REMARKABLE SIMILARITIES OF PEPTIDE FINGERPRINTS OF HISTONE 2A AND NONHISTONE CHROMOSOMAL PROTEIN A24

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Protein A24 is of interest because of the rapid reduction in its content in hypertrophic liver nucleoli. Although its amino acid composition is similar to that of histone 2A, protein A24 has a higher molecular weight and a higher content of glutamic acid, threonine and proline. Inasmuch as the tryptic and chymotryptic peptide fingerprints of protein A24 contain almost all the peptides of the corresponding fingerprints of histone 2A, protein A24 may be either a unique protein composed in large part of amino acid sequences of histone 2A or a "prohistone" for histone 2A.

INTRODUCTION

The nonhistone chromosomal protein A24, initially found by two-dimensional gel electrophoresis of nucleolar proteins (1), was found in markedly reduced amounts in nucleoli during nucleolar enlargement of thioacetamide-treated (2) and regenerating rat liver (3). Protein A24 was isolated and its amino acid composition and amino terminal amino acid were determined. Protein A24 differs from histones in its high content of glutamic acid and its NH_2-terminal amino acid, methionine. However, its solubility and its binding to chromatin were like those of histones 2A, 2B, 3 and 4 (4,5).

The present comparison of protein A24 and histone 2A indicates that protein A24 contains almost all the tryptic and chymotryptic peptides of histone 2A. Accordingly, it may be either a "prohistone" or a nonhistone chromosomal protein con-

taining a large part, if not all, of the amino acid sequence of histone 2A.

MATERIALS AND METHODS

Preparation of highly purified protein A24 and histone 2A - Protein A24 migrated as a single spot on two-dimensional polyacrylamide gel electrophoresis after purification by gel filtration and preparative electrophoresis and had a single NH_2 terminal amino acid (4). Histone 2A, prepared in highly purified form as previously described (6), was generously provided by Mr. Charles W. Taylor and Dr. Mark O. J. Olson.

Amino acid composition, NH_2-terminal amino acid analysis and molecular weight of protein A24 and histone 2A - For protein A24 the amino acid composition was determined by hydrolysis with 5.7 N HCl (4,7) and mercaptoethane sulfonic acid (4,8). The NH_2-terminal amino acid was determined by dansylation (4,9,10) and the molecular weight by gel electrophoresis in sodium dodecyl sulfate (4,11). The corresponding data for histone 2A were reported earlier (12).

Peptide fingerprint analysis of protein A24 and histone 2A - Protein A24 (250-500 μg) or histone 2A (250-500 μg) were digested twice for 2 hours at 40°C with 5-10 μg each time of either TPCK trypsin (4) or α chymotrypsin. The digests were lyophylized, dissolved in 25 μl of 50% acetic acid and applied onto Whatman No. 1 filter paper (4,13). Chromatography of the peptides was carried out in butanol, acetic acid and water (4:1:5, V:V:V) for 16-17 hours and electrophoresis was at pH 3.6 (4,13) at 3000 volts for 45-50 minutes in pyridine, acetic acid and water (1:10:189,V:V:V). The cadmium-ninhydrin stain was used to visualize the peptides (14).

RESULTS

A comparison of the amino acid composition of protein A24 and histone 2A (Table I) shows similarities in content of lysine, histidine, aspartic acid, leucine and phenylalanine. Protein A24 contained less arginine and alanine than histone 2A. On the other hand, the contents of threonine, glutamic acid and proline were higher in protein A24 and it had a higher acidic/basic amino acid molar ratio than histone 2A. Neither protein contained tryptophan. Histone 2A contained no methionine. The single methionine residue in protein A24 was the amino terminal amino acid of this protein. In addition, the molecular weight of protein A24 was approximately twice that of histone 2A (4,5).

The striking similarities in tryptic peptide fingerprints are shown in Figures 1 and 2. The tryptic peptide fingerprints of histone 2A (Fig. 1a and 2a) contained approximately 27 peptides, of which 15 were readily identifiable and identical in mobility to peptides in the patterns of protein A24 (Fig. 1b and 2b). The corresponding tryptic peptide fingerprints of protein A24 (Fig. 1b and 2b) contained approximately 45 peptides of which 8 were definitely not present in histone 2A (arrows, Fig. 2b).

The chymotryptic peptide fingerprint of histone 2A (Fig. 3a) contained approximately 20 peptides, 12 of which were readily identifiable and identical in mobility to peptides in the pattern of protein A24 (Fig. 3b). The chymotryptic peptide fingerprint of protein A24 (Fig. 3b) contained approximately 30 peptides, 3 of which were definitely not found in histone 2A (arrows, Fig. 3b).

