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Biochemistry

Isopeptide linkage between nonhistone and histone 2A polypeptides of chromosomal conjugate-protein A24

(chromatin/amino acid sequence/tryptic peptides)

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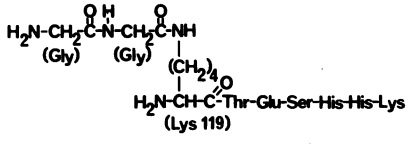
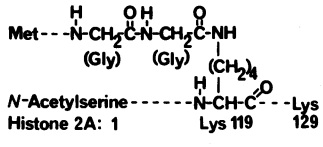
ABSTRACT **Chromosomal protein A24 has a unique structure inasmuch as it contains histone 2A and a nonhistone polypeptide the sequence of which has been partially determined. Comparative analysis of the ninhydrin-insensitive amino-terminal tryptic peptides of protein A24 and histone 2A and a quantitative analysis of their carboxyl-terminal amino acid indicated that protein A24 has two amino termini and one carboxyl terminus. The amino acid sequence analysis of tryptic peptide 17′ of protein A24:**



showed it contains tryptic peptide 17 of histone 2A, Lys-Thr-Glu-Ser-His-His-Lys. Lysine 119, the amino terminus of this peptide, which is derived from the histone 2A portion of protein A24, is linked by an isopeptide bond to the carboxyl group of a glycine residue. Accordingly, the branched structure of protein A24 proposed is:



Initial interest in chromosomal protein A24 arose from a marked reduction in its nucleolar levels during the liver nucleolar hypertrophy and increased rRNA synthesis induced either by the administration of thioacetamide (1) or by partial hepatectomy (2). Isolation and initial chemical characterization indicated that protein A24 was a nonhistone chromosomal protein with approximately equal amounts of acidic and basic amino acids; it constituted approximately 1.9% of the sum of histones 2A, 2B, 3, and 4 (3). Protein A24 was found to contain the tryptic and chymotryptic peptides of histone 2A as well as additional peptides and, accordingly, it was suggested that protein A24 contained a "nonhistone-like" polypeptide linked to a histone 2A molecule (4). More recently (5, 6), protein A24 has been shown to contain the amino-terminal sequence:

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 1                   5                  10
Met-Gln-Ile-Phe-Val-Lys-Thr-Leu-Thr-Gly-
 11                  15                 20
Lys-Thr-Ile-Thr-Leu-Glu-Val-Glu-Pro-Ser-
 21                  25                 30
Asp-Thr-Ile-Glu-Asn-Val-Lys-Ala-Lys-Ile-
 31                  35
Gln-Asp-Lys-Glu-Gly-Ile-Pro-
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This sequence is not homologous to histone sequences. On the other hand, the carboxyl-terminal amino acid sequence of protein A24 was identical to that of histone 2A (5, 6).

The present study indicates that protein A24 has two amino termini and one carboxyl terminus. In addition, the amino acid sequence of the branched tryptic peptide 17′ indicates that the ε-amino group of lysine 119 of the histone 2A portion of protein A24 is linked to glycine by an isopeptide bond. Therefore, a branched structure is proposed for protein A24.

MATERIALS AND METHODS

Protein Isolation. Protein A24 and histone 2A, isolated from calf thymus tissue as described (3, 7), were obtained in highly purified form, as determined by two-dimensional polyacrylamide gel electrophoresis (8).

Preparation of Tryptic Peptides. Protein A24 and histone 2A (4.5 mg each) were simultaneously subjected to two digestions for 2 hr each with 1% by weight of trypsin as described (3, 4). Aliquots of each protein sample were also hydrolyzed and subjected to amino acid analysis as described (9) to determine yields of individual peptides. The digests were then subjected to chromatography in butanol:acetic acid:water, 4:1:5 (vol/vol) (10) followed by electrophoresis in pyridine:acetic acid:water, 1:10:189 (vol/vol), pH 3.6 (10). The separations were monitored by staining with Sakaguchi reagent (11), fluorescamine (12), ninhydrin cadmium, or starch iodide reagents (11). Separated peptides were eluted from the paper with 30% acetic acid as described (11).

