THE ROLE OF THE N- AND C-TERMINAL AMINO ACIDS OF ACTIVATED PEPTIDE ESTERS IN THEIR POLYCONDENSATION

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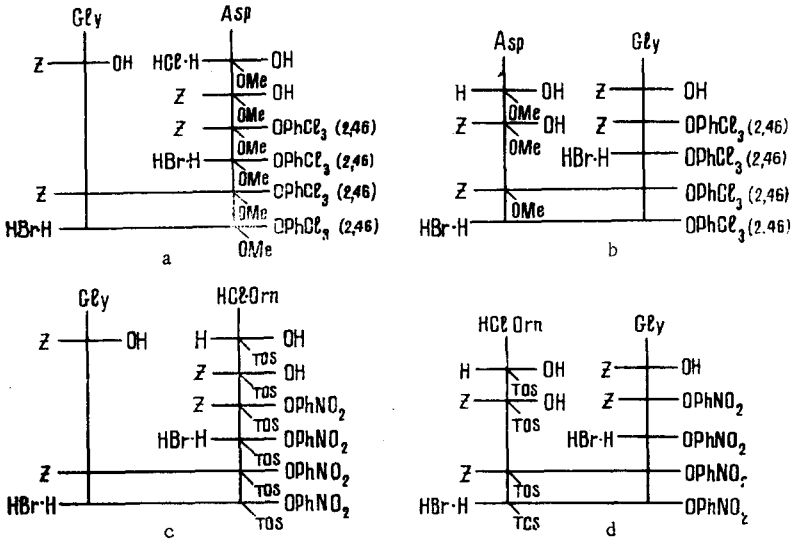
We have previously reported a study of the polycondensation of activated esters of certain amino acids [1], dipeptides [2], and tripeptides [3]. In the present paper we give the results of an investigation of the polycondensation of the activated esters of a number of dipeptides with various amino acid sequences in order to determine the role of the N- and C-terminal amino acids in this reaction.

As the monomeric compounds, we used HBr · Gly-Asp(OMe)-$OPhCl_3$-(2,4,6), HBr · H-Asp(OMe)-Gly-$OPhCl_3$-(2,4,6), HBr · H-Gly-Orn(Tos)-ONP and HBr · H-Orn(Tos)-Gly-ONP. The starting materials for obtaining these monomers were Cbo-GlyAsp(OMe)-$OPhCl_3$-(2,4,6), Cbo-Asp(OMe)-Gly-$OPhCl_3$-(2,4,6), Cbo-Gly-Orn(Tos)-ONP and Cbo-Orn(Tos)-Gly-ONP, respectively. The constants of the compounds synthesized are given in Table 1.

The synthesis of the initial compounds was performed by schemes a-d.

The activated esters used had approximately equivalent activities (for p-nitrophenol, pK 9.41, and for 2,4,6-trichlorophenol, pK 9.65) [4].



Schemes of the synthesis of the activated esters H-Gly-Asp(OMe)-OH (a), H-Asp(OMe)-Gly-OH (b), H-Gly-Orn(Tos)-OH (c), and H-Orn (Tos)-Gly-OH (d).

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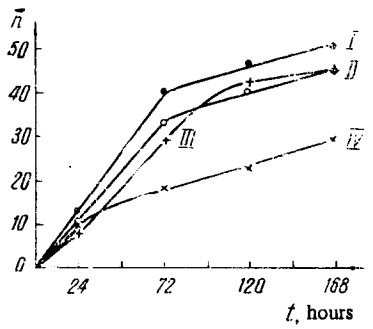


Fig. 1. Dependence of the degree of polymerization of the polypeptides on the time: I) H-Asp(OMe)-Gly-$OPhCl_3$; II) H-Gly-Asp(OMe)-$OPhCl_3$; III) H-Gly-Orn(Tos)-ONP; IV) H-Orn (Tos)-Gly-ONP.

