SYNTHESIS OF REGULAR POLYPEPTIDES THAT CONTAIN TYROSINE AND GLUTAMIC ACID RESIDUES

V. A. Shibnev, M. I. Ismoilov, and Sh. Kh. Khalikov

UDC 542.91:547.964.4

The copolymers of tyrosine and glutamic acid have a certain catalytic activity for the hydrolysis of p-nitrophenyl acetate [1, 2]. The catalytic activity of the polypeptides with a regular structure, which contain tyrosine and glutamic acid [3], is approximately equal to that of the copolymer [1]. Consequently, it was expedient to examine the effect of the regularity of the primary structure and the change in the ratios of these amino acids (AA) in the polypeptide on its catalytic effect.

For this we synthesized the polypeptides with the sequence: -Glu-Tyr-, -Tyr-Glu-, -Glu-Glu-Tyr, -Glu-Tyr-Tyr, -Glu-Glu-Glu-Tyr, -Gly-Tyr-Tyr-Tyr-, and Tyr-Glu-Glu-Glu-Glu-Glu. The polypeptides were obtained by the polycondensation of the 2,4,5-trichlorophenyl esters of the appropriate peptides as described in [4]. The o-nitrophenylsulfenyl (NPS) protective group was used in the synthesis of the latter. The hydroxyl group of the Tyr residue and the γ-carboxyl group of glutamic acid were protected by the benzyl group (Bzl). OBzl-Tyr-OH was obtained as described in [5], but with some modifications: the copper complex was not isolated in advance, while the reaction was run in aqueous medium. The NPS-amino acids were obtained as the dicyclohexylammonium (DCHA) salts [6]. The starting monomers (Table 1) were synthesized by the carbodiimide method from the NPS-amino acids and the appropriate hydrohalides of the 2,4,5-trichlorophenyl esters of the AA or peptides (Table 2). The polymerization was run in DMF, and in benzene in the presence of triethylamine (TEA). The average molecular weight (M_{av}) of the polypeptides was determined by titrating the terminal NH_2 groups of the polypeptides with 0.01 N $HClO_4$ solution in the presence of Crystal Violet in abs. dioxane, and in glacial AcOH, as described in [1]. The polymerization data are given in Table 3. A noticeable decrease in the degree of polymerization (n) of the polypeptides when the polymerization is run in benzene can be explained by the lower solubility of the monomers in it. An increase in the amount of TEA from 1 to 2.5 equiv in the polymerization of HCl · H-Glu(OBzl)]-[Tyr(OBzl)]$_2$-$OPhCl_3$ led to an increase in n, and to its decrease in the case of HCl · H-[Glu(OBzl)]$_2$—Tyr-(Bzl)-$OPhCl_3$. The protective groups were removed by catalytic hydrogenation over Pd black until the absorption bands, corresponding to the ester groups (1750 cm^{-1}) and monosubstituted benzene ring (750 and 700 cm^{-1}), disappeared in the IR spectrum.

EXPERIMENTAL METHOD

The purity of the obtained compounds was checked by TLC in a bound layer of silica gel (250 mesh, 7.5 × 2.5 cm) in the following solvent systems: 3% NH_3—sec-butanol (44:100) (A), toluene—dioxane—cyclohexane—AcOH (10:6:3:1) (B), and toluene—dioxane—cyclohexane—ethanol (10:6:3:1) (C). The developers were iodine vapors and ninhydrin solution in 95% acetone.

H-Tyr(OBzl)-OH (I). To a solution of 18.1 g of tyrosine and 12.5 ml of 2 N NaOH solution, heated to 70°C, was added 17 g of $CuSO_4 \cdot 5H_2O$. The mixture was cooled to ~20° and then, with vigorous stirring, a solution of 20 ml of $C_6H_5CH_2Cl$ in 50 ml of acetone was added in portions in 1 h, after which the mixture was stirred at ~20° for 2 h and at 60-70° for 2 h, cooled to 20°, and the obtained precipitate was filtered and washed with water, and then with ether, until the filtrate was colorless. After drying we obtained 25 g of the Cu complex of benzyltyrosine, which was reduced with 28 g of Trilon B in a mixture of 200 ml of

Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow. V. I. Lenin Tadzhik State University, Dushanbe. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 9, pp. 2082-2088, September, 1975. Original article submitted October 31, 1974.

