SYNTHESIS OF TWO PROTECTED HEXADECAPEPTIDES CORRESPONDING TO THE Gly^{10}- AND THE Gly^1, Lys^2, Gly^3-ANALOGS OF THE FRAGMENT OF SEQUENCE 1-16 OF THE N-TERMINAL SEGMENT OF THE HISTONE OF FRACTION F2aI OF CALF THYMUS

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Histones are proteins with a characteristic amino-acid composition which are distinguished by the presence of modified – especially acetylated – amino-acid residues [1]. The arginine-rich fractions of the histones (F 2aI) are the most highly acetylated. According to Meisler et al. [3], the acetylation of the histones is the earliest chemical change detectable on the activation of the genes in relation to the synthesis of RNA, between the rate of synthesis of which and the level of acetylation of the histones a correlation is observed [4].

The investigation of the primary structure of the histones of identical fractions from different sources (of plant and animal origin) has shown a surprising similarity of composition and amino-acid sequence [5].

The presence of acetylated amino-acid residues and the conservativeness in the amino-acid sequence of similar fractions of histones from different sources possibly corresponds to their biological specificity. Having obtained analogs of natural fragments of histones, it is possible to attempt to follow the changes in their biological properties as functions of changes in their primary structures. With this aim, we have synthesized two protected hexadecapeptides corresponding to the Gly^{10}- and Gly^1, Lys^2, Gly^3- analogs of a natural fragment of the sequence 1-16 of the N-terminal segment of F 2aI histone. In the case of the Gly^{10}-analog, the leucine residue in position 10 was replaced by glycine, but in the case of the Gly^1,Lys^2, Gly^3-analog the three amino-acid residues were substituted directly, the acetylated serine being lost.

To obtain the Gly^1,Lys^2,Gly^3-analog (III), we used tert-butoxycarbonylglycyl-N^{ε}-benzyloxycarbonyllysylglycine (I) and the trifluoroacetate of the methyl ester of the tridecapeptide glycyl-N^{ε}-benzyloxycarbonyllysylglycylglycyl-N^{ε}-benzyloxycarbonyllysylglycylleucylglycyl-N^{ε}-benzyloxycarbonyllysylglycylglycylalanyl-N^{ε}-benzyloxycarbonyllysine (II), which we had prepared previously (Scheme 1). It was found that this analog has different physical constants: its solubility in organic solvents had fallen sharply, its melting point had risen from 100°C to 133°C, and the R_f values in the same system (system 1) were 0.78 and 0.09, respectively.

The Gly^{10}-analog was obtained by the same method as the hexadecapeptide corresponding to the natural fragment [6] (Scheme 2) using the mixed-anhydride method to obtain the peptides (IV, V, and VI) and the carbodiimide method for the peptide (VII). As the initial blocks we used the previously-prepared [6] protected hexapeptide corresponding to sequence 11-16 and the tripeptides tert-butoxycarbonylglycyl-N^{ε}-benzyloxycarbonyllysylglycine and O,N-diacetylseryglycyl-N^{G}-nitroarginine with free carboxy groups.

EXPERIMENTAL

Amino acids of the L series were used for the synthesis. In the process, tert-butoxycarbonyl groups were removed by the action of trifluoroacetic acid, the crystalline trifluoroacetates being obtained. The purity of the peptides obtained was checked by chromatography on standard plates of the "Silufol UV_{254}" brand marketed by the "Kavalier" enterprise (Czechoslovakia) in the following systems: 1) methanol–

