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Academician of the Academy of Sciences of the Tajik SSR K. T. Poroshin, Yu. I. Khurgin, and M. G. Dmitrieva

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Abstract

Full Text

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HYDROLYSIS OF *p*-NITROPHENYL ESTERS OF GLYCINE, GLYCYLGLYCINE, AND DIGLYCYLGLYCINE AND OF THEIR CARBOBENZOXY DERIVATIVES

Cyanomethyl, *p*-nitrophenyl, thiophenyl, and other "activated" esters of α-amino acids and peptides are widely used in peptide synthesis ([1],[2]). Through activated esters of α-amino acids and peptides, a considerable number of complex and biologically important peptides have been synthesized. In this way, in particular, a nonapeptide possessing oxytocic activity was synthesized (oxytocin ([3])). Intramolecular condensation of activated peptide esters proved to be a convenient method for obtaining cyclic peptides ([5]). This method was used, for example, in the synthesis of gramicidin C[4], an antibiotic having the structure of a cyclic decapeptide. In addition, activated peptide esters can be used as monomers in a polycondensation reaction to obtain high-molecular-weight peptides with a regular alternation of amino acids ([6]).

Owing to their comparatively simple preparation and a number of other features, *p*-nitrophenyl esters of amino acids and peptides have recently become increasingly widespread in peptide synthesis. Additional interest in these compounds was also aroused by the fact that *p*-nitrophenyl esters of amino acids and other organic acids proved to be nonspecific substrates of a number of hydrolytic enzymes. *p*-Nitrophenyl esters of N-carbobenzoxy derivatives of glycine and tyrosine, acetic acid, and other organic acids are at the same time substrates of trypsin, chymotrypsin, thrombin, and other esterases ([7]); *p*-nitrophenyl esters of sulfuric and phosphoric acids are intensively hydrolyzed by certain phosphatases ([8]) and sulfatases ([9]).

The use of *p*-nitrophenyl esters as substrates for esterases proved especially convenient because of the sharp differences in the positions of the absorption bands of the ester and of the product of its hydrolysis (*p*-nitrophenol), which makes it possible to carry out a spectrophotometric study of the kinetics of enzymatic hydrolysis. The relative simplicity and accuracy of the spectrophotometric method made it possible to accumulate a considerable amount of data on the kinetics of the action of esterases, which contributed to recent advances in the study of the mechanism of action of these enzymes and of the structure of their active center ([10]), containing serine and histidine. As a result, the mechanism of imidazole catalysis of ester hydrolysis was discovered and studied ([11]-[13]).

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Fig. 1

Figure 1: Fig. 1

One of the main stages of protein biosynthesis, as in peptide synthesis, is activation of the carboxyl group, but it proceeds in an aqueous medium under considerably milder conditions. In biosynthesis, activation of the carboxyl group occurs through the decomposition of aminoacyladenylates—compounds related to activated esters of α-amino acids and close to them in their chemical properties. Therefore, the study of the reactions of activated esters of α-amino acids and peptides in an aqueous medium under conditions close to physiological ones can be used in modeling biosynthetic processes.

The present work is devoted to an investigation of the stability of *p*-nitrophenyl esters of glycine, glycylglycine, and diglycylglycine and of their carbobenzoxy derivatives with respect to basic hydrolysis in an aqueous medium.

In connection with the very low solubility of *p*-nitrophenyl esters in water, hydrolysis was studied in an aqueous–alcoholic medium (50% by volume), at a constant concentration of hydroxyl ions, which was ensured by the use of buffer solutions. The course of hydrolysis with time was studied by a spectrophotometric method. From the data obtained, the rate constants for hydrolysis of the activated esters were calculated; these were used to estimate their relative reactivity. Alcoholic solutions of the *p*-nitrophenyl esters of the hydrobromides of glycine, glycylglycine, their carbobenzoxy derivatives, and carbobenzoxydiglycine were mixed with an equal volume of phosphate buffer ($M/15$), pH 7.20, so that the final concentration of ester was 10^{-4} mole.

Fig. 1. Hydrolysis of *p*-nitrophenyl esters of hydrobromides (a) and carbobenzoxy derivatives (b): 1 –glycine; 2 –glycylglycine; 3 –diglycylglycine; 4 –*p*-nitrophenyl acetate; in a phosphate buffer system (pH 7.2) –alcohol (1 : 1); at 25° $e = 1 - e'$ –concentration of *p*-nitrophenyl esters relative to their initial concentration

Before mixing, the absorption spectrum of the alcoholic solution of the *p*-nitrophenyl ester was measured, and the preservation of the ester was checked by the absence of an absorption band at λ 315 mμ. To dissolve the hydrobromide of the *p*-nitrophenyl ester of diglycylglycine, preliminary heating is necessary; this is accompanied by some hydrolysis, the extent of which, as well as the concentration of the unhydrolyzed ester in alcohol, was measured from the absorption spectrum. When the alcoholic solution of the activated ester is mixed with the buffer solution, warming occurs; therefore mixing was carried out at a temperature of 24.5°, which ensured that the entire experiment was performed at 25°.