TABLE 1. Characteristics of 2,4,5-Trichlorophenyl Esters of NPS-Protected Peptides

2,4,5-Trichlorophenylesters of NPS-peptides	Type of coupling*	mp, °C (solvent)	$[\alpha]_D^t$	R_f			Yield, %	Found, %		Empirical formula	Calculated, %	
				A	B	C		C	H		C	H
NPS-Tyr(OBzl)-Glu(OBzl)-OPhC'$_3$·$^1/_3$CCl$_4$	1—1	88—89 (CCl$_4$)	$[\alpha]_D^{22}$ + 6,65 (*C* 1,65, CHCl$_3$)	0,92	0,97	0,95	108,5	58,99	4,25	$C_{40}H_{34}O_8N_3SCl_3$	58,91	4,15
NPS-Glu(OBzl)-Tyr(OBzl)-OPhC'$_3$	1—1	159—159,5 (CHCl$_3$—ether)	$[\alpha]_D^{24}$ + 14,5 (*C* 1,1, CHCl$_3$)	0,86	0,88	0,89	74,6	58,97	4,21	$C_{40}H_{34}O_8N_3SCl_3$	58,91	4,15
NPS-Glu(OBzl)-[Tyr(OBzl)]$_2$ OPhCl$_3$	2—1	141—144 (CHCl$_3$—ether)	$[\alpha]_D^{25}$ — 23,1 (*C* 0,82, CHCl$_3$)	0,94	0,97	0,97	70	62,61	4,67	$C_{56}H_{49}O_{10}N_4SCl_3$	62,18	4,58
NPS-[Glu(OBzl)]$_2$-Tyr(OBzl)-OPhCl$_3$	2—1	112—115 (CHCl$_3$—ether)	$[\alpha]_D^{25}$ — 33,9 (*C* 0,77, CHCl$_3$)	0,94	0,97	0,95	51	60,51	4,59	$C_{52}H_{47}O_{11}N_4SCl_3$	59,91	4,54
NPS-Tyr(OBzl)-Tyr(OBzl)-OPhCl$_3$	1—1	165—166 (CHCl$_3$—ether)	$[\alpha]_D^{24}$ + 14,5 (*C* 1,1, CHCl$_3$)	0,88	0,84	0,86	88,7	61,92	4,48	$C_{44}H_{36}O_7N_3SCl_3$	61,48	4,23
NPS-[Tyr(OBzl)]$_3$-OPhCl$_3$	1—2	>200	$[\alpha]_D^{21}$ + 6,01 (*C* 1,33, CHCl$_3$)	0,93	0,93	0,89	65	65,90	4,61	$C_{59}H_{51}O_8N_4SCl_3$	65,51	4,74
NPS-[Glu(OBzl)]$_3$-Tyr(OBzl)-OPhCl$_3$	3—1	Amorphous	$[\alpha]_D^{21}$ — 30,8 (*C* 1,28, CHCl$_3$)	0,88	0,90	0,90	71,1	61,33	5,07	$C_{61}G_{69}O_{14}N_5SCl_3$	60,96	4,77
NPS-Glu(OBzl)-[Tyr(OBzl)]$_3$-OPhCl$_3$	1—3	»	$[\alpha]_D^{18}$ — 47,05 (*C* 0,61, CHCl$_3$)	0,90	0,91	0,89	97,5	—	—	—	—	—
NPS-Tyr(OBzl)-[Glu(OBzl)]$_5$-OPhCl$_3$	1—5	»	$[\alpha]_D^{18}$ + 11,05 (*C* 1,2, CHCl$_3$)	0,89	0,90	0,90	83	62,88	5,29	$C_{88}H_{86}O_{20}N_7SCl_3$	62,45	5,05

*The numbers respectively indicates the number of amino acid residues in the N-acyl and amino components.

TABLE 2. Characteristics of Hydrochlorides of 2,4,5-Trichlorophenyl Esters of Peptides