TABLE 1

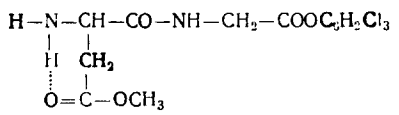
Compound	Yield, %	mp, °C	R_f^*
Cbo-Gly-(β-OMe)-Asp-OH	35,0	101	0,49 (3)
Cbo-Gly-(β-OMe)-Asp-$OPhCl_3$	40,0	110	0,88 (3)
Cbo-(β-OMe)-Asp-Gly-$OPhCl_3$	53,0	103	0,95 (3)
Cbo-Orn (Tos)-$OPhNO_2$	59,3	143–145	0,84 (1)
HBr·H-Orn (Tos)-$OPhNO_2$	83,0	121–126	0,34 (1) 0,17 (2)
Cbo-Gly-Orn (Tos)-$OPhNO_2$	64,0	132–134	0,85 (1) 0,83 (2)
Cbo-Orn (Tos)-Gly-$OPhNO_2$	74,6	140–142	0,80 (1)
HBr·H-Gly-(β-OMe)-Asp-$OPhCl_3$	94,0	163–166	0,71 (3)
HBr·H-(β-OMe) Asp-Gly-$OPhCl_3$	80,7	111–113	0,59 (3)
HBr·H-Orn (Tos)-Gly-$OPhNO_2$	94,0	109–114	0,62 (1) 0,50 (2)
HBr·H-Gly-Orn (Tos)-$OPhNO_2$	78,9	183–185	0,19 (1) 0,24 (2)

* Chromatography was performed in the following solvent systems: butan-1-ol–water–acetic acid (4 : 5 : 1) (1); butan-2-ol–3% ammonia (100 : 44) (2); and butan-1-ol–water–acetic acid (4 : 1 : 1) (3).

Polycondensation was performed in sealed tubes at 20°C. The monomeric hydrobromides of the activated peptide esters were dissolved in dimethylformamide (DMF) (concentration 50%). After the addition of an equivalent amount of carefully purified triethylamine (TEA), the reaction mixture was kept for a predetermined time. The polypeptides were isolated by precipitation with methanol. To free them from low-molecular-weight impurities and from the TEA salt, they were washed with methanol and DMF. The mean molecular weight (M_{av}) of the polypeptides were determined by the Van Slyke method. The results of a study of the dependence of M_{av} on the time are given in Fig. 1

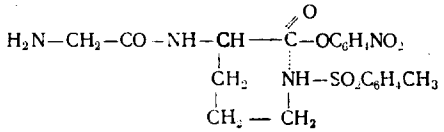
As can be seen from Fig. 1, in the case of HBr · H-Asp(OMe)-Gly-$OPhCl_3$ and HBr · H-Gly-Asp-(OMe)-$OPhCl_3$, (curves I and II), M_{av} is greater for the former. A similar pattern has been observed in a comparison of HBr · H-Gly(OMe)-Gly-$OPhCl_3$ and HBr · H-Gly-Glu(OMe)-$OPhCl_3$ [2]. The greater activity of HBr · H-Asp(OMe)-Gly-$OPhCl_3$ and HBr · H-Glu(OMe)-Gly-$OPhCl_3$ can be explained in the following way. When residues of aspartic and glutamic acids are present at the C end of the peptide; mutual repulsion of the carboxy group takes place, as a consequence of which steric hindrance arises which interferes with the attack of the carbonyl oxygen of the ester group by the NH_2 group of the other peptide molecule.

When aspartic and glutamic acid residues are present at the N end of the peptide, the formation of an intramolecular hydrogen bond is possible, which increases the nucleophilicity of the nitrogen



It must also be mentioned that in this case the steric hindrance mentioned above disappears.

On comparing the other pair of monomers HBr · H-Gly-Orn(Tos)-ONP and HBr · H-Orn(Tos)-Gly-ONP (Fig. 2, curves III and IV) it can clearly be seen that the activity of HBr · H-Gly-Orn(Tos)-ONP is higher, and therefore the polypeptide formed has a larger value of M_{av} than in the case of HBr · H-Orn(Tos)-Gly-ONP. Such behavior of these monomers can be explained by the formation of an intramolecular coordination bond between the NH group and the carbonyl carbon in the COOR group when an ornithine residue is present at the C end of the peptide:



Because of this, the positive charge on the carbonyl carbon is increased, which facilitates the nucleophilic attack of its NH_2 group by another molecule of the monomer. In addition, it leads to the formation of a cyclic structure having a planar arrangement, which also facilitates this attack.