Quantitative Hydrazinolysis. Protein A24 and histone 2A (0.5 mg each, quantitated by amino acid analysis as noted above) were subjected to hydrazinolysis at 85° for 28 hr to minimize destruction of lysine as described (13). For determination of carboxyl-terminal amino acids, the hydrazinolysates were extracted with benzaldehyde to remove hydrazides and the free amino acids were analyzed by amino acid analysis. Purified peptides were subjected to hydrazinolysis in the same fashion. Peptides containing acetyl groups yielded acetyl hydrazide upon hydrazinolysis. Accordingly, to analyze for the presence of acetyl hydrazide, we subjected the hydrazinolysates to chromatography in collidine:H_2O,10:2 (vol/vol) (14). Hydrazides were detected by the staining method of Andrae (15). Standard acetyl hydrazide was prepared by hydrazinolysis of acetyl valine (Schwarz/Mann).

Edman Degradation. The amino acid sequences of peptides were determined by a modification of the Edman degradation (16, 17). For analysis of Edman degradation products, thiazolinones were either hydrolyzed under reduced pressure in hydrogen iodide (18) and subjected to amino acid analysis, or they were first converted to phenylthiohydantoins in 1 M HCl at 80° for 10 min, extracted in ethyl acetate, and analyzed by gas chromatography (19). The amounts of amino acids released from thiazolinones were corrected for destruction during HI hydrolysis as described (5).