Hydrochlorides of 2, 4, 5-trichlorophenyl esters of peptides	Reaction time, min	$[\alpha]_D^t$	mp, °C (solvent)	R_f			Yield, %
				A	B	C	
HCl·H-Glu(OBzl)-Tyr(OBzl)-OPhCl$_3$	4	$[\alpha]_D^{21}$ + 4,05 (*C* 1,23, CH_3OH)	146—147 (CH_3OH — ether)	0,72	0,69	0,71	97
HCl·H-Tyr(OBzl)-Glu(OBzl)-OPhCl$_3$	4	$[\alpha]_D^{21}$ — 15,1 (*C* 0,48, CH_3OH)	Amorphous	0,75	0,72	0,75	60,4
HCl·H-[Glu(OBzl)]$_2$-Tyr(OBzl)-OPhCl$_3$	5	$[\alpha]_D^{23}$ — 5,42 [*C* 1,19, $CHCl_3$—CH_3OH (3:1)]	168—170 [$CHCl_3$—CH_3OH (2 : 1)]	0,78	0,75	0,74	40
HCl·H-Glu(OBzl)-[Tyr(OBzl)]$_2$-OPhCl$_3$	5	$[\alpha]_D^{21}$ — 11,0 [*C* 0,6, $CHCl_3$—CH_3OH (3:2)]	159—160 [$CHCl_3$—CH_3OH—ether (2 : 1)]	0,70	0,86	0,75	89,7
HCl·H-[Tyr(OBzl)]$_2$-OPhCl$_3$	4	—	182—185 (CH_3OH — ether)	0,79	0,74	0,73	97
HCl·H-[Tyr(OBzl)]$_2$-OPhCl$_3$	4	$[\alpha]_D^{21}$ — 15,1 (*C* 1,92, ДМФ)	270	0,78	0,75	0,72	93,2
HCl·H-Tyr(OBzl)-[Glu(OBzl)]$_3$-OPhCl$_3$	5	$[\alpha]_D^{17}$ + 1,32 (*C* 1, $CHCl_3$)	260	0,65	0,68	—	94,5
HCl·H-Glu(OBzl)-[Tyr(OBzl)]$_3$-OPhCl$_3$	5	$[\alpha]_D^{19}$ — 20,6 [*C* 0,55, $CHCl_3$—CH_3OH (3 : 2)]	Amorphous	0,56	0,62	0,65	87
HCl·H-[Glu(OBzl)]$_2$-Tyr(OBzl)-OPhCl$_3$	4	$[\alpha]_D^{19}$ —8,25 (*C* 0,8, $CHCl_3$)	»	0,86	—	—	99,5

TABLE 3. Conditions for Polymerization of Hydrochlorides of 2,4,5-Trichlorophenyl Esters of Peptides

Monomers	Amount of monomer, g	Amount of solvent ml	Monomer concentration, mmole/ml	TEA, equiv	Polymerization time, days	Yield of protected polypeptide, g	M_{av}	Degree of polymerization (n)
HCl·H-Tyr(OBzl)-Glu(OBzl)-OPhCl$_3$	0,2109	0,30, DMF	1	1	10	0,122	12 000	25
HCl·H-Glu(OBzl)-Tyr(OBzl)-OPhCl$_3$	0,1997	0,37, DMS	0,76	1	15	0,099	31 200	66
HCl·H-Glu(OBzl)-[Tyr(OBzl)]$_2$-OPhCl$_3$	0,1926	0,20, DMF	1	1	12	0,080	6 670	9
	0,1924	0,20, DMF	1	2,5	12	0,145	55 500	76
HCl·H-[Glu(OBzl)]$_2$-Tyr(OBzl)-OPhCl$_3$	0,1317	0,14, DMF	1	1	15	0,096	44 500	64
	0,1200	0,13, DMF	1	2,5	15	0,080	33 000	49
HCl·H-[Glu(OBzl)]$_3$-Tyr(OBzl)-OPhCl$_3$	0,2452	0,21, DMF	1	2,5	15	0,134	88 300	98
HCl·H-Glu(OBzl)-[Tyr(OBzl)]$_3$-OPhCl$_3$	0,2198	0,18, C_6H_6	1	2,5	12	0,120	41 700	43
HCl·H-Tyr(OBzl)-[Glu(OBzl)]$_3$-OPhCl$_3$	0,2237	0,14, C_6H_6	1	2,5	15	0,156	65 000	48
	0,2354	0,30, C_6H_6	0,5	1	15	0,136	13 400	10

water and 200 ml of n-butanol by refluxing for 5 h. A three-layer system is formed, which was filtered, and then washed in sequence with water and ether until the filtrate was colorless. The yield of (I) was 22.4 g (46.8%); mp 228-229°,* R_f 0.49 (A).

NPS-Tyr(OBzl)-OH · DCHA (II). With vigorous stirring, to a solution of 12.38 g of (I) in a mixture of 44 ml of 1 N NaOH and 60 ml of dioxane were simultaneously added in 15 min 9.2 g of NPS chloride and in drops 26.5 ml of 2 N NaOH (pH ~7-9), after which 100-150 ml of ethyl acetate was added, and the mixture was acidified with 1 N H_2SO_4 to pH 5-4. The obtained two-layer solution was filtered from the unreacted (I), and the aqueous layer was extracted with ethyl acetate (EA) (4 × 100 ml). The combined ethyl acetate extracts were washed with water and dried over Na_2SO_4. After separating the drying agent the solution was treated with 9 ml of DCHA and let stand in the refrigerator for 5-10 h. The obtained crystalline precipitate was filtered, washed with ether, and dried in vacuo at 40-50°. The yield of (II) was 26.6 g (84.2%); mp 166° ($CHCl_3$—ether); $[\alpha]_{D^{24}}$ + 30.7 (C 1.27, $CHCl_3$); R_f 0.79 (C).