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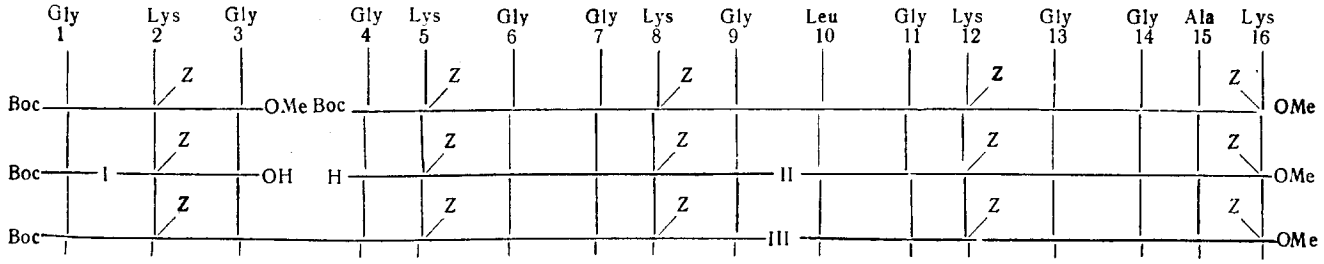
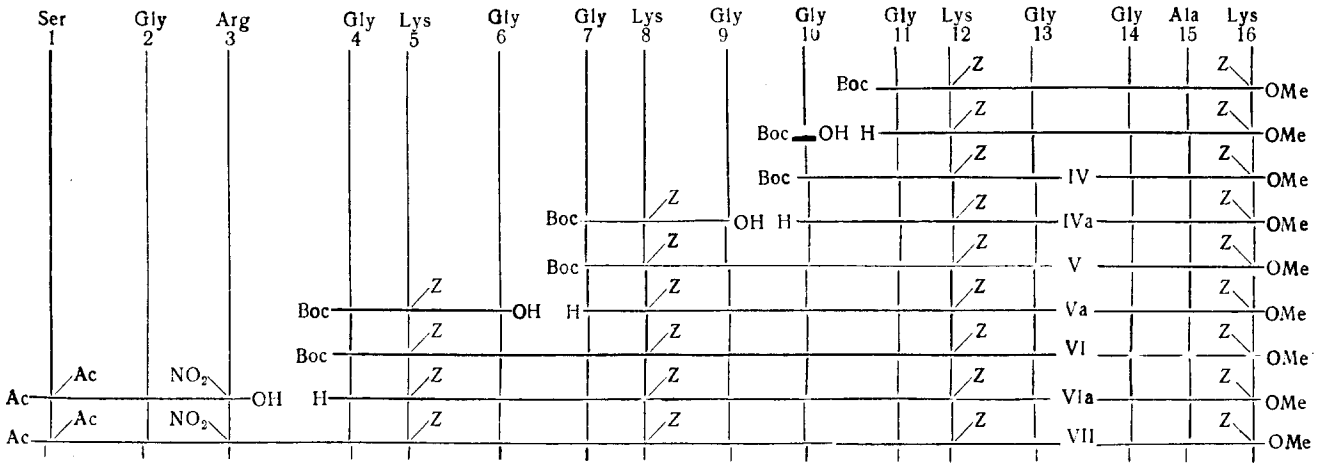
chloroform (1 : 8); 2) water–formic acid–tert-butyl alcohol (1.5 : 1.5 : 7.0); 3) benzene–ethanol–water (2 : 1 : 0.1). The analyses of all the compounds corresponded to the calculated figures.

Methyl Ester of tert-Butoxycarbonyl(glycyl-N^{ε}-benzyloxycarbonyllysylglycyl)$_3$leucylglycyl-N^{ε}-benzyloxycarbonyllysylglycylglycylalanyl-N^{ε}-benzyloxycarbonyllysine (III). To a solution of 0.1 g (excess) of tert-butoxycarbonylglycyl-N^{ε}-benzyloxycarbonyllysylglycine (I) in 20 ml of chloroform cooled to −20°C were added 0.03 ml of triethylamine and 0.04 ml of isobutyl chloroformate. After 15 min, 0.26 g of the trifluoroacetate of the methyl ester of the tridecapeptide (II) in the dry form and 0.03 ml of triethylamine were added to the reaction mixture. After 3 h, the temperature was gradually raised to that of the room, and stirring was continued for 48 h. Then the reaction mixture was diluted with 50 ml of chloroform and was washed in the usual way. The chloroform solution was left viscous, gel-like. After drying with sodium sulfate, the solvent was distilled off to dryness. The residue was treated with several portions of ether, whereupon the product crystallized. Yield 0.28 g (90.3%), mp 133°C (decomp.), R_f 0.09 (system 1), 0.83 (system 2). Because of the poor solubility of the peptide itself, the specific rotation of its trifluoroacetate was determined: $[\alpha]_D^{25}$ −25.5° (c 0.62; dimethylformamide).

Scheme 1



Scheme 2



Methyl Ester of tert-Butoxycarbonylglycylglycyl-N^{ε}-benzyloxycarbonyllysylglycylglycyllalanyl-N^{ε}-benzyloxycarbonyllysine (IV). Compound (IV) was obtained by the mixed-anhydride method, like (I) from 0.1 g (excess) of tert-butoxycarbonylglycine, 0.35 g of the trifluoroacetate of the methyl ester of the hexapeptide glycyl-N^{ε}-benzyloxycarbonyllysylglycylglycylalanyl-N^{ε}-benzyloxycarbonyllysine [6], and 0.08 ml of triethylamine in chloroform, using 0.1 ml of isobutyl chloroformate and 0.08 ml of triethylamine. The reaction lasted 48 h. The reaction mixture was washed in the usual way and dried, and the solvent was distilled off in vacuum. The residue was treated with ether, whereupon the product solidified. Yield 0.32 g (87.5%), mp 101°C (decomp.), R_f 0.32 (system 1) $[\alpha]_D^{25}$ −19.2° (c 0.81; chloroform).

