Relative Rates of Peptide Synthesis

Table 1

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Abstract

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(Aminolysis of *p*-Nitrophenyl Esters)

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At the present time, the synthesis of increasingly complex peptides containing a large number of amino acids in a specified sequence is being undertaken. Industrial synthesis of peptides is also beginning to develop. However, no systematic quantitative study has yet been made of peptide-bond synthesis reactions as a function of the position and nature of the amino acids.

Table 1

Rate constants for aminolysis of *p*-nitrophenyl esters (k, sec^{-1}). Concentration of the C-component $1 \cdot 10^{-4}$ M and of the N-component $1 \cdot 10^{-2}$ M

C-component	N-component	N-component	N-component
	H-glyc-—OC_2H_5	H-glyc$_2$-—OC_2H_5	H-glyc$_3$-—OC_2H_5
p-nitrophenyl acetate	$1.33 \cdot 10^{-5}$	$6.40 \cdot 10^{-5}$	$6.59 \cdot 10^{-5}$
Cbz-glyc-Onp	$1.07 \cdot 10^{-4}$	$4.68 \cdot 10^{-4}$	$5.70 \cdot 10^{-4}$
Cbz-glyc$_2$-Onp	$2.55 \cdot 10^{-4}$	$1.27 \cdot 10^{-3}$	$1.22 \cdot 10^{-3}$
Cbz-glyc$_3$-Onp	$2.12 \cdot 10^{-4}$	$1.10 \cdot 10^{-3}$	—

In this communication, the results are presented of measuring the relative rates of synthesis of a series of simple glycine peptides. In all cases, the peptide bond was formed through the interaction of the *p*-nitrophenyl ester of an N-carbobenzoxy derivative of an amino acid or peptide (C-component) with the ethyl ester of an amino acid or peptide (N-component):

$$\text{Cbz-glyc}_m\text{-Onp} + \text{H-glyc}_n\text{-}OC_2H_5 \rightarrow$$

$$\rightarrow \text{Cbz-glyc}_{m+n}\text{-}OC_2H_5 + HOC_6H_4NO_2 *$$

Fig. 1

Figure 1: Fig. 1

For a comparative study of the rates of peptide-bond formation, aminolysis of *p*-nitrophenyl esters has a number of advantages over other methods of synthesis.

The aminolysis reaction does not require the use of condensing agents and therefore proceeds in one stage. In an anhydrous medium, side reactions are absent. For studying the kinetics of the reaction, it is possible to use a continuous spectrophotometric method for measuring the concentrations of the initial C-component and of the reaction product—*p*-nitrophenol. The interaction of *p*-nitrophenyl esters of acetic acid and N-carbo-

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* The following abbreviations are adopted: Cbz—carbobenzoxy group ($C_6H_5CH_2OCO-$); glyc—glycine residue ($-NHCH_2-CO-$); Onp—*p*-nitrophenyl group ($-OC_6H_4NO_2$); $m, n = 1, 2, 3$ and $m+n = 2, 3, 4, 5$—number of amino acid residues.

benzyloxy derivatives of glycine, glycylglycine, and diglycylglycine with the ethyl esters of glycine, glycylglycine, and diglycylglycine.

All reactions were carried out under identical conditions: at 37.5° in a thermostated cuvette of an SF-4 spectrophotometer in dioxane solution. The course of the reaction was followed both by the appearance of *p*-nitrophenol ($\lambda = 305\,\mathrm{m}\mu$) and by the consumption of *p*-nitrophenyl esters ($\lambda = 270\,\mathrm{m}\mu$). The data obtained by measurement at both wavelengths coincide (Fig. 1). The end of the reaction was checked spectrophotometrically. The amine component was taken in large excess, and the reaction proceeded according to pseudo-first order (Fig. 1). The rate constants of all the reactions studied are given in Table 1.

Fig. 1. Kinetics of aminolysis of the *p*-nitrophenyl ester of N-carbobenzoxyglycylglycine (Cbz-gly$_2$-ONp + gly$_2$-OC$_2$H$_5$) in dioxane at 37.5°. Relative concentrations of Cbz-gly$_2$-ONp (C/C_0) calculated from optical densities measured at $\lambda = 305\,\mathrm{m}\mu$ (2) and $\lambda = 270\,\mathrm{m}\mu$ (1).

Comparison of the rates of peptide synthesis can be carried out in three directions: 1. Comparison of the rates of acylation of the N-component, i.e., comparison of the acylating ability of the C-components. 2. Comparison of the rates of aminolysis of the C-component, or comparison of the nucleophilicity of the N-components. 3. Comparison of the rates of synthesis of one and the same peptide from different C- and N-components.

1. Let us first consider the interaction of various *p*-nitrophenyl esters with the ethyl ester of glycylglycine (Tables 1 and 2). On going from *p*-nitrophenyl acetate to the *p*-nitrophenyl ester of glycine, the rate of N-acylation of the ethyl ester of glycylglycine increases noticeably. An increase in the elec-

tropositivity of the α-carbon atom of the acyl group facilitates nucleophilic attack by the amino group, which leads to an increase in the reaction rate. Removal of the carbobenzoxy group from the reaction center (the carbonyl carbon of the C-terminal amino acid) leads to a further facilitation of attack: peptide derivatives are more reactive than amino-acid derivatives. An increase in the reaction rate on going from *p*-nitrophenyl acetate to a glycine derivative, and then to its peptides, was observed by us earlier (1), in studying the reaction of alkaline hydrolysis of the same C-components (Table 2).

