SOME LAWS OF THE POLYCONDENSATION OF ACTIVATED ESTERS OF AMINO ACIDS AND PEPTIDES

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UDC 547.466.1

In recent years, polypeptides of regular structure have acquired great importance as convenient models for the study of the structure and functions of a number of proteins [1-3]. Polypeptides of this type can be obtained by the activated-ester method. The following groups are the most widely used for activating the carboxy group of a peptide: 2,4,5- and 2,4,6-trichlorophenyl, pentachlorophenyl, and some others [4].

However, the quality of the polypeptide obtained, including its molecular weight, depends not only on the selection of the corresponding activating group but also on the optimum relationship of such factors as the temperature and time of polycondensation, the concentration of the monomer, etc.

In view of the great difficulty in obtaining regular polypeptides and also the absence from the literature of appropriate recommendations, we have made an attempt to systematize our results on the influence of various factors on the degree of polymerization of polypeptides. These factors may also include the time, the type of solvent, the concentration of the monomer in the solvent, the amino acid sequence in the monomer, and the nature of the N- and C-terminal amino acids.

For this purpose, the activated esters of amino acids and of di- and tripeptides were subjected to polycondensation. Table 1 gives the constants of the compounds obtained.

Polycondensation was performed in sealed tubes in dimethyl sulfoxide (DMS) or dimethylformamide (DMF) solution in the presence of an equivalent amount of triethylamine. The polypeptides obtained on

TABLE 1. Physicochemical Constants of Activated Esters of Amino Acids and Peptides

Compound	Yield, %	mp, °C	R_f (system)*	$[\alpha]^{20}_D$, deg
Cbo-Gly-OPhCl$_3$(2,4,6)	81,9	107–108	0,69(2)	—
Cbo-Lys(Tos)-OPhCl$_5$	81,6	153–154	0,85(2)	−11,3(c 1,5; NaHCO$_3$)
Cbo-Glu(γ-Bzl)-OPhCl$_5$	49,6	121–122	0,90(2)	−23,4(c 1,4; CHCl$_3$)
Cbo-Glu(γ-Bzl)-OPhCl$_3$(2,4,6)	84,1	77–78	0,9 (3)	−21,3(c 0,7; CH$_3$OH)
Cbo-Gly-OPhCl$_5$	55,72	185–187	—	—
Cbo-Glu(γ-OMe)-Gly-OPhCl$_5$	67,5	129–130	0,83(2)	−11,6(c 1,4 ;THF)
Cbo-Glu(γ-OMe)-Gly-OPhCl$_3$ (2,4,6)	66,6	117–118	0,54(1)	− 7,9(c 1,4 CHCl$_3$)
Cbo-Gly-Lys(Tos)-OPhCl$_5$	74	135–136	0,82(2)	−10,5(c 2; CHCl$_3$)
Cbo-Gly-Glu(γ-Bzl)-OPhCl$_5$	65	118 121	0,80(2)	− 7,2(c 1; DMF)
Cbo-Gly-Glu(γ-Bzl)-OPhCl$_3$(2,4,6)	56	137–138	0,74(2)	− 6,3(c 1,1 DMF)
Cbo-Lys(Tos)-Gly-OPhC$_5$	81	156–157	0,89(2)	−17,8(c 0,8 DMF)
Cbo-Glu(γ-Bzl)-Gly-Ala-OPhNO$_2$	68	98–99	0,91(2)	−37,6(c 0,9 CH$_3$OH)
Cbo-Glu(γ-Bzl)-Gly-Lys(Tos)-OPhCl$_5$	64	107–108	—	−9,1(c 0,3; CHCl$_3$
Cbo-Gly-Ala-Hypro-OPhCl$_3$	78	170–172	0,74(1)	—

*Systems: 1) butan-1-ol−H_2O−CH_3COOH (4:5:1); 2) butan-1-ol−H_2O−CH_3COOH (4:1:1); 3) butan-1-ol−3% NH_4OH (100:44).

V. I. Lenin Tadzhik State University. Institute of Molecular Biology, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 773-779, November-December, 1973. Original article submitted September 26, 1972.