Methyl Ester of tert-Butoxycarbonylglycyl-N^{ε}-benzyloxycarbonyllysylglycylglycylglycyl-N^{ε}-benzyloxycarbonyllysylglycylglycylalanyl-N^{ε}-benzyloxycarbonyllysine (V). This compound was obtained in a similar manner to (I) from 0.20 g (excess) of tert-butoxycarbonylglycyl-N^{ε}-benzyloxycarbonyllysylglycine [6] and 0.32 g of the trifluoroacetate of the methyl ester of the heptapeptide (IVa) in chloroform with the addition of 0.06 ml of triethylamine, 0.08 ml of isobutyl chloroformate, and 0.06 ml of triethylamine. The reaction took 48 h,

and then the reaction mixture was washed in the usual way, dried, and evaporated in vacuum. After treatment with ether, the residue crystallized. Yield 0.35 g (77.7%), mp 120°C (decomp.), R_f 0.25 (system 1), $[\alpha]_D^{25}-25.6°$ (c 0.64; chloroform).

Methyl Ester of tert-Butoxycarbonyl(glycyl-N^{ε}-benzyloxycarbonyllysylglycyl)$_2$glycylglycyl-N^{ε}-benzyloxycarbonyllysylglycylglycylalanyl-N^{ε}-benzyloxycarbonyllysine (VI). This compound was also obtained by the mixed-anhydride method from 0.15 g (excess) of tert-butoxycarbonylglycyl-N^{ε}-benzyloxycarbonyllysylglycine, 0.04 ml of triethylamine, and 0.35 g of the trifluoroacetate of the methyl ester of the decapeptide (Va) with the addition of 0.05 ml of isobutyl chloroformate and 0.04 ml of triethylamine at −20°C in chloroform for 48 h. The reaction mixture had the form of a viscous gel. After the reaction, it was evaporated to dryness. The oily residue was treated with ethyl acetate, and the product crystallized. It was carefully washed with ethyl acetate and with ether. After drying in vacuum, 0.32 g (72.7%) of a white crystalline product was obtained. Beginning from 150°C, it decomposed without melting; R_f 0.18 (system 1), 0.62 (system 2), 0.20 (system 3). Because of the poor solubility of the peptide, the specific rotation of its trifluoroacetate was determined: $[\alpha]_D^{25}-27.0°$ (c 0.8; dimethylformamide).

Methyl Ester of O,N-Diacetylserylglycyl-N^{G}-nitroarginyl(glycyl-N^{ε}-benzyloxycarbonyllysylglycyl)$_2$glycylglycyl-N^{ε}-benzyloxycarbonyllysylglycylglycylalanyl-N^{ε}-benzyloxycarbonyllysine (VII). The tripeptide O,N-diacetylserylglycyl-N^{G}-nitroarginine [6] (0.03 g) was dissolved in 5 ml of dimethylformamide, and 0.02 g of N-hydroxysuccinimide and, after the mixture had been cooled to −15°C, 0.02 g of dicyclohexyldicarbodiimide were added. After 15 min, 0.1 g of the trifluoroacetate of the methyl ester of the tridecapeptide and 0.01 ml of triethylamine were added. The reaction mixture was stirred with cooling for 2 h, and it was kept in the refrigerator for 2 days and at room temperature for 5 h. The solvent was evaporated to dryness. The residue was triturated with several portions of ethyl acetate to eliminate the urea that had formed, and then with several portions of ether. The product was dried in vacuum. Yield 0.08 g (72.7%), mp. 140°C (decomp.), R_f 0.60 (system 1), 0.76 (system 2), 0.84 (system 3), $[\alpha]_D^{25}-27.5°$ (c 0.62; dimethylformamide).

SUMMARY

Two protected hexadecapeptides corresponding to the Gly^{10}- and the Gly^1,Lys^2,Gly^3-analogs of the natural fragment of sequence 1-16 of the N-terminal segment of the histone of fraction F 2aI of calf thymus have been synthesized; their physical constants differ sharply from those of the hexadecapeptide corresponding to the natural fragment.

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