DISCUSSION

The striking similarities in amino acid composition, tryptic

TABLE I

COMPARISON OF PROTEIN A24 AND HISTONE 2A

	Protein A24(4)	Histone 2A (12)
	Mole Percent	Mole Percent
Trp	0.0	0.0
Lys	11.3	10.9
His	2.4	3.1
Arg	7.4	9.3
Asx	7.3	6.2
Thr	6.5	3.9
Ser	4.5	3.1
Glx	12.3	9.3
Pro	5.6	3.9
Gly	9.2	10.9
Ala	9.6	13.4
Val	4.9	6.2
Met	0.3	0.0
Ileu	5.8	4.7
Leu	10.9	12.4
Tyr	1.3	2.3
Phe	0.9	0.8
Lys+His+Arg	21.1	23.3
Glx+Asx	19.6	15.5
Glx+Asx/Lys+His+Arg	0.93	0.67
NH_2-terminal	Methionine	Acetylserine
Molecular Weight	27,000	14,000

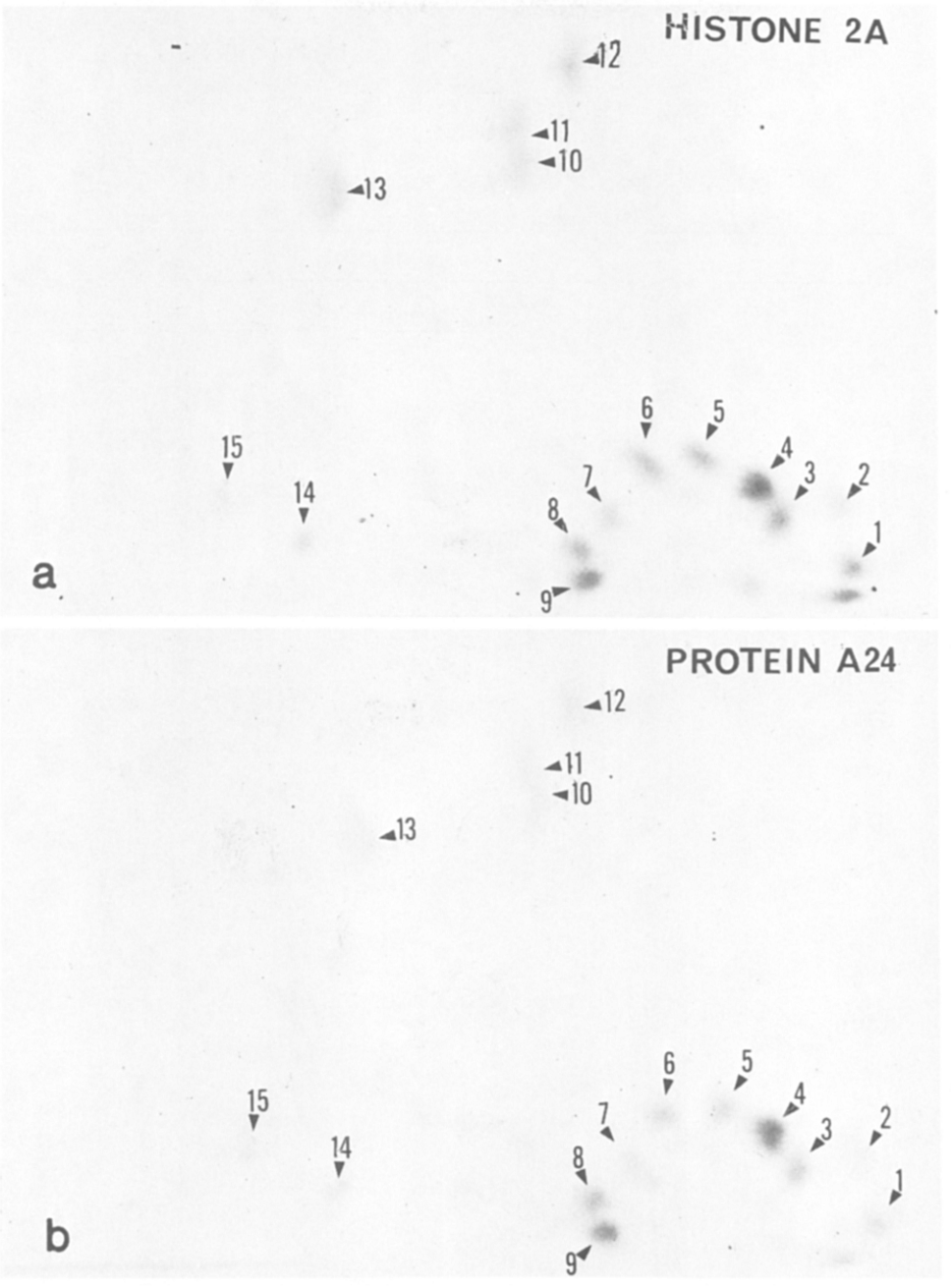

Figure 1 - Tryptic peptide maps of (a) histone 2A and (b) protein A24. The 15 major peptides in common are marked with small pointers and numbers. 250 μg of each protein were digested simultaneously with trypsin (see Materials and Methods) and chromatographed simultaneously.

