SYNTHESIS OF SUBSTITUTED PEPTIDES – FRAGMENTS (9-14), (4-9), AND (8-14) OF THE N-TERMINAL SECTION OF THE HISTONE OF FRACTION F2aI FROM CALF THYMUS

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Among natural proteins, considerable interest is presented by nuclear proteins of basic nature [1-3]. A possible participation in biological processes connected with the mechanism of the regulation of the gene activity of DNA is ascribed to these proteins [2, 3].

Definite advances have been achieved in the interpretation of the primary structure of the histones. De Lange et al. [4] have established the amino-acid sequence of an individual histone of fraction F2aI of calf thymus, and this has been confirmed by other workers [5]. A feature of the N-terminal section of this histone is the frequently recurring sequence of amino acids -Gly-Lys-Gly-. Of the 13 amino-acid residues of the (1-13) fragment, nine belong the the sequence -Gly-Lys-Gly-. According to this, the N-terminal segment of the chain of the F2aI histone contains a large number of basic amino-acid residues, and, possibly, for F2aI, this segment is primarily responsible for its electrostatic binding to DNA [1, 6-8].

We have previously [9, 10] synthesized a polypeptide with the amino-acid sequence -Gly-Lys-Gly- as a model of this histone fragment. The present paper gives the results of the synthesis of a protected heptapeptide and of two protected hexapeptides forming fragments of the N-terminal part of the nonspiralized section of the glycine-arginine-rich F2aI histone. The following hexapeptides have been obtained: the methyl ester of benzyloxycarbonylglycylleucylglycyl(N^{ε}-tosyl)lysylglycylglycine (IX), fragment (9-14); the methyl ester of benzyloxycarbonylglycyl(N^{ε}-tosyl)lysylglycylglycyl(N^{ε}-tosyl)lysylglycine (XIII), fragment (4-9); and the methyl ester of benzyloxycarbonyl(N^{ε}-tosyl)lysylglycylleucylglycyl(N^{ε}-tosyl)lysylglycylglycine (XI) (fragment 8-14).

To obtain the peptides we used the mixed-anhydride method with isobutyl chloroformate and the azide method. To protect the ε-amino group of lysine we used the tosyl group (Tos– = $CH_3C_6H_4SO_2$–). The α-amino groups of the amino acids and peptides were protected by the benzyloxycarbonyl group (Z– = $C_6H_5CH_2OCO$–), and the carboxy groups of the amino acids and peptides by conversion into the methyl esters. Amino acids of the L series were used for the synthesis. Below we give the scheme for the synthesis of the protected hexapeptide (IX).

The hexapeptide (IX) was obtained by condensing the dipeptide (VI) with the hydrobromide of the tetrapeptide (VIII), also using isobutyl chloroformate; the tetrapeptide (VII) was prepared by condensing the azide of benzyloxycarbonylglycyl(N^{ε}-tosyl)lysine (II) with the hydrobromide of the methyl ester of glycylglycine (IV). We also give the schemes of the syntheses of the protected heptapeptide (XIV) and the protected hexapeptide (XVIII).

The pentapeptide (XII) was synthesized by condensing benzyloxycarbonylleucine with the hydrobromide of the tetrapeptide (VIII) by the mixed-anhydride method. From benzyloxycarbonyl(N^{ε}-tosyl)lysylglycine (XI), obtained by the saponification of the product of the condensation of benzyloxycarbonyl(N^{ε}-tosyl)lysine with the hydrochloride of the methyl ester of glycine, and the hydrobromide of the pentapep-