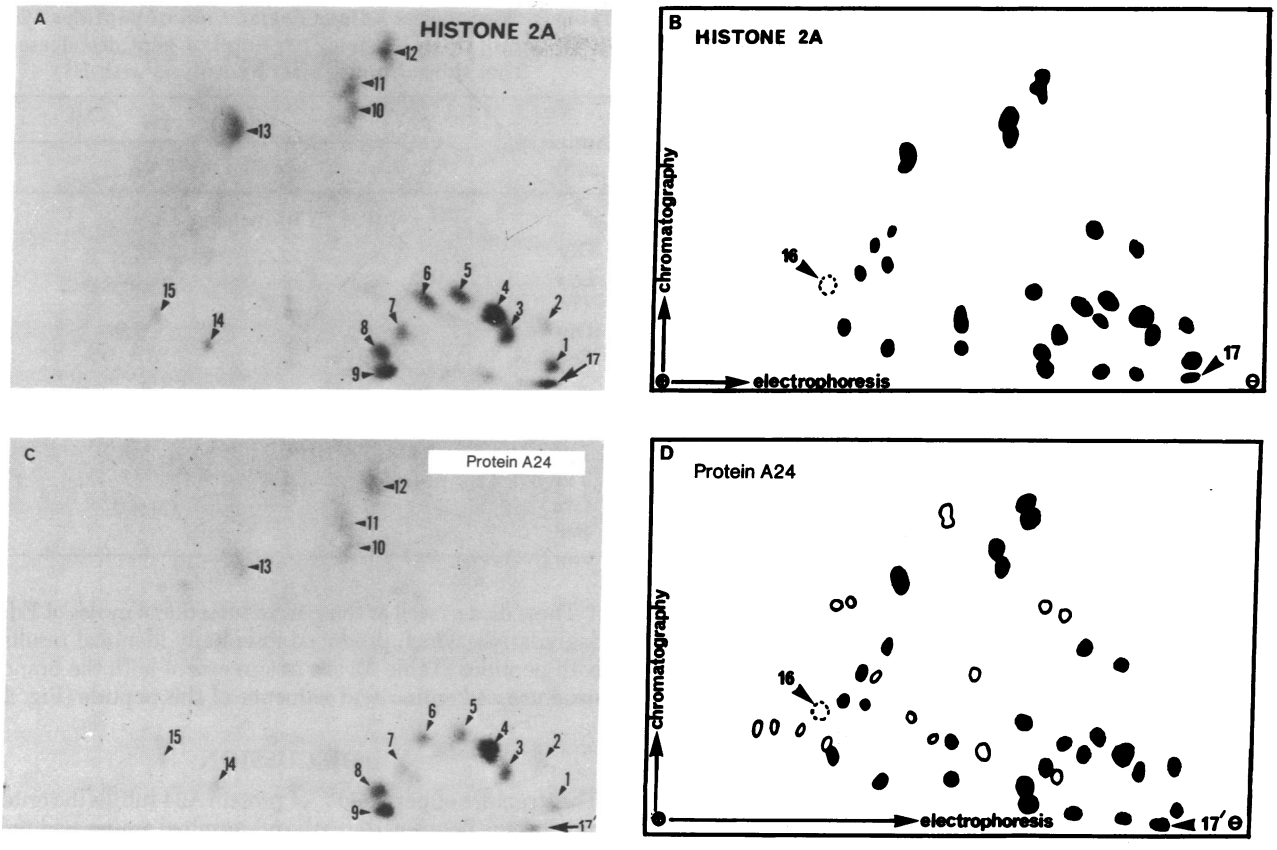

FIG. 1. Two-dimensional peptide maps and c rresponding diagrams of (A and B) histone 2A and (C and D) protein A24 tryptic digests. Filled spots are common to both proteins. Note the positions of peptides 16 (diagrams), which were negative to the ninhydrin cadmium stain used in the maps. Note also the position of peptides 17 of histone 2A and 17′ of protein A24, which differ slightly in electrophoretic mobility.

RESULTS

The two amino termini of protein A24

As reported previously, a comparison of tryptic peptide maps of histone 2A and protein A24 revealed remarkable similarities (4) as indicated in Fig. 1. However, the peptide designated 16 (diagrams, Fig. 1) was not previously detected in protein A24 because it did not stain with the ninhydrin-cadmium (peptide maps, Fig. 1) or fluorescamine procedures, which require the presence of a primary amino group (Table 1). This peptide had been reported to be present in histone 2A (7) and in fact contained the blocked amino terminus of the histone (20, 21). Accordingly, an analysis was performed of purified peptides 16 from histone 2A and protein A24 (Table 1). Both peptides had identical staining characteristics, amino acid composition, and positive tests for the acetyl group. This was consistent with the structure previously determined (20, 21) for the blocked amino-terminal tryptic peptide of histone 2A:

N-Acetylserine-Gly-Arg.

Thus, in addition, to the nonhistone-like free amino-terminal amino acid sequence previously detected (3, 5, 6), protein A24 also contained the blocked amino terminus of histone 2A.

The single carboxyl terminus of protein A24

Carboxypeptidase A and B digestion indicated that protein A24 contains the carboxyl-terminal sequence (5, 6) identical to that of histone 2A (22, 23):

-His -His -Lys -Ala -Lys-Gly-Lys
123 124 125 126 127 128 129
(histone 2A residue number)

In the present study a quantitative analysis of carboxyl-terminal amino acids was undertaken to determine if protein A24 had an additional carboxyl terminus as well. Quantitative hydrazinolysis released molar yields of carboxyl-terminal lysine of 1.01 and 0.88 for protein A24 and histone 2A, respectively;* no other carboxyl-terminal amino acids were detected in protein A24.

The branched tryptic peptide of protein A24

The detection of two amino termini and one carboxyl terminus suggested that the protein A24 molecule was branched and that the nonhistone polypeptide was linked to histone 2A in a manner that prevented detection of its carboxyl terminus. A search was made for an altered tryptic peptide of protein A24. Although the other peptides had similar electrophoretic mobilities, tryptic peptide 17′ from protein A24 had a slightly different electrophoretic mobility from peptide 17 of histone 2A (Fig. 1). Accordingly, these peptides were subjected to amino acid analysis, quantitative hydrazinolysis, and sequential Edman degradation.