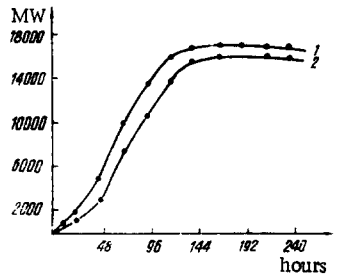

Fig. 1

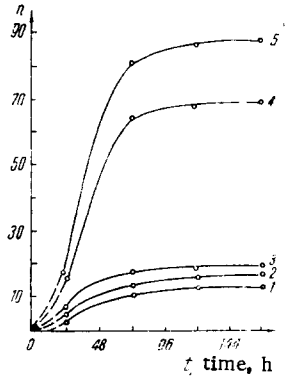

Fig. 2

Fig. 1. Molecular weights of the polypeptides $(Gly\text{-}Ala\text{-}Hypro)_n$ as a function of the nature of the solvent: 1) DMF; 2) DMS.

Fig. 2. Degree of polymerization of the polypeptides as a function of the time of polycondensation: 1) H-Glu-(γ-OMe)-$OPhCl_3$ (2,4, 6); 2) H-Glu(γ-OMe); $OPhCl_5$; 3) H-Lys(Tos)-$OPhCl_5$; 4) H-Gly-$OPhCl_3$ (2, 4, 6); 5) H-Gly-$OPhCl_5$.

polycondensation were isolated by treating the reaction mixture with methanol, whereupon the low-molecular-weight fractions dissolved in the methanol and the high-molecular-weight fractions precipitated. The mean molecular weights (M_{av}) were determined by the Van Slyke method. Although in this method the permissible error is ±10-15%, it is convenient because it is quick to perform. A certain error is of no significance, since what are important for our purposes are the relative values of M_{av} and not their absolute magnitudes. Information on the polypeptides obtained is given in Table 2.

The influence of the nature of the solvent on the polycondensation process was studied with two monomers: H-Gly-Ala-OPro-$OPhCl_5$ and H-Gly-Glu-(γ-OMe)-Gly-$OPhNO_2$. The polycondensation of the first monomer was performed in DMS and DMF solutions (Fig. 1). As can be seen from the figure, the curves almost coincide, which shows the similar nature of the processes taking place. In the polycondensation of the second monomer in DMF and dioxane solutions it was found that dioxane does not greatly favor the polycondensation process: M_{av}) 3173, n = 12. In dimethylformamide in the same time, M_{av} = 9108, n = 36.

We determined the dependence of M_{av} of the polypeptides on the time of polycondensation in 50% solution at 20°C. As was expected, the polycondensation of amino acid esters takes place more vigorously than that of dipeptide and tripeptide esters (Figs. 2-4). Consequently, after the same time the polypeptides obtained from amino acid esters have higher degrees of polymerization.

TABLE 2. Polypeptides Obtained by the Polycondensation of Activated Esters of Amino Acids and Peptides

Elementary unit of the polypeptide	Ester	Time of polycondensation, h	M_{av}	n	Literature references
$(\text{-Gly-})_n$	$-C_6Cl_5$		4950	87	
$(\text{-Gly-})_n$	$-C_6H_2Cl_3(2,4,6)$		3965	69	
$[\text{-Glu}(\gamma\text{-OMe})\text{-}]_n$	$-C_6Cl_5$		3640	16	[7]
$[\text{-Glu}(\gamma\text{-OMe})\text{-}]_n$	$-C_6H_2Cl_3(2,4,6)$		2828	13	
$[\text{-Lys}(N^{\varepsilon}\text{-Tos})\text{-}]_n$	$-C_6Cl_5$		5518	20	
$[\text{-Gly-Glu}(\gamma\text{-Bzl})]_n$	$-C_6Cl_5$	168	2814	16	
$[\text{-Gly-Glu}(\gamma\text{-Bzl})]_n$	$-C_6H_2Cl_3(2,4,6)$		2800	10	
$[\text{-Glu}(\gamma\text{-OMe})\text{-Gly-}]_n$	$-C_6Cl_5$		2450	14	
$[\text{-Glu}(\gamma\text{-OMe})\text{-Gly-}]_n$	$-C_6H_2Cl_3(2,4,6)$		2275	12	[8]
$[\text{-Gly-Lys}(N^{\varepsilon}\text{-Tos})\text{-}]_n$	$-C_6Cl_5$		7000	20	
$[\text{-Lys}(N^{\varepsilon}\text{-Tos})\text{-Glu-}]_n$	$-C_6Cl_5$		4218	10	
$[\text{-Glu}(\gamma\text{-OMe})\text{-Gly-Ala}]_n$	$-C_6H_4NO_2$		7838	22	[9]
$[\text{-Gly-Gly}(\gamma\text{-OMe})\text{-Gly-}]_n$	$-C_6H_4NO_2$	144	9108	35	

