THE AMINO TERMINAL SEQUENCE OF CALF THYMUS HISTONE III

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Received October 13, 1971

Summary: The amino terminal 26 residues of the arginine-rich histone III are Ala-Arg-Thr-Lys-Gln-Thr-Ala-Arg-Lys-Ser-Thr-Gly-Gly-Lys-Ala-Pro-Arg-Lys-Glu-Leu-Ala-Thr-Lys-Ala-Ala-Arg. Two short sequences in this structure are homologous with sequences found in the AL and GAR histones.

INTRODUCTION

Structural studies on the GAR (1,2) and AL (3,4) histones* have led to proposals that the amino terminal regions of these molecules are DNA binding sites (5,6). It is therefore of interest to know if the amino terminal sequence of another highly purified histone, histone III*, has similarities to the AL and GAR histones.

Ala-	Arg-	Thr-	Lys-	Gln-	Thr-	Ala-	Arg-	Lys-	Ser-
1	2	3	4	5	6	7	8	9	10
Thr-	Gly-	Gly-	Lys-	Ala-	Pro-	Arg-	Lys-	Glu-	Leu-
11	12	13	14	15	16	17	18	19	20
Ala-	Thr-	Lys-	Ala-	Ala-	Arg-				
21	22	23	24	25	26				

Fig. 1 Amino terminal sequence of the histone III.

*The abbreviations used are: GAR, glycine and arginine rich histone or histone IV; AL, arginine and lysine-rich histone or histone IIb_1; histone III, the arginine-rich histone or histone F_3.

The carboxyl terminal sequence of histone III has been previously reported, as well as the positions of methyl and acetyl groups on several tryptic peptides (7). This report presents the sequence of the amino terminal 26 residues of histone III as deduced by analysis on the automatic protein sequencer.

MATERIALS AND METHODS

Calf thymus histone III was prepared by exclusion chromatography as previously described (8). The dimer form, or fraction AR-5, was used for this study.

Samples of protein (5-8 mg) were treated in the Beckman 890 protein sequencer using a program similar to that described by Edman and Begg (9). The resulting PTH amino acids were analyzed by gas chromatography (10). In some instances, the PTH amino acids were hydrolyzed with 5.7 N HCl containing mercaptoethanol in a ratio of 1:2000 at 130° for 24 hrs. The free amino acids were analyzed on a Beckman Model 120 amino acid analyzer.

RESULTS AND DISCUSSION

The results of twenty-six cycles of Edman degradation are shown in Table I. Most residues were identified directly on the gas chromatograph as PTH amino acids. Residues 4,5,9,18, 19 and 23 were determined as trimethyl-silyl derivatives of the phenylthiohydantoins. All arginine residues were identified as free arginine on the amino acid analyzer after hydrolysis of the PTH derivative. Other residues were confirmed by hydrolysis and amino acid analysis.

Residues 9 through 17 and 18 through 26 have been previously reported by DeLange *et al* (7) as nearly identical sequences

TABLE I

IDENTIFICATION OF PTH AMINO ACIDS

Step Number	Retention Time[1]		Amino Acid Analysis[3]	Residue
	Direct	TMS[2]		
1	6.2	-	-	Ala
2	-	-	Arg	Arg
3	7.0,8.4[4]	-	-	Thr
4	-	13.9	Lys	Lys
5	-	12.7,14.4[5]	-	Gln
6	7.1,8.4	-	-	Thr
7	6.2	-	-	Ala
8	-	-	Arg	Arg
9	-	13.9	Lys	Lys
10	6.4,6.6,7.0[6]	-	-	Ser
11	7.0,8.4	-	-	Thr
12	7.1	-	Gly	Gly
13	7.1	-	Gly	Gly
14	-	-	Lys	Lys
15	6.2	-	Ala	Ala
16	8.4	-	Pro	Pro
17	-	-	Arg	Arg
18	-	13.9	Lys	Lys
19	-	12.7	Glu	Glu
20	8.9	8.9	Leu	Leu
21	6.2	-	Ala	Ala
22	7.1,8.4	-	-	Thr
23	-	13.9	Lys	Lys
24	6.2	-	Ala	Ala
25	6.2	-	Ala	Ala
26	-	-	Arg	Arg