After the alcoholic solution had been mixed with the buffer, the mixture was vigorously shaken and immediately transferred to the cuvette of an SF-4 spectrophotometer. The second cuvette contained a mixture of equal volumes of

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alcohol and phosphate buffer. Both cuvettes were placed in advance in a copper block thermostated at 25°, located in the cuvette compartment of the spectrophotometer. The time from mixing the components of the reaction mixture to taking the first reading did not exceed 30–40 sec. Measurements were made at the wavelength λ 405 mμ, corresponding to the absorption maximum of the *p*-nitrophenolate ion. The extent of hydrolysis was calculated from the formula $e = (E_m - E(t))/E_m$, where E is the optical density of the solution, and the subscript m denotes completion of hydrolysis (maximum optical density). A semilogarithmic transformation of the dependence of e' on time shows that hydrolysis of *p*-nitrophenyl esters, as expected for solutions with a constant concentration of hydroxyl ions, proceeds according to first order. From the data shown in Fig. 1, the experimental hydrolysis rate constants were calculated (Table 1). Under the same conditions, the hydrolysis of *p*-nitrophenyl acetate (NPA) was studied and the rate constant was measured: $k_{\mathrm{NPA}} = 5.75 \cdot 10^{-5}$ sec^{-1}.

The data obtained by us on the hydrolysis rate of *p*-nitrophenyl acetate are in agreement with the data of other authors. Bender and Turnquest (11) obtained the value $k_{\mathrm{NPA}} = 1.46 \cdot 10^{-5}$ sec^{-1}, studying the hydrolysis of *p*-nitrophenyl acetate in imidazole buffer, pH 7.15, at 26.2° in 5%

dioxane. Koltun, Clark et al. [13] obtained the value $k_{\mathrm{NFA}} = 1.92 \cdot 10^{-5}$ sec^{-1} in phosphate buffer, pH 7.27, at 25° in 2% alcohol. Bruice and Schmir [12], for phosphate buffers in 28.5% alcohol at 25°, found values $k_{\mathrm{NFA}} = 1.14 \cdot 10^{-5}$ sec^{-1} at pH 6.9 and $k_{\mathrm{NFA}} = 4.50 \cdot 10^{-5}$ sec^{-1} at pH 7.4. The somewhat larger value

$$k_{\mathrm{NFA}} = 5.75 \cdot 10^{-5} \text{ sec}^{-1},$$

obtained in the present work, is probably explained by an increase in the rate of hydrolysis of *n*-nitrophenyl acetate with increasing alcohol concentration in the reaction mixture.

Table 1

Relative rate of hydrolysis of *n*-nitrophenyl esters in the alcohol–phosphate buffer ($M/15$), pH 7.20 (1 : 1), at 25°

n-Nitrophenyl ester	k, sec^{-1}	k/k_{NFA}
Hydrobromide of glycine	$1.46 \cdot 10^{-2}$	$2.54 \cdot 10^{2}$
Hydrobromide of glycylglycine	$5.90 \cdot 10^{-3}$	$1.09 \cdot 10^{2}$
Hydrobromide of diglycylglycine	$7.84 \cdot 10^{-4}$	$0.136 \cdot 10^{2}$
N-Carbobenzoxyglycine	$1.51 \cdot 10^{-4}$	2.63

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n-Nitrophenyl ester	k, $\sec^{-1}$	k/k_{NFA}
N-Carbobenzoxyglycylglycine	$3.19 \cdot 10^{-4}$	5.57
N-Carbobenzoxydiglycylglycine	$3.84 \cdot 10^{-4}$	6.67

It has repeatedly been noted in the literature that the stability of ester groups (unactivated) decreases on passing from carboxylic acids to amino acids, although quantitative data on this question are still not available. The data obtained by us for activated n-nitrophenyl esters of amino acids and peptides are in agreement with the qualitative observations mentioned. Of all the compounds studied, as was to be expected, the hydrobromide of the n-nitrophenyl ester of glycine is hydrolyzed at the highest rate; removal of the amino group in the hydrobromide of the glycylglycine ester is accompanied by a decrease in the rate of hydrolysis by approximately 2.5 times and by approximately 19 times on passing to the tripeptide.

Thus, with growth of the peptide chain the influence of the amino group decreases, and the stability of the ester grouping increases, approaching the stability of esters of carboxylic acids. In the series of N-carbobenzoxy derivatives the reverse dependence is observed: on removal of the carbobenzoxy group the strength of the n-nitrophenyl esters decreases (on passing from glycine to the dipeptide and tripeptide). On passing from glycine to peptides, the rate of hydrolysis increases noticeably (though this difference is expressed less markedly than for the hydrobromides), while the difference in the hydrolysis rates for the peptides is comparatively small. It is important to note that, with growth of the chain, the difference in the hydrolysis rates of the n-nitrophenyl esters of the hydrobromides and of the corresponding N-carbobenzoxy derivatives decreases. If for glycine the ratio of the corresponding rate constants is 96.5, then already for glycylglycine it is 18.3 and for diglycylglycine only 2.05.

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