Table 2

Relative rates $(k/k_0)^*$ of N-acylation with the ethyl ester of glycylglycine and of hydrolysis** of *p*-nitrophenyl esters

C-component	k/k_0 N-acylation	k/k_0 hydrolysis
p-Nitrophenyl acetate	1	1
Cbz-gly-ONp	7.32	2.63
Cbz-gly$_2$-ONp	19.9	5.57
Cbz-gly$_3$-ONp	17.2	6.67

* k_0 is the rate constant of N-acylation and hydrolysis of *p*-nitrophenyl acetate, respectively.
** Hydrolysis in an ethanol–phosphate buffer system ($M/15$), pH 7.20 (1 : 1), at 25° (1).

The transition from dipeptide to tripeptide in the C-component is accompanied by a slight decrease in the rate of N-acylation, probably owing to steric hindrance in the *p*-nitrophenyl ester of N-carbobenzoxytripeptide upon formation of a tetrahedral intermediate complex with the rather bulky amine molecule. These steric hindrances, which are easily detected by means of Stuart atomic models, should not play a noticeable role in the formation of the intermediate complex with small molecules. Therefore, previously no decrease in the rate of alkaline hydrolysis was observed on going from dipeptide to tripeptide (Table 2). Consequently, for comparison of the reactivity

the C-component in the N-acylation reaction, one cannot use data obtained by measuring the relative rates of hydrolysis.

The difference in steric hindrance on going from a dipeptide to a tripeptide is possibly associated with the configuration of only the C-components, since the relative rates of N-acylation of the N-components are very close in magnitude (Table 3).

Table 3

Relative rates of N-acylation (k/k_0) of various N-components

C-component	N-component: H-gly-O $_2$H$_5$	N-component: H-gly$_2$-O $_2$H$_5$	N-component: H-gly$_3$-O $_2$H$_5$	σ_C^*
n-Nitrophenyl acetate	1	1	1	0
Cbz-gly-ONp	8.05	7.32	8.65	0.903
Cbz-gly$_2$-ONp	19.1	19.9	18.5	1.283
Cbz-gly$_3$-ONp	15.9	17.2	—	1.217

The constancy of the relative rates of N-acylation shows that this reaction obeys the Hammett–Taft rule of proportionality of changes in the free energy of activation ([2]) ($\lg k/k_0 = \sigma^*\rho^*$). Consequently, the reactivity of each of the C-components can be characterized by the value $\sigma_C^* = \lg k/k_0$, which is analogous in meaning to the Taft constant σ^*, a quantitative measure of transmission of polar influence by the induction mechanism. In calculating σ_C^*, it was assumed that ρ_C^* for the N-acylation reaction is equal to unity. The equality of σ_C^* for different N-components shows that the state of the amino groups in all N-components is the same.

2. In an analogous way, one can compare the rates of aminolysis of each of the nitrophenyl esters with all the N-components. If the ethyl ester of glycine is taken as the standard amine component, then from the data in Table 1 one can calculate the relative rates of aminolysis for the N-components (Table 4). The agreement of the relative rates of aminolysis with different C-components makes it possible to introduce the value σ_N^*, characterizing the relative reactivity of the N-components.

Table 4

Relative rates of aminolysis $(k/k_0')^*$ of various C-components

C-component	N-component: H-gly-O $_2$H$_5$	N-component: H-gly$_2$-O $_2$H$_5$	N-component: H-gly$_3$-O $_2$H$_5$
n-Nitrophenyl acetate	1	4.81	4.95
Cbz-gly-ONp	1	4.37	5.32
Cbz-gly$_2$-ONp	1	4.98	4.30
Cbz-gly$_3$-ONp	1	5.28	—
σ_N^*	0	0.687	0.701

* k'_0 is the rate constant of N-acylation of the ethyl ester of glycine for each C-component, respectively.

It is important to note that, on going from a dipeptide to a tripeptide, the rate of aminolysis does not decrease. This corresponds to our assumption that there are no differences in steric hindrance for the N-component. The rates of acylation of glycine, glycylglycine, and diglycylglycine by acetic anhydride and fluorodinitrobenzene in aqueous medium were measured by Havinga and co-workers ([3]). Assuming for these reactions $\rho^*_N = -1$, the calculated values of σ^*_N for glycine, glycylglycine, and diglycylglycine are, respectively, 0, 0.66, and 0.65, and agree fairly well with our data.

The assumption that the reactivity of the amino group in the N-component increases on going from an amino acid to a peptide had earlier been made on the basis of indirect data, in an analysis of the kinetics of polycondensation of the ethyl ester of glycine [4]. Measurement of the relative rates of peptide synthesis by the method of aminolysis of *n*-nitrophenyl esters made it possible to confirm this assumption directly.

3. Any peptide containing no fewer than three amino acid residues can be obtained in different ways. For example, a tripeptide can be synthesized in the reactions: Cbz-glyc-ONp + H-glyc$_2$-OC$_2$H$_5$ and Cbz-glyc$_2$-ONp + H-glyc-OC$_2$H$_5$. The rate constants of these two reactions are in the ratio 1.76 : 1. A tetrapeptide can be synthesized by three routes: Cbz-glyc-ONp + H-glyc$_3$-OC$_2$H$_5$, Cbz-glyc$_2$-ONp + H-glyc$_2$-OC$_2$H$_5$, and Cbz-glyc$_3$-ONp + H-glyc-OC$_2$H$_5$. The rate constants of these reactions are in the ratio 2.59 : 6.01 : 1.

These data show that the rate of synthesis of a peptide bond depends strongly on the choice of the initial C- and N-components. The addition of peptide derivatives both as the N-component and as the C-component proceeds at a higher rate than the addition of the corresponding amino acid derivatives.

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