The amino acid composition and carboxyl terminus of peptide 17 of histone 2A (Table 2) were found to be the same as that reported previously (22) for a peptide containing amino acid residue 119–125 of the histone 2A sequence (23). Edman degradation (Table 3) confirmed the identity and amino acid sequence of this peptide (Fig. 2A).

The amino acid composition of peptide 17′ of protein A24 (Table 2) was the same as that of peptide 17 of histone 2A except for the presence of two additional glycine residues. The yield

* The molar yield calculations were based on the molecular weights of 27,000 and 14,000 for protein A24 (3) and histone 2A (23), respectively.

Table 1. Analysis of tryptic peptide 16 of protein A24 and histone 2A

	Protein A24	Histone 2A
Staining reactions:		
Fluorescamine	–	–
Ninhydrin	–	–
Sakaguchi	+	+
Rydon-Smith	+	+
Amino acid ratios:		
Ser (= 1.00)	1.00	1.00
Gly	1.09	1.15
Arg	1.12	1.21
Molar yield*	0.42	0.46
Acetyl group†	+	+

* Nanomoles recovered from tryptic peptide maps per nanomole of protein digested.
† Detected as acetyl hydrazide as in *Materials and Methods*.

of the carboxyl-terminal lysine was essentially the same in peptide 17′, indicating both peptides were homogeneous. The first cycle of sequential Edman degradation (Table 3) of this peptide produced thiazolinones containing 1.6 nmol of glycine and 0.4 nmol of lysine per nmol of peptide. The remaining degradation cycles were similar to those of the histone peptide except for 0.5 nmol of glycine and 0.1 nmol of lysine per nmol of peptide in cycle 2, which presumably resulted from overlap from cycle 1.

As outlined in Fig. 3, the results from the first cycle of Edman degradation of the protein A24 peptide are consistent with a branched structure arising from the additional two glycines at the amino terminus being attached to the rest of the peptide in an isopeptide linkage to the ϵ-amino group of lysine. Coupling with phenylisothiocyanate (A) occurred at the α-NH_2 groups of both lysine and glycine. Cyclization (B) produced two thiazolinones, one containing glycine and one containing lysine with a glycine residue attached to its ϵ-NH_2 group. HI hydrolysis of these thiazolinones (C) released two glycine residues and one lysine residue. In addition, conversion of the thiazolinones to phenylthiohydantoins (D) produced sufficient PTH-glycine to account for half of the glycine in the thiazolinones. In fact a total of 2.1 nmol of glycine per nmol of peptide were released from thiazolinones obtained from cycles 1 and 2 of the Edman degradation of peptide 17′ (Table 3). Gas chromatographic analysis of phenylthiohydantoins (Fig. 3E) detected 0.9 nmol of PTH-glycine per nmol of peptide or approximately one-half of the amount obtained from hydrolysis of thiazolinones.

Table 2. Amino acid ratios and carboxyl termini of peptides 17 of histone 2A and 17′ of protein A24

	17	17′
Gly	—	1.64
Lys	1.67	1.68
His	1.71	1.61
Thr	0.91	0.93
Ser (= 1.00)	1.00	1.00
Glu	0.95	0.94
COOH terminus*	Lys (0.57)†	Lys (0.64)†
Molar yield‡	0.67	0.74

* Data obtained by hydrazinolysis; only lysine was found.
† Data in parentheses are molar yield of carboxyl-terminal lysine.
‡ Nanomoles of peptide recovered from tryptic peptide maps per nanomole of protein digested.