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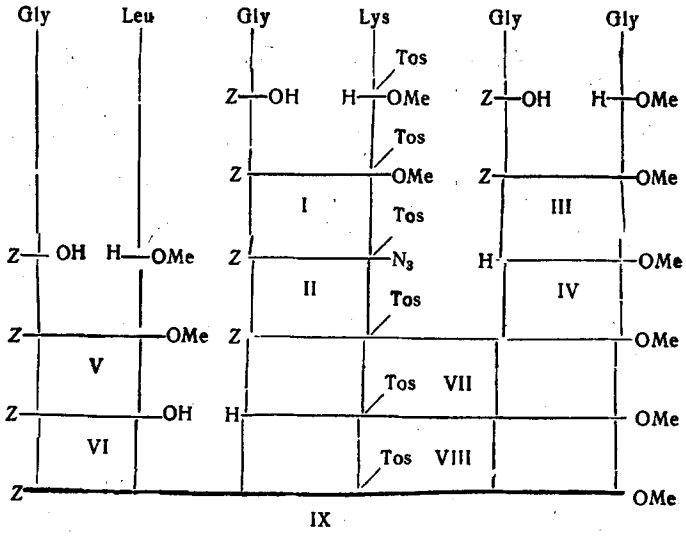
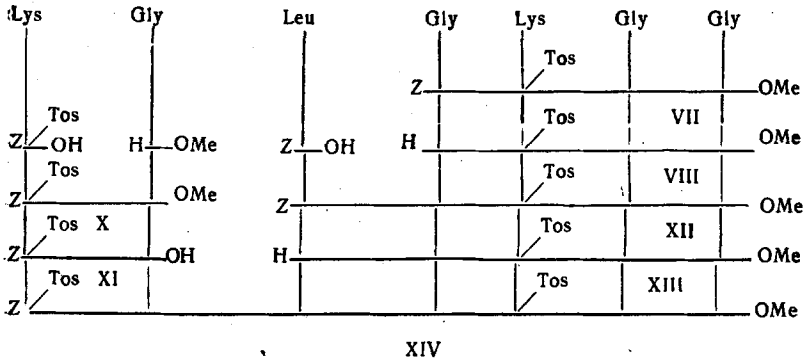
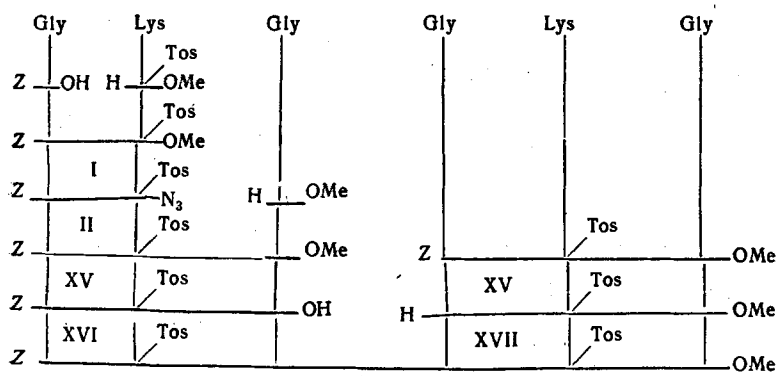
tide (XIII) we synthesized the heptapeptide (XIV), using isobutyl chloroformate. The hexapeptide (XVIII) was obtained similarly from the tripeptide (XVI) and the hydrobromide of the tripeptide (XVII).



IX



XIV



XVIII

EXPERIMENTAL

In the synthesis of the peptides, the benzyloxycarbonyl groups were removed with a 40% solution of hydrogen bromide in glacial acetic acid. The hydrobromides of the peptides were precipitated with absolute ether and were purified by reprecipitation from absolute ethanol with ether. In this way chromatographically pure hydrobromides of the peptides (IV), (VIII), (XIII), and (XVII) were obtained.

The methyl esters were saponified in dioxane with a 1 N solution of caustic potash in 10% excess for 1 h. This gave the peptides (VI), (XI), and (XVI).

The individuality of the compounds was checked by thin-layer chromatography in a fixed layer of silica gel in the systems: 1) benzene–ethanol (2:0.3), and 2) butan-1-ol–acetic acid–water (100:10:30).

The hydrochlorides of the methyl esters of the amino acids were obtained by Brenner's method [11] using thionyl chloride and methanol. The methyl ester of benzyloxycarbonylglycyl(N^{ε}-tosyl)lysine (I) and its hydrazide (II) and the methyl ester of benzyloxycarbonylglycyl(N^{ε}-tosyl)lysylglycine (XV) were synthesized as described by Bustin et al. [7]. The methyl ester of benzyloxycarbonylglycylleucine (V) was synthesized as described previously [10]. The elementary analyses of all the compounds obtained corresponded to the calculated figures.

Methyl Ester of Benzyloxycarbonylglycyl(N^{ε}-tosyl)lysylglycylglycine (VII). To a solution of 3.8 g of the hydrazide of benzyloxycarbonylglycyl(N^{ε}-tosyl)lysine in water–glacial acetic acid–hydrochloric acid (16 : 12 : 2) was added 50 ml of chloroform, and the mixture was cooled to −5° and with stirring a cooled solution of 0.46 g of sodium nitrite in water was added. The mixture was stirred for another 5 min, and then the chloroform solution was washed with cold 5% sodium bicarbonate solution and with water and was dried with magnesium sulfate. A chloroform solution of the methyl ester of glycylglycine was prepared in parallel by adding 1.12 ml of triethylamine to the hydrobromide of the methyl ester of glycylglycine (IV), and this was cooled to −5°C and was added to the dried and filtered solution of the azide. The mixture was left at 0°C for 12 h and then at room temperature for 24 h. Then it was washed with water, 0.5 N hydrochloric acid, water again, 5% sodium bicarbonate solution, and water again and was dried with magnesium sulfate. The solvent was distilled off to dryness. The residue was washed with ether and dried in vacuum over caustic potash. Yield 3.57 g (75.4%); mp 58-60°C, $[\alpha]_D^{20}$ −17.0° (c 1; chloroform); R_f 0.35 (1).

