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SYNTHESIS OF A FRAGMENT OF HISTONE H4 WITH THE SEQUENCE 14-21

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UDC 542.91 : 547.466.46 : 547.466.1

In recent years, intensive investigations have been carried out on the structural and functional features of histones [1-3]. In spite of the considerable advances in this field, there still remain urgent problems of the elucidation of the molecular organization of the higher levels of the structure of histones, and also their role in the organization of nuclear structures. A knowledge of the structural features of histones will permit a further deepening of investigations of their biological role and, in particular, will reveal the role of histones in the regulation of the activity of the genetic apparatus of the cell.

With the aim of structural and functional investigations, we have synthesized the N-terminal section of histone H4 with the sequence 14-21. The selected section of the histone is interesting by virtue of the fact that it is saturated with basic amino acids – lysine and arginine – which are arranged symmetrically in relation to a histidine residue. It is not excluded that this section may play an important role in the blocking of a DNA template [4].

The synthesis of the octapeptide was carried out by means of the scheme shown below. The choice of this scheme predicated the exclusion of the possibility of the occcurrence of side reactions. With this aim, at all stages of the synthesis CDI was used as the condensing agent and methylmorpholine as the base. The Cbo and NO_2 protective groups were used for blocking the side functions of lysine and arginine, respectively, and t-BOC protection for blocking α-amino groups. (See scheme on following page.)

A number of difficulties arose in the introduction of histidine into the peptide chain. An attempt to synthesize a peptide with the sequence 18-21 using the N^{α}-BOC-N^{im}-FDN derivative of histidine was unsuccessful. Satisfactory results were obtained when the methyl ester of histidine was introduced from the N-end of the peptide chain. The formation of byproducts was observed at the stages of the synthesis of the pentapeptide (14-18) and of the octapeptide (14-21). (The results of the elementary and amino acid analyses of these products showed the impossibility of the formation of acylureas [5]). The yields of peptides in these stages fell to 60%. In all the other stages the peptides were isolated with fairly good yields and were chromatographically pure. The peptides were purified by repeated crystallization from a mixture of ethanol and ethyl acetate and also by chromatography on Sephadex LH-20. The Cbo and NO_2 groups were removed from the octapeptide with anhydrous hydrogen fluoride. The absence of absorption bands in the UV spectrum in the 257 and 271 nm regions showed the completeness of the elimination of the protective groups. The free octapeptide was purified on a column of Sephadex G-15. The peptides synthesized and their constants are given in Table 1.

EXPERIMENTAL

Amino acids of the L series were used in experiments. All the products obtained were analyzed for C, H, and N. In all cases the results of the elementary analysis corresponded to the calculated figures. The course

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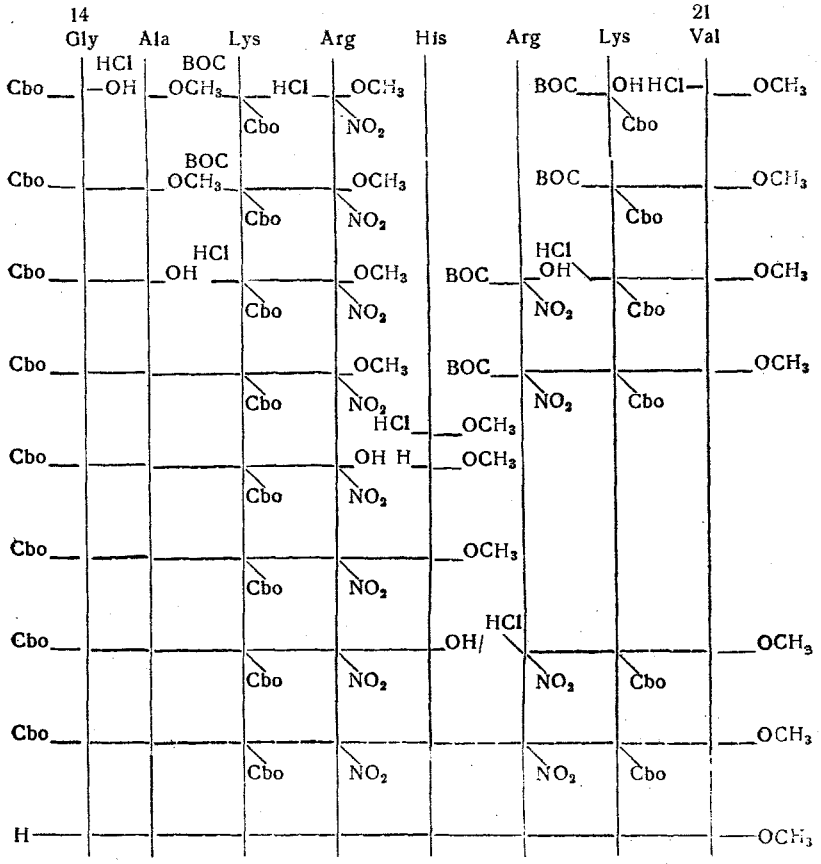
0009-3130/79/1501-0039$07.50