Table 3. Sequential Edman degradation of peptides 17 and 17′ (nmol of amino acids per nmol of peptide released from thiazolinones after hydrolysis with HI)

Amino acid	Edman cycle 1	2	3	4
	Peptide 17 of histone 2A			
Gly				
Lys	0.5			
Thr		1.0		
Glu			0.9	
Ser				0.3
	Peptide 17′ of protein A24			
Gly	1.6	0.5		
Lys	0.4	0.1		
Thr		1.1		
Glu			0.8	
Ser				0.3

These data as well as those from subsequent cycles of Edman degradation, which produced essentially identical results for both peptides (Table 3), are in agreement with the branched structure and amino acid sequence of this peptide (Fig. 2B).

DISCUSSION

The structure of peptide 17′ of protein A24 fulfills the criterion of a linkage between the carboxyl-terminal amino acid residue of the nonhistone polypeptide chain and the ϵ-amino group of lysine 119 of the histone 2A polypeptide by an isopeptide linkage. The proposed overall structure of protein A24 (Fig. 4) contains the blocked amino terminus of histone 2A, the free amino terminus of the nonhistone polypeptide, the single carboxyl-terminal lysine contributed by histone 2A, and a glycine carboxyl-terminal amino acid from the nonhistone polypeptide chain which would not be detected as a free amino acid upon hydrazinolysis because it is in an isopeptide linkage.

Although isopeptide crosslinks between polypeptide chains have been found in collagen (24), fibrin (25, 26), peptidoglycans (27), and hair medulla protein (28), these linkages have not been previously observed in histones or nonhistone chromosomal proteins (29). Such a linkage between histone 2A and a nonhistone chromosomal protein suggests that the functions of these two types of nuclear proteins may be combined in the bifunctional structure of protein A24 (4).

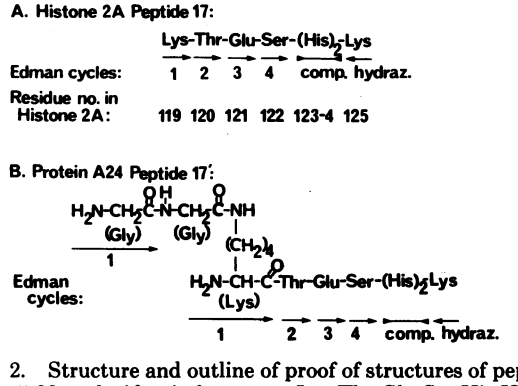

FIG. 2. Structure and outline of proof of structures of peptides 17 and 17′. Note the identical sequence Lys-Thr-Glu-Ser-His-His-Lys in both peptides and the position number of these amino acid residues in the histone 2A sequence. Comp. = amino acid composition; hydraz. = hydrazinolysis.