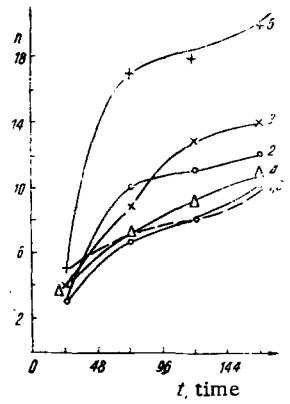

Fig. 3

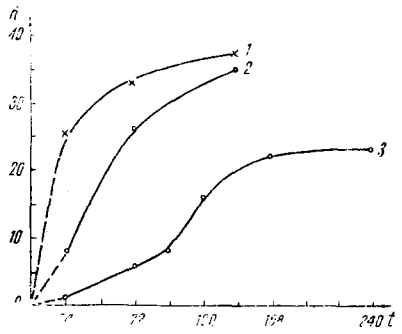

Fig. 4

Fig. 3. Degree of polymerization of the polypeptides as a function of the time of polycondensation: 1) H-Gly-Glu(γ-Bzl)-OPhCl$_3$ (2, 4, 6); 2) H-Glu(γ-OMe)-Gly-OPhCl$_3$ (2, 4, 6); 3) H-Glu(γ-OMe)-Gly-OPhCl$_5$; 4) H-Gly-Glu(γ-Bzl)-OPhCl$_5$; 5) H-Gly-Lys(Tos)-OPhCl$_5$; 6) H-Lys(Tos)-Gly-OPhCl$_5$.

Fig. 4. Degree of polymerization of the polypeptides as a function of the time of polycondensation: 1) Glu(γ-OMe)-Gly-AlaOPhNO$_2$; 2) Gly-Glu(γ-OMe)-Gly-OPhNO$_2$ without the addition of nitrophenol; 3) Gly-Glu(γ-OMe)-GlyOPhNO$_2$ with the addition of nitrophenol.

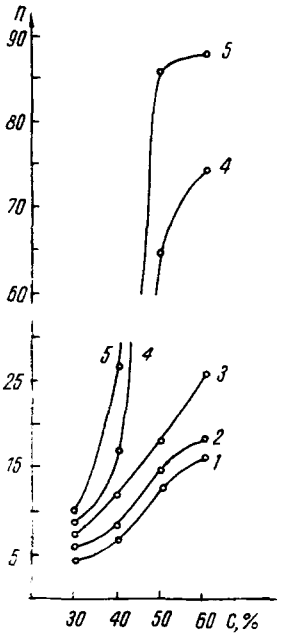

Fig. 5

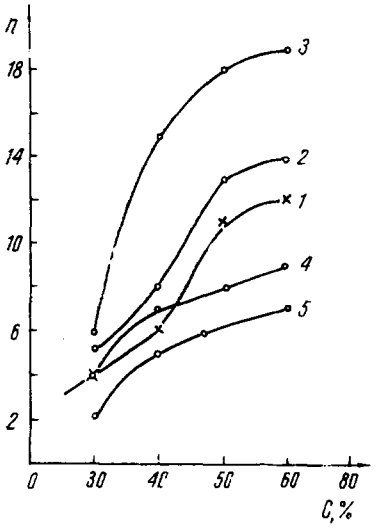

Fig. 6

Fig. 5. Degree of polymerization of polypeptides as a function of the initial concentration of monomer in solution: 1) H-Glu(γ-OMe)-OPhCl$_3$ (2, 4, 6); 2) H-Glu(γ-OMe)-OPhCl$_5$; 3) H-Lys(Tos)-OPhCl$_5$; 4) H-Gly-OPhCl$_3$ (2, 4, 6); 5) H-Gly-OPhCl$_5$.

Fig. 6. Degree of polymerization of polypeptides as a function of the initial concentration of the monomer in solution: 1) H-Glu(γ-OMe)-Gly-OPhCl$_3$ (2, 4, 6); 2) H-Glu(γ-OMe)-Gly-OPhCl$_5$; 3) H-Gly-Lys(Tos)-OPhCl$_5$; 4) H-Lys(Tos)-Gly-OPhCl$_5$; 5) H-Glu(γ-Bzl)-Gly-Lys(Tos)-OPhCl$_5$.