NPS-Tyr(OBzl)-$OPhCl_3$ (III). To separate the DCHA a suspension of 10.9 g of (II) in 150 ml of EA was treated with 22.0 ml of 1 N H_2SO_4 and the mixture was shaken until solution was complete. The aqueous layer was separated, while the ethyl acetate layer was washed with water, dried over Na_2SO_4, and evaporated in vacuo. To the obtained NPS-Tyr-(OBzl)-OH in 75 ml of abs. EA was added 3.6 g of 2,4,5-trichlorophenol, the mixture was cooled to −15°, 3.8 g of dicyclohexylcarbodiimide (DCHCD) was added, and the mixture was stirred at −15 to 0° for 2 h and at ~20° for 1 h, and left to stand overnight. Then the reaction mass was diluted with EA, 1 ml of 50% AcOH solution was added, and after 15 min the dicyclohexylurea was filtered. The filtrate was washed with 1 N H_2SO_4, then with water, and dried over Na_2SO_4. The drying agent was separated and the solution was evaporated to dryness. The product is contaminated with a little NPS-Tyr(Obzl)-OH, which is easily separated by passing a solution of (III) in $CHCl_3$ through a silica gel column; (III) is eluted with chloroform, while the impurity remains behind. After evaporating the eluate the residue was rubbed with hexane, the solvent was decanted, and the residue was dried, dissolved again in $CHCl_3$, and precipitated with ether. The yield of (III) was 10.7 g (92.0%), mp 114-114.5° ($CHCl_3$—ether); $[\alpha]_{D^{22}}$—33.7 (C 0.92, $CHCl_3$); R_f 0.95 (B), 0.97 (C).

HCl · H-Tyr(OBzl)-$OPhCl_3$ (IV). To 11.47 g of (III) in 80 ml of abs. $CHCl_3$ was added 19.4 g of 3 N HCl in EA, and the mixture was kept at ~20° for 4 min and at −4 to −5° for 60 min. The obtained suspension was diluted with abs. ether, filtered, and the product was washed with ether until the filtrate was colorless. After reprecipitation from MeOH solution with ether we obtained 8.52 g (92.6%) of (IV), mp 190° (MeOH—ether); $[\alpha]_{D^{23}}$ + 17.9 (C 1.29, CH_3OH); R_f 0.72 (A), 0.69 (B).

NPS-Tyr(OBzl)-Glu(OBzl)-$OPhCl_3$ (V). To a mixture of 3.73 g of (II) [freed of DCHA in the same manner as (III)] and 2.8 g of HCl · H—Glu(OBzl)-$OPhCl_3$ in 45 ml of EA at −15 to −20° were added 1.3 g of DCHCD and 0.83 ml of TEA. The mixture was stirred at −15 to 0° for 2 h, at ~20° for 2 h, and then left to stand overnight, after which it was diluted with EA, 0.5 ml of 50% AcOH was added, and after 15 min the DCHA was separated. The filtrate was washed with 1 N H_2SO_4, then with water, and dried over Na_2SO_4. After separating the drying agent the solvent was evaporated, while the residue was dissolved in CCl_4, and purified on a silica gel column the same as (III). After evaporating the CCl_4 we obtained 5.58 g of crystalline (V) that contains 0.5 mole of solvated CCl_4, which corresponds to a yield of 108.8%; mp 88-89° (CCl_4); $[\alpha]_{D^{22}}$ + 6.65 (C 1.65 $CHCl_3$); R_f 0.97 (B). The other protected peptides were synthesized in a similar manner (see Table 1).

HCl · H-Tyr(OBzl)-Glu(OBzl)-$OPhCl_3$ (VI). To 2.48 g of (V) in 56 ml of abs. EA was added 2.8 ml of 3 N HCl in EA, and the mixture was kept at ~20° for 4 min and at −4 to −5° for 25 min. Half of the solvent was evaporated and the product was precipitated with a 1 : 2 ether—hexane mixture. After decantation, the precipitate was dried and reprecipitated from MeOH solution with ether. We obtained 0.68 g (60.4%) of amorphous (VI); $[\alpha]_{D^{21}}$—15.1 (C 0.48, CH_3OH); R_f 0.75 (B). The other hydrochlorides of the 2,4,5-trichlorophenyl esters of the peptides were obtained in a similar manner (see Table 2).