TABLE LEGENDS

1. Gas chromatography on the Beckman GC-45. A column (130 x 0.2 cm) of 10% DC-560 on Chromosorb W was used with the following temperature program: 2 min isothermal at 165° followed by a linear rise to 275° over a time of 16 min. Retention times are within 0.1 min of those of corresponding standard PTH or TMS-PTH amino acids.

2. TMS - Trimethylsilyl.

3. The hydrolysis procedure is given in text.

4. The elution of two peaks with these retention times is characteristic of PTH-threonine. As another confirmation of threonine, a lower conversion temperature, 50° rather than 80°, changed the relative proportions of the peaks.

5. The first peak corresponds to PTH-Glu.

6. PTH-serine normally contains three peaks with these retention times.

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in the tryptic peptides T(M)-IIa and T(M)-IIb$_1$, respectively. The residue corresponding to glutamic acid 19 was reported as glutamine. Only glutamic acid was found at this position. However, this discrepancy could arise from deamidation during conversion from the anilinothiazolinone to the PTH amino acid.

Although residues corresponding to lysines 14 and 23 have been reported to be partially acetylated (7), acetylation was not detected at these points. However, the yields of these residues were low and present automated sequencing methodology may not permit determination of partially acetylated residues.

An interesting aspect of this sequence is the presence of two Arg-Lys sequences. It has been suggested that adjacent basic residues such as these are involved in binding phosphates of opposite strands of the DNA double helix (10). Such a hypothesis must await results of more detailed structural and physical studies.

III			Ala	Arg	Thr	Lys	Gln	Thr	Ala	Arg	Lys
AL	AcetylSer		Gly	Arg	Gly	Lys	Gln	-	-	-	-
GAR	AcetylSer		Gly	Arg	Gly	Lys	-	-	-	-	-
III	-	Ser	Thr	Gly	Gly	Lys	Ala	Pro	Arg	Lys	Glu
AL	-	-	-	Gly	Gly	Lys	Ala	Arg	Ala	Lys	Ala
GAR	-	-	-	Gly	Gly	Lys	Gly	Leu	Gly	Lys	Gly

Fig. 2 Comparison of amino terminal sequence of histone III with AL and GAR calf thymus histones.

Figure 2 compares the amino terminal sequences of histone III, AL (6) and GAR (1,2) histones. After making appropriate deletions, some homology is apparent as indicated by the blocked in residues. The amino terminal acetylserine residue appears to be deleted from histone III. The first residue in the AL and GAR histones is phosphorylated under certain conditions (5,11) and is possibly involved in removal of the histone from DNA. If histone III combines with DNA in a similar manner, the specificity of removal could be partially directed by the absence of this site of phosphorylation. A somewhat analogous situation exists in the very lysine rich histone subfractions (12). Fraction 4 contains a serine which is readily phosphorylated. In fraction 3, this site is replaced by alanine, thereby implying functional differences in these two subfractions.

The sequence -Gly-Gly-Lys-Ala- is highly homologous in the three histones. The less bulky side chains of glycine or alanine adjacent to lysine may be required for formation of a DNA-histone complex and may facilitate interaction of ϵ-amino groups with DNA phosphates.

Further homologies between the AL histone and histone III have recently been observed from partial sequences of these

proteins (13). The actual extent of similarity must await the elucidation of the total sequences of these histones.

ACKNOWLEDGMENTS

These studies were supported in part by USPHS Grant CA-10892, P-3 and the American Cancer Society Grant ACS NP-28F. The authors wish to thank Dr. W. C. Starbuck for helpful discussions and Mr. Charles Taylor for preparation of protein samples.

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