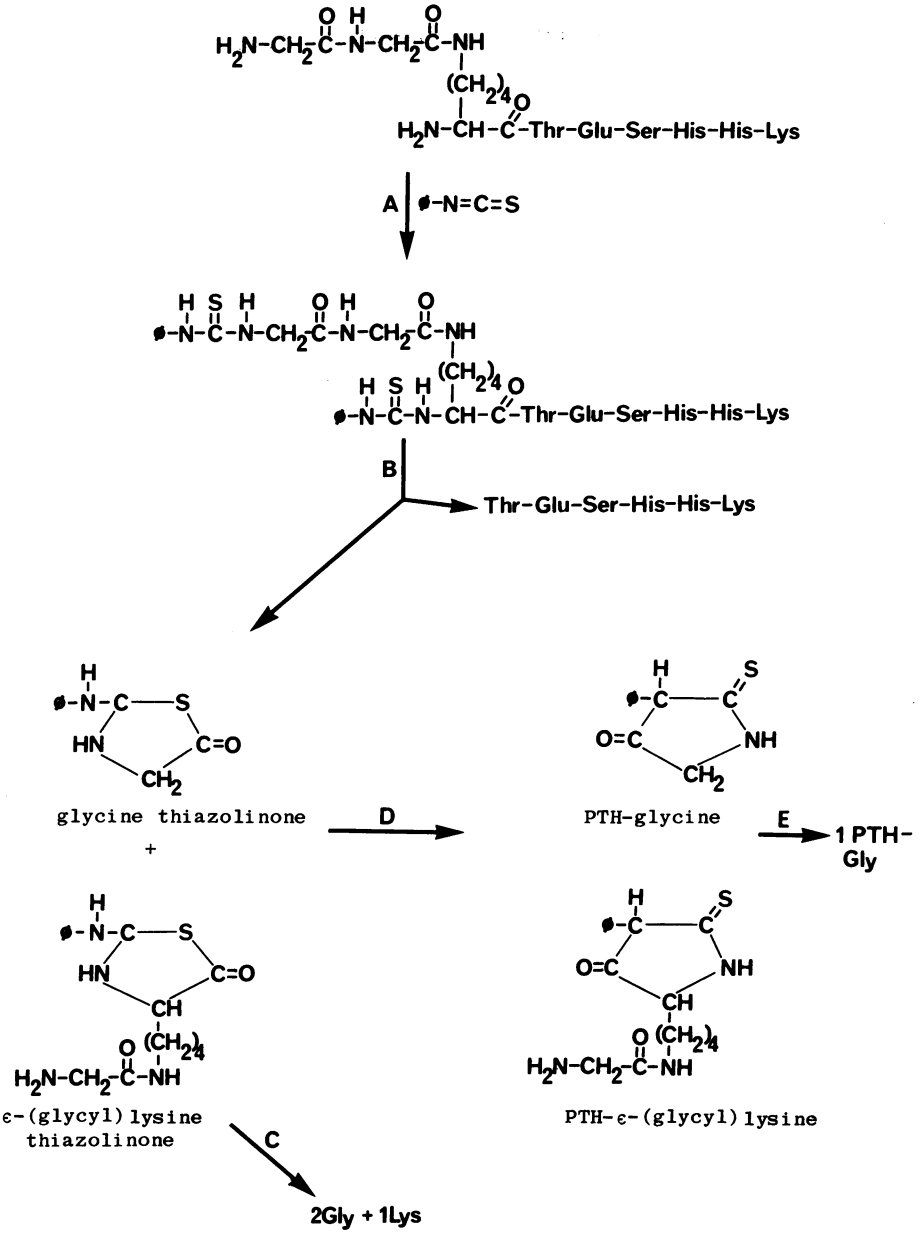

FIG. 3. Diagram of chemical reactions involved in the first cycle of Edman degradation of branched peptide 17′ of protein A24. (A) Coupling of peptide with phenylisothiocyanate. (B) Cyclization and extraction of thiazolinones. (C) Hydrolysis of the thiazolinones. (D) Conversion of the thiazolinones to the corresponding phenylthiohydantoin (PTH) amino acids. (E) Analysis of PTH amino acids by gas chromatography.

The complete amino acid sequence of histone 2A (23) and the first 37 amino acids on the amino terminus of the nonhistone polypeptide of protein A24 (5) have already been determined. On the basis of its molecular weight of 27,000 and its amino acid composition (3), the sequence of approximately 80 amino acids needs to be determined to complete the primary structure of protein A24.

Recent models of chromatin structure place histone 2A in the chromatin subunits or "Nu bodies" (30–32). Currently there is uncertainty about whether "Nu bodies" are associated with both actively and inactively transcribed chromatin (33, 34), and the presence of different types of "Nu bodies" in chromatin has been suggested (35–37). Protein A24 was extracted from chromatin with the histones (3, 38) and it may be associated with a particular type of chromatin subunit. Consequently, determination of the function of protein A24 may provide information about general relationships between structure and function in chromatin.

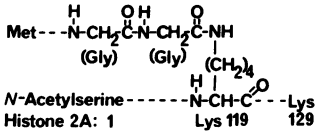

FIG. 4. Proposed overall structure of protein A24.

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