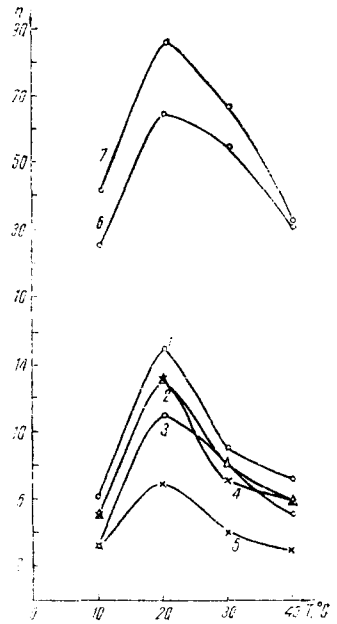

Fig. 7. Degree of polymerization of polypeptides as functions of the temperature of polycondensation: 1) H-Glu(γ-Bzl)-$OPhCl_6$; 2) H-Glu(γ-Bzl)-$OPhCl_3$ (2,4,6); 3) H-Glu(γ-OMe)-Gly-$OPhCl_3$ (2,4,6); 4) H-Glu(γ-OMe)-Gly-$OPhCl_5$; 5) H-Glu-(γ-Bzl)-Gly-Lys(Tos)-$OPhCl_5$; 6) H-Gly-$OPhCl_3$ (2,4,6); 7) H-Gly-$OPhCl_5$.

In a comparison of the polycondensation of esters of various amino acids (see Fig. 2), attention is attracted by the fact that the activity of the esters depends on the nature of the amino acids, in addition to other factors. Thus, 2,4,6-trichlorophenyl esters of glycine are more active than pentachlorophenyl esters of lysine and of glutamic acid (on polycondensation the glycine esters give polypeptides with a higher degree of polymerization), while for the same amino acids and peptides the pentachlorophenyl esters are the most active [5]. Esters of lysine are more active than esters of glutamic acid.

If esters of the same radical with different peptides are compared, it is possible to trace the relationship between the activity of the ester and the nature of the N- and C-terminal amino acids (see Fig. 3). These relationships can be well seen on comparing the pentachlorophenyl esters H-Glu(γ-OMe)-Gly-$OPhCl_5$, H-Lys(Tos)-Gly-$OPhCl_5$, H-Gly-Lys(Tos)-$OPhCl_5$, and H-Lys(Tos)-Gly-$OPhCl_5$, H-Glu(γ-OMe)-Gly-$OPhCl_5$ and H-Gly-Glu(γ-Bzl)-$OPhCl_5$. In the polycondensation of the esters H-Glu(γ-OMe)-Gly-$OPhCl_5$ and H-Lys(Tos)-Gly-$OPhCl_5$ (curves 3 and 6 of Fig. 3), the C-terminal amino acid is lysine. Hence, the difference in the activities of the monomeric compounds can be ascribed to the N-terminal amino acid.

On comparing the polycondensation of another pair of monomers – H-Gly-Lys(Tos)-$OPhCl_5$ and H-Gly-Glu(γ-Bzl)-$OPhCl_5$ (curves 5 and 4 in Fig. 3), a different characteristic can be clearly seen. In the molecules of these monomers the N-terminal amino acids are glycine and the C-terminal amino acids are glutamic acid and lysine. This obviously shows the great importance for the polycondensation process of the nature of the C-terminal amino acid.

On comparing the activities of esters of dipeptides consisting of the same amino acid residues with different sequences, it can be seen that H-Gly-Lys(Tos)-$OPhCl_5$ (curve 5 of Fig. 3) and H-Lys(Tos)-Gly-$OPhCl_5$ (curve 6) differ sharply, and H-Glu(γ-OMe)-Gly-$OPhCl_5$ (curve 3) and H-Gly-Glu(γ-Bzl)-$OPhCl_5$ (curve 4) have no such difference. While in the first pair of monomers the more active is the ester H-Glu-Lys(Tos)-$OPhCl_5$, in the second pair it is H-Glu(γ-OMe)-Gly-$OPhCl_5$, which shows the role of the C-terminal amino acid in an activated peptide ester.

In addition to the polycondensation of esters of amino acids and dipeptides, we studied the polycondensation process of a number of activated esters of tripeptides – H-Glu(γ-OMe)-Gly-Ala-$OPhNO_2$ and H-Gly-Glu(γ-OMe)-Gly-$OPhNO_2$ (see Fig. 4).