TABLE 1. Peptides Synthesized and Their Constants

Peptide	mp, °C	Yield, %	$[\alpha]_D$	R_f A	R_f B	R_f C	R_f D
1. Cbo-Gly-Ala-Lys (Cbo)-Arg (NO_2)-OCH_3	90	80	−33.3 c 0.9	0.83	0.80	0.75	0.70
2. Cbo-Gly-Ala-Lys (Cbo)-Arg (NO_2)-OH	81–83	85	−28 c 0.9	0.53	0.62		0.68
3. Cbo-Gly-Ala-Lys(Cbo)-Arg (NO_2)-His-OCH_3	99–100	64	−20 c 1.0	0.73	0.50	0.50	0.80
4. Cbo-Gly-Ala-Lys(Cbo)-Arg(NO_2)-His-OH	125–127	70		0.50	0.36		
5. BOC-Lys(Cbo)-Val-OCH_3	73	90		0.93	0.95	0.96	0.95
6. BOC-Arg(NO_2)-Lys (Cbo)-Val-OCH_3	110	94	−35 c 1.0	0.80	0.86	0.65	0.78
7. Cbo-Gly-Ala-Lys(Cbo)-Arg(NO_2)-His-Arg(NO_2)-Lys(Cbo)-Val-OCH_3	121–122	62	−40 c 1.0	0.74	0.57	0.39	



Scheme of synthesis.

of the reactions and the purity of the products were checked by chromatography in a thin layer of silica gel fixed with gypsum in the following systems: sec-butanol–3% NH_4OH (100 : 44) (A); butan-1-ol–water–acetic acid (100 : 30 : 10) (B); methanol–chloroform (13 : 60) (C); and methanol–benzene (2 : 0.3) (D).

The purity of the final product was also checked electrophoretically in 30% acetic acid. The chromatograms were revealed with ninhydrin and in iodine vapor. Qualitative amino acid analysis was carried out after hydrolysis (6 N HCl, 120°C, 20 h) by paper chromatography with two runs in the butan-1-ol–water–acetic acid (5 : 4 : 1) system.

Melting points were determined on a Kofler block. Values of $[\alpha]_D$ were measured at 25°C on a Russian-made polarimeter in ethanol. After each stage of condensation, the reaction mixture was washed with 10% citric acid, 0.5 N $NaHCO_3$, and water, and was dried over Na_2SO_4. The solvent was distilled off in a rotary evaporator under reduced pressure at 40°C. The peptides were purified on columns of Sephadexes G-15 and LH-20. The

following symbols are used: CDI – N,N'-dicyclohexylcarbodiimide; Cbo – the benzyloxycarbonyl group; t-BOC – the tert-butoxycarbonyl group; THF – tetrahydrofuran; DMFA – dimethylformamide; and FDN – fluoro-2,4-dinitrobenzene.

Synthesis of BOC-Lys(Cbo)-Val-OCH_3 (5). With cooling (–5 to –10°C), 0.006 mole of HCl·Val-OCH_3 in DMFA and 0.006 mole of methylmorpholine were added to a mixture of 0.005 mole of BOC-Lys(Cbo)-OH in THF and 0.005 mole of CDI. The mixture was stirred at the same temperature for 3-4 h and was left overnight. Then it was acidified with acetic acid, the solvent was evaporated off completely, and the residue was dissolved in ethyl acetate and was washed free from unchanged starting materials. The product was crystallized from a mixture of diethyl ether and petroleum ether.

Peptides 1, 3, 6, and 7 were obtained similarly. These peptides were crystallized from ethanol or mixtures of ethanol and ethyl acetate.

Saponification of Cbo-Gly-Ala-Lys(Cbo)-Arg(NO_2)-OCH_3 (1). A solution of 0.004 mole of Cbo-Gly-Ala-Lys(Cbo)-ArgNO_2)-OCH_3 in the minimum volume of ethanol was made up with acetone to 10 ml. Then the calculated amount of 1 N NaOH was added. The mixture was kept at room temperature for 1 h. Then the solvent was evaporated off to a small volume, the residue was diluted with water, and the unchanged initial compound was eliminated by extraction with ethyl acetate. After this, the reaction mixture was acidified to pH 2 and the main product was extracted with ethyl acetate. It was recrystallized from ethyl acetate. The saponification of peptide (3) was performed similarly.

Elimination of the t-BOC Protective Group from BOC-Arg(NO_2)-Lys(Cbo)-Val-OCH_3 was carried out by treatment with 2 N HCl/ethyl acetate at 0°C for 25 min. After the end of the reaction, the solvent was evaporated off and the residue was treated with ether. The product was crystallized from ethanol and ethyl acetate. The t-BOC protective groups were eliminated from the other peptides in the same way.

Elimination of the Protective Groups from the Octapeptide 14-21. A Teflon cylinder was charged with 0.002 mole of the protected octapeptide in anisole, and then, with cooling, liquid hydrogen fluoride was added. The reaction was performed at 0°C for 1 h 25 min. Then the excess of HF was evaporated off in vacuum, and the oily product was dissolved in water and was passed through a column of Amberlite IRA-401 (OH^- form). The peptide was purified on a column of Sephadex G-15 (eluent 0.001 N HCl).

The octapeptide was identified by amino acid analysis and electrophoretically.

SUMMARY

A fragment of histone H4 with the sequence -Gly-Ala-Lys-Arg-His-Arg-Lys-Val- has been synthesized.

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