Methyl Ester of Benzyloxycarbonylglycylleucylglycyl(N^{ε}-tosyl)lysylglycylglycine (IX). To a solution of 0.25 g of benzyloxycarbonylglycylleucine (VI) in 5 ml of dimethylformamide cooled to −15°C were added 0.11 ml of triethylamine and 0.12 ml of isobutyl chloroformate. The mixture was stirred for 25 min, and then a solution of 0.43 g of the hydrobromide of the tetrapeptide (VIII) mixed with 0.11 ml of triethylamine and cooled to −15°C was added, and stirring was continued for another 2 h. Then the solution was left at 20°C for 24 h. The dimethylformamide was evaporated off in vacuum, and the residue was dissolved in chloroform and treated as described for compound (VII). The product was reprecipitated from chloroform with ether and was dried in vacuum over caustic potash. Yield 0.30 g (50.0%), mp 136-139°C, $[\alpha]_D^{20}$ −20.0° (c 1; chloroform), R_f 0.22 (1).

Methyl Ester of Benzyloxycarbonyl(N^{ε}-tosyl)lysylglycine (X). A mixture of 2.0 g of benzyloxycarbonyl(N^{ε}-tosyl)lysine and 0.65 ml of triethylamine in tetrahydrofuran was cooled to −15°C. The mixed anhydride was obtained over 25 min with 0.65 ml of isobutyl chloroformate. The hydrochloride of the methyl ester of glycine (0.58 g) in tetrahydrofuran was mixed with 0.65 ml of triethylamine, and this mixture was cooled to −15°C. Then it was added to the solution of the mixed anhydride and stirring with cooling was carried out for 2 h. The mixture was left at room temperature for 24 h. The tetrahydrofuran was evaporated off in vacuum, and the residue was dissolved in chloroform. The chloroform solution was washed in the usual way, as for compound (VII). The product was recrystallized from a mixture of chloroform and diethyl ether. Yield 1.15 g (50.0%), mp 119-121°C, $[\alpha]_D^{20}$ −6.0° (c 1; chloroform), R_f 0.54 (1).

Methyl Ester of Benzyloxycarbonylleucylglycyl(N^{ε}-tosyl)lysylglycylglycine (XII). The substance was obtained by the mixed-anhydride method in ethyl acetate, using isobutyl chloroformate (0.20 ml) and 0.4 g of benzyloxycarbonylleucine with the addition of 0.20 ml of triethylamine and the methyl ester of the tetrapeptide (VIII) obtained from 0.85 g of the hydrobromide of this peptide by the action of 0.20 ml of triethylamine. Then the mixture was worked up in the usual way (see compound (IX)). Yield 0.65 g (59.1%), mp 54-56°C, $[\alpha]_D^{20}$ −12.5° (c 1.6; chloroform), R_f 0.31 (1).

Methyl Ester of Benzyloxycarbonyl(N^{ε}-tosyl)lysylglycylleucylglycyl(N^{ε}-tosyl)lysylglycylglycine (XIV). A solution of 0.10 g of benzyloxycarbonyl(N^{ε}-tosyl)lysylglycine in 5 ml of absolute chloroform was treated with 0.03 ml of triethylamine, and the mixture was cooled to −15°C. The mixed anhydride was produced with 0.03 ml of isobutyl chloroformate over 30 min. To this was added a solution of the hydrobromide of the pentapeptide (XIII) mixed with 0.03 ml of triethylamine and cooled to −15°C. Stirring was continued with cooling for 2 h, and then the mixture was left at 20°C for 48 h. The chloroform solution was washed in the usual way and was dried with magnesium sulfate, and the solvent was distilled off in vacuum. The product was washed several times with ether and was dried in vacuum over caustic potash. Yield 0.12 g (60.0%), mp 98-100°C, $[\alpha]_D^{20}$ −21.0° (c 1; chloroform), R_f 0.09 (1).

Methyl Ester of Benzyloxycarbonylglycyl(N^{ε}-tosyl)lysylglycylglycyl(N^{ε}-tosyl)lysylglycine (XVIII). The substance was obtained in chloroform by the mixed-anhydride method in a similar manner to substance (XIV) starting from 0.18 g of benzyloxycarbonylglycyl(N^{ε}-tosyl)lysylglycine (XVI), 0.06 ml of triethylamine,

0.06 ml of isobutyl chloroformate, 0.17 g of the hydrobromide of the tripeptide (XVII) and 0.06 ml of triethylamine. Yield 0.2 g (60.6%), mp 117-120°C, $[\alpha]_D^{20}$ −31.7° (c 0.6; dioxane), R_f 0.09 (1).

SUMMARY

The synthesis has been effected of the following protected hexa- and heptapeptides: the methyl ester of benzyloxycarbonylglycylleucylglycyl(N^{ε}-tosyl)lysylglycylglycine (IX), the methyl ester of benzyloxycarbonylglycyl(N^{ε}-tosyl)lysylglycylglycyl(N^{ε}-tosyl)lysylglycine (XVIII), and the methyl ester of benzyloxycarbonyl(N^{ε}-tosyl)lysylglycylleucylglycyl(N^{ε}-tosyl)lysylglycylglycine (XIV), corresponding to the sequences (9-14), (4-9), and (8-14) of the N-terminal section of the histone of fraction F2aI of calf thymus.

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