The results of an analysis of the curves of the tripeptides show that the polycondensation of tripeptide esters takes place in such a way that sections showing a low rate of the process appear on the curves, these probably being connected with the accumulation of phenol derivatives in the reaction mixture. Then a marked acceleration of the process is observed. After 7-10 days, polycondensation ceases completely or slows down sharply. To investigate the hypothesis of the influence of phenol derivatives on the process, the action of various amounts of p-nitrophenol was studied. When p-nitrophenol was added to the reaction mixture in a ratio to the monomer of 0.1 : 1 (molar), the process sharply accelerated in the first 24 h, i.e., during the period when the rate of the process was low in the absence of added p-nitrophenol. The effect of p-nitrophenol can be explained by the formation of a salt at the amino group of the peptide, which increases the positive charge on the carbonyl carbon of the ester group (as a result of the elimination of the influence of the amino group, which lowers this charge).

In order to study the dependence of M_{av} on the initial concentration of the monomer solution, we used 30, 40, 50, and 60% solutions. Initial concentrations of less than 30% were not investigated, since, as De Tar et al. have already shown [6], at these concentrations intramolecular processes take place, especially cyclization.

Concentrations greater than 60% are undesirable; the solutions become too viscous with a considerable worsening of the possibility of stirring the reaction mixture. It can be seen from a consideration of the curves of molecular weights as functions of the concentrations of the solutions (Figs. 5 and 6) that a change in concentration has the greatest effect on the polycondensation of activated glycine esters. At a concentration of 30%, the degree of polymerization of, for example, the pentachlorophenyl ether is 10 while at a concentration of 60% it is 88. This is obviously connected with the ease of conversion of glycine esters into cyclic products at low concentrations.

The change in the rate of polycondensation of dipeptides remains independent of the nature of the amino acids in them when the concentration is increased 2- to 3-fold (from 30% to 60%). Among the dipeptide esters, the concentration of monomer in the solution most strongly affects the pentachlorophenyl ester H-Gly-Lys(Tos)-OPhCl_5. This effect of a change in the concentration of the monomer is probably due to the better solubility of the monomer and the slower increase in the viscosity of the reaction mixture.

Figure 7 shows the degree of polymerization of polypeptides as a function of the temperature of the polycondensation processes. This relationship was studied with the other parameters of the process kept constant (polycondensation was performed for 126 h with 50% solutions of six monomers). As can be seen from the figure, it is of the same type for all the monomers. Of the four temperatures studied (10, 20, 30, and 40°C) the optimum is +20°C. As was stated above, the other relationships were considered at just this temperature.

At +10°C, the rates of all the processes are low and therefore the chain grows insignificantly. With a rise in the temperature, the number of active polycondensation centers increases and therefore the growth of the chain about each center decreases, which is in harmony with the experimental results obtained.

EXPERIMENTAL

The work was performed with L-amino acids. The individuality of the compounds synthesized was checked by chromatography on paper and in a thin layer of silica gel–gypsum (for solvent systems see Table 1).

The polycondensation of all the monomers – hydrobromides of amino acids and of peptides – was performed by the same method. A weighed sample of monomer was introduced into a tube and the appropriate amount of solvent was added. If the monomer was insoluble in the cold, the mixture was heated to 40-50°C. Then carefully purified triethylamine was added in an equimolecular or 1.1 : 1 ratio to the monomer. The reaction mixture was stirred, and the tube was sealed. Then it was placed in a thermostat at the required temperature (the fluctuations did not exceed ±1°C).

After the end of the time of polycondensation, the polymer was precipitated with methanol. The methanol-insoluble fraction was separated off and washed with methanol. For more careful purification, the polypeptides were washed with methanol and dimethylformamide (successively) in Soxhlet apparatuses for 4-5 h. The yield of high-molecular-weight fraction was 13-30%.

The molecular weights were determined by the Van Slyke method (Ioanisiani's modification) [10].

SUMMARY

The polycondensation of activated esters (p-nitrophenyl, 2,4,6-trichlorophenyl, and pentachlorophenyl esters) of a number of amino acids and di- and tripeptides has been studied. The dependence of the degree of polymerization of the polypeptides on the time of polycondensation, on the initial concentration of monomer in the solution, and on the temperature has been established. The roles of the N- and C-terminal amino acids in the monomers in determining their capacity for polycondensation have been studied.

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