NPS-Glu(OBzl)-Glu(OBzl)-OH · DCHA (VII). To a solution of 4.31 g of NPS-Glu(OBzl) · $OPhCl_3$, obtained the same as (III), in 5 ml of CH_2Cl_2 were added 2.75 g of H-Glu(OBzl)-OH, 2.1 ml of DCHA, and 0.2 g of 2-hydroxypyridine, after which the mixture was stirred at −20° for 2 days and then filtered from the unreacted H-Glu(OBzl)-OH. The filtrate was evaporated to dryness, and the residue was rubbed with abs. ether. The obtained crystalline product was filtered, washed with abs. ether, and reprecipitated from $CHCl_3$ solution with ether. We obtained 2.77 g (46.7%) of crystalline (VII), mp 145-146° ($CHCl_3$—ether); $[\alpha]_{D^{21}}$—22.8 (C 1.64, $CHCl_3$); R_f 0.67 (B). In a similar manner were synthesized NPS-Glu-(OBzl)-Tyr(OBzl)-OH · DCHA in 58.8% yield, mp 157-158° ($CHCl_3$—ether); $[\alpha]_{D^{24}}$ + 42.4 (C 1.71, $CHCl_3$);

*From [5], mp 223°.

R_f 0.82 (B), and amorphous NPS-[Glu(OBzl)]$_3$-OH · DCHA in 79.2% yield; $[\alpha]_D^{21}$—19.72 (C 1.52, $CHCl_3$); R_f 0.75 (A).

H-(Tyr-Glu)$_n$-OH. To 0.2109 g of (VI) in 0.3 ml of DMF was added 0.040 ml of TEA (1 equiv). The ampul was sealed and kept at ~20° for 10 days, after which it was heated at 60-65° for 1 h. The ampul contents were dissolved in 3-5 ml of dioxane and then ether was added to the obtained solution to precipitate the polycondensate. The organic layer was separated by decantation, the precipitate was rubbed well with MeOH, and the solvent was removed by decantation. The polypeptide was dissolved in dioxane and the solution was concentrated in vacuo at 65-70°, cooled to ~20°, diluted with MeOH, and the obtained polypeptide was rubbed twice with MeOH, and dried. The yield of H-[Tyr(OBzl)-Glu(OBzl)]$_n$—OH, freed of low-molecular-weight compounds, was 0.122 g (86.6%). The average molecular weight of the protected polypeptide, determined by titration in abs. dioxane with 0.01 N $HClO_4$ in glacial AcOH in the presence of Crystal Violet, was ~12,000.

To remove the protective Bzl groups we hydrogenated 0.065 g of the polypeptide in a 1 : 1 : 2 dioxane —CH_3COOH—DMF mixture in the presence of Pd black. The catalyst was removed, the solvent was evaporated, while the residue was rubbed in sequence with dioxane and ether. We obtained 0.0357 g (26.5%) of (VII). The cleavage of the benzyl groups was checked by the disappearance of the absorption bands at 1740, 750, and 700 cm^{-1} in the IR spectrum. The polycondensation of the other monomers was carried out in a similar manner (see Table 3).

CONCLUSIONS

The polymerization of the 2,4,5-trichlorophenyl esters of peptides gave polypeptides with the sequence: -(Glu-Tyr)$_n$-, -(Tyr-Glu)$_n$-, -(Glu-Tyr-Tyr)$_n$-, -(Glu-Glu-Tyr)$_n$-, -(Glu-Glu-Glu-Tyr)$_n$-, -(Tyr-Tyr-Tyr-Glu)$_n$-, and -(Tyr-Glu-Glu-Glu-Glu-Glu)$_n$-, with an average degree of polymerization of respectively 66, 25, 76, 64, 98, 43, and 48.

LITERATURE CITED

1. J. Noguchi and H. Yamamoto, J. Biochem., 65, 123 (1969).
2. H. Yamamoto and J. Noguchi, J. Biochem., 67, 103 (1970).
3. M. I. Ismoilov and V. A. Shibnev, Izv. Akad. Nauk SSSR, Ser. Khim., 1872 (1973).
4. V. A. Shibnev, T. P. Chuvaeva, G. N. Martynova, and K. T. Poroshin, Izv. Akad. Nauk SSSR, Ser. Khim., 637 (1969).
5. E. Wünsch, G. Fries, and A. Zwick, Chem. Ber., 91, 542 (1958).
6. A. Kapoor and E. J. Davis, Experientia, 23, 253 (1967).