225 nm with a maximum at 215 nm ($[\theta]_{215} \sim -3.3 \cdot 10^{-4}$) and a positive band in the 220-240-nm region with a maximum at 230 nm ($[\theta]_{230} \sim 0.26 \cdot 10^{-4}$) (Fig. 6). With a change in the pH of the medium to 6.2, the negative band became broader and a shoulder appeared at 225-227 nm. The positive band decreased and its maximum shifted to 238 nm. A rise in the temperature to 45°C led to a uniform decrease in the amplitudes of the negative and positive band with no other appreciable change in the spectrum.

The form of the spectrum under the most favorable conditions for hydrolysis is characteristic for a structure of the random coil type in which elements of β-structure are present. A proof of its existence may be provided by the following discussion: the CD spectrum of poly-L-tyrosine in the β- form has a positive band in the 200-nm region just like the unordered structure, but the value of $[\theta]_{230}$ for the β- form is negative ($[\theta]_{230} \sim -2.0 \cdot 10^{-4}$) while that for the "coil" is positive ($[\theta]_{230} \sim 1.6 \cdot 10^{-4}$). In the spectrum of H-[Glu-Tyr$_2$]-OH, $[\theta]_{230}$ is an order of magnitude smaller than the values given in the literature [20]. Consequently, one may assume the presence in these polypeptides not of α-helical but of

pleated structures. This negative band in poly-L-tyrosine appears only when it is present in the α-helical state but if any other amino acid residue is added to the polypeptide sequence this band will be observed in the β-form and other structural forms.

H-[Glu_3-Tyr]-OH catalyzes the reaction. The spectrum taken at 20°C and pH 7.12 (Fig. 4) shows a negative band at 190-210 nm with a maximum at 197 nm ($[\Theta]_{197} \sim 2.08 \cdot 10^{-4}$) and a positive band at 218 nm ($[\Theta]_{218} \sim 0.16 \cdot 10^{-4}$). With a rise in the temperature to 45°C, a uniform decrease in the negative band was observed. A change in the pH of the medium from 7.12 to 5.2 was accompanied by the appearance of a broad negative band with maxima at 222 nm ($[\Theta]_{222} \sim 0.5 \cdot 10^{-4}$) and 208 nm ($[\Theta]_{208} \sim -0.8 \cdot 10^{-4}$), which shows the appearance of α-helical sections in the secondary structure.

H-[Glu_3-Tyr]$_n$-OH does not catalyze the reaction. The CD spectrum taken at 20°C and pH 7.0 shows a negative band in the 190-210-nm region with a maximum at 197 nm and $[\Theta]_{197} \sim 0.94 \cdot 10^{-4}$ and a positive band in the 215-240-nm region with a maximum at 230 nm and $[\Theta]_{230} \sim 0.18 \cdot 10^{-4}$ due to the presence of tyrosine residues in the polypeptide chain. A rise in temperature to 45°C involved a decrease in the value of [Θ] with no change in the form of the spectrum. A change in the pH of the medium to 4.8 led to the appearance of a negative band with a maximum at 203 nm and $[\Theta]_{203} \sim -0.2 \cdot 10^{-4}$, which may indicate the appearance of α-helical sections in the secondary structure of the polypeptide (Fig. 3).

SUMMARY

1. The catalytic properties of polypeptides of regular structure containing glutamic acid and tyrosine residues with respect to the hydrolysis of p-NPA have been investigated as functions of the pH, the temperature, and the concentration of the substrate.

2. It has been shown that the polypeptides H-[Glu-Tyr]$_n$-OH, H-[Glu-Tyr_3]$_n$-OH, and H-[Glu_3-Tyr]$_n$-OH have an enzyme-like type of catalysis of the hydrolysis of p-NPA.

3. The structural features of the polypeptides have been investigated by the circular dichroism method and it has been shown that at the optimum values of the pH and the temperature when the polypeptide possesses its maximum activity a structure of the random coil type exists in it. On the appearance of elements of orderedness (α-helical and β-structural sections) in the structure of the polypeptides, their catalytic activity falls or disappears completely.

4. It has been shown that the maximum catalytic activity is exhibited by the polypeptide H-[Glu-Tyr]$_n$-OH.

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