When there is an ornithine residue at the N end of the peptide, the long side chain ($-CH_2-CH_2-CH_2-NH-SO_2C_6H_4CH_3$) creates steric hindrance to the interaction of the NH_2 group with the ester group of another molecule of the monomer.

Similar results were obtained in a study of the polycondensation of HBr · H-Lys(Tos)-Gly-$OPhCl_5$ and HBr · H-Gly-Lys(Tos)-$OPhCl_5$ (2).

EXPERIMENTAL

The work was carried out with L-amino acids. Chromatography was performed in a thin layer of silica gel (300 mesh) fixed with gypsum on plates 75×25 mm in solvent systems 1, 2, and 3 (see Table 1). The chromatograms were revealed with ninhydrin and iodine vapor.

The starting materials were obtained by known methods: Cbo-Gly-Cl [5], HCl · H-Asp(OMe)-OH [6], Cbo-Asp(OMe)-OH [7], H-Orn(Tos)-OH [8], Cbo-Orn(Tos)-OH [9], Cbo-Gly-$OPhCl_3$-(2,4,6) [10], Cbo-Gly-ONP, and HBr • H-Gly-ONP [11].

The analyses of all the compounds corresponded to the calculated figures.

1. Cbo-Gly-Asp(OMe)-OH (I). With vigorous stirring, 5 g of Cbo-Gly-Cl [5] was added to 4.04 g of HCl · H-Asp(OMe)-OH [6] in 100 ml of water in the presence of $NaHCO_3$ (pH 8). Stirring was continued at −1°C for 1 h and at ~ 20°C for 2 h. After extraction with ether, the aqueous layer was acidified to pH 1-2, and the oil that separated out was extracted with ethyl acetate. The solution was washed with water and dried. After evaporation of the solvents, 3.15 g (34.9%) of compound (I) was obtained with the composition $C_{15}H_{18}O_7N_2$, mp 111°C, R_f (2) 0.49.

2. Cbo-Gly-Asp(OMe)-$OPhCl_3$-(2,4,6) (II). To a solution of 1 g of (I) in 10 ml of ethyl acetate cooled to 0°C was added 0.6 g of dicyclohexylcarbodiimide (DCHCD). After the mixture had been stirred for 15 min, 0.58 g of 2,4,6-trichlorophenol was added to it. Stirring was continued at 0°C for 3 h, and then the reaction mixture was left overnight. The excess of DCHCD was decomposed with glacial acetic acid, and the precipitate of dicyclohexylurea (DCHU) was filtered off. The filtrate was evaporated, and the residue was dissolved in ethyl acetate. The new precipitate of DCHU that formed was filtered off, and the filtrate was washed with water, 1 N HCl, 5% $NaHCO_3$, and water again, and was dried over Na_2SO_4. The solvent was driven off, and the residue was crystallized by trituration with ether. This gave 0.6 g (40.0%) of (II) with the composition $C_{21}H_{19}O_7N_2Cl_3$, mp 110°C, R_f (2) 0.88; $[\alpha]_D^{27}$ −36.4 (c 1.5; DMF).

3. Cbo-Asp(OMe)-Gly-$OPhCl_3$-(2,4,6) (III). To a solution of 2.81 g of Cbo-Asp(OMe)-OH [7] and 1.4 ml of triethylamine (TEA) in 6.2 ml of chloroform cooled to −10°C was added 1.31 g of isobutyl chloroformate (IBCF). The mixture was stirred at −10°C for 40 min and at −5°C for 15 min and was then cooled again to −10°C. Then a suspension of 2.6 g of HBr · H-Gly-$OPhCl_3$-(2,4,6) [10] in 1.4 ml of TEA and 6 ml of chloroform cooled to −10°C was added. The mixture was stirred at −10°C for 40 min, at 0°C for 15 min, at 20°C for 2 h, and at 50°C for 15 min, after which it was diluted twofold with chloroform. The solution was washed with water, 1 N HCl, water again, 0.5 N $NaHCO_3$, and water again, and was dried over Na_2SO_4. The solvent was evaported until the residue had the state of a viscous syrup, and this was dissolved in ethyl acetate. After precipitation with ether, 2.65 g (53%) of (III) with the composition $C_{21}H_{19}O_7N_2Cl_3$ was obtained mp 103°C; R_f (3) 0.95; $[\alpha]_D^{27}$ −48.7 (c 1.5; DMF).

4. Cbo-Orn(Tos)-ONP (IV). By the method of Para. 3, 1 g of Cbo-Orn(Tos)-OH (III), 0.3 g of p-nitrophenol, 0.22 ml of ethyl chloroformate (ECF), and 0.32 ml of TEA yielded 0.76 g (59.3%) of (IV), mp 143-145°C, R_f (I) 0.84.

5. HBr · H-Orn(Tos)-ONP (V). To a solution of 0.6 g of (IV) in 3 ml of glacial CH_3COOH was added 3 ml of a solution of HBr in glacial CH_3COOH (3.5%). The mixture was kept at 20°C for 30 min. Then the hydrobromide was precipitated with cooled ether. After recrystallization from a mixture of methanol and ether, 0.4 g (83%) of (V) was obtained with mp 121-126°C, R_f (1) 0.34, R_f (2) 0.17.

6. Cbo-Gly-Orn(Tos)-ONP (VI). By the method of Para. 3, 1 g of Cbo-Gly-OH, 1.9 g of (V), 0.46 ml of ECF, and 0.64 ml of TEA yielded 1.83 g (64%) of (VI) with the composition $C_{29}H_{32}O_9N_4S$, mp 132-134°C, R_f (1) 0.85, $[\alpha]_D^{27}$ −21.4° (c 0.4; $CHCl_3$).

7. Cbo-Orn(Tos)-Gly-ONP (VII). By the method of Para. 3, 1 g of Cbo-Orn(Tos)-OH [9], 0.66 g of HBr · H-Gly-ONP [11], 0.22 ml of ECF, and 0.64 ml of TEA yielded 1 g (74.6%) of (VII) with the composition $C_{29}H_{32}O_9N_4S$, mp 140-142°C, R_f (1) 0.80 $[\alpha]_D^{20}$ −45.1° (c 0.7; $CHCl_3$ − CH_3COCH_3, 15:10).

8. HBr · H-Gly-Asp(OMe)-OPHCl_3-(2,4,6) (VIII). Compound (II) (0.35 g) was treated with 0.32 ml of a 30% solution of HBr in glacial CH_3COOH. After 40 min, the hydrobromide was precipitated with cooled ether. This gave 0.29 g (94%) of (VIII), mp 165°C, R_f (III) 0.71.

9. HBr · H-Asp(OMe)-Gly-OPhCl_3-(2,4,6) (IX). Compound (III) (1 g) was treated with 1.2 ml of a 30% solution of HBr in glacial CH_3COOH. After 30 min, the hydrobromide was precipitated with ether. This gave 0.71 g (80.7%) of (IX), mp 113°C, R_f (III) (0.59).

10. HBr · H-Gly-Orn(Tos)-ONP (X). To a solution of 0.5 g of (VI) in 3 ml of glacial CH_3COOH was added 3 ml of a 35% solution of HBr in glacial CH_3COOH. After 30 min, the hydrobromide was precipitated with cooled ether. This gave 0.36 g (78.9%) of (X), mp 181-183°C, R_f (1) 0.19.

11. HBr · H-Orn(Tos)-Gly-ONP (XI). By the method of Para. 10, 0.5 g of (VII) yielded 0.43 g (94%) of (XI), mp 109-114°C, R_f (2) 0.50.

12. H[Gly-Asp(OMe)]$_n$OH (XII). To a solution of 0.6 g of (VIII) in 0.62 ml of DMF was added 0.18 ml of TEA, and the mixture was kept at 20°C for 168 h. The polypeptide was precipitated with methanol, washed with methanol and DMF, and dried. This gave 0.21 g (35%) of a product of M_{av} 8315.

All the other polypeptides were obtained similarly.

SUMMARY

1. The polycondensation of activated esters of some dipeptides has been studied. The dependence of M_{av} on the time has been established.

2. It has been shown that the degree of polycondensation of the polypeptides depends on the nature of the N- and C-terminal amino acids of the activated ester of the peptide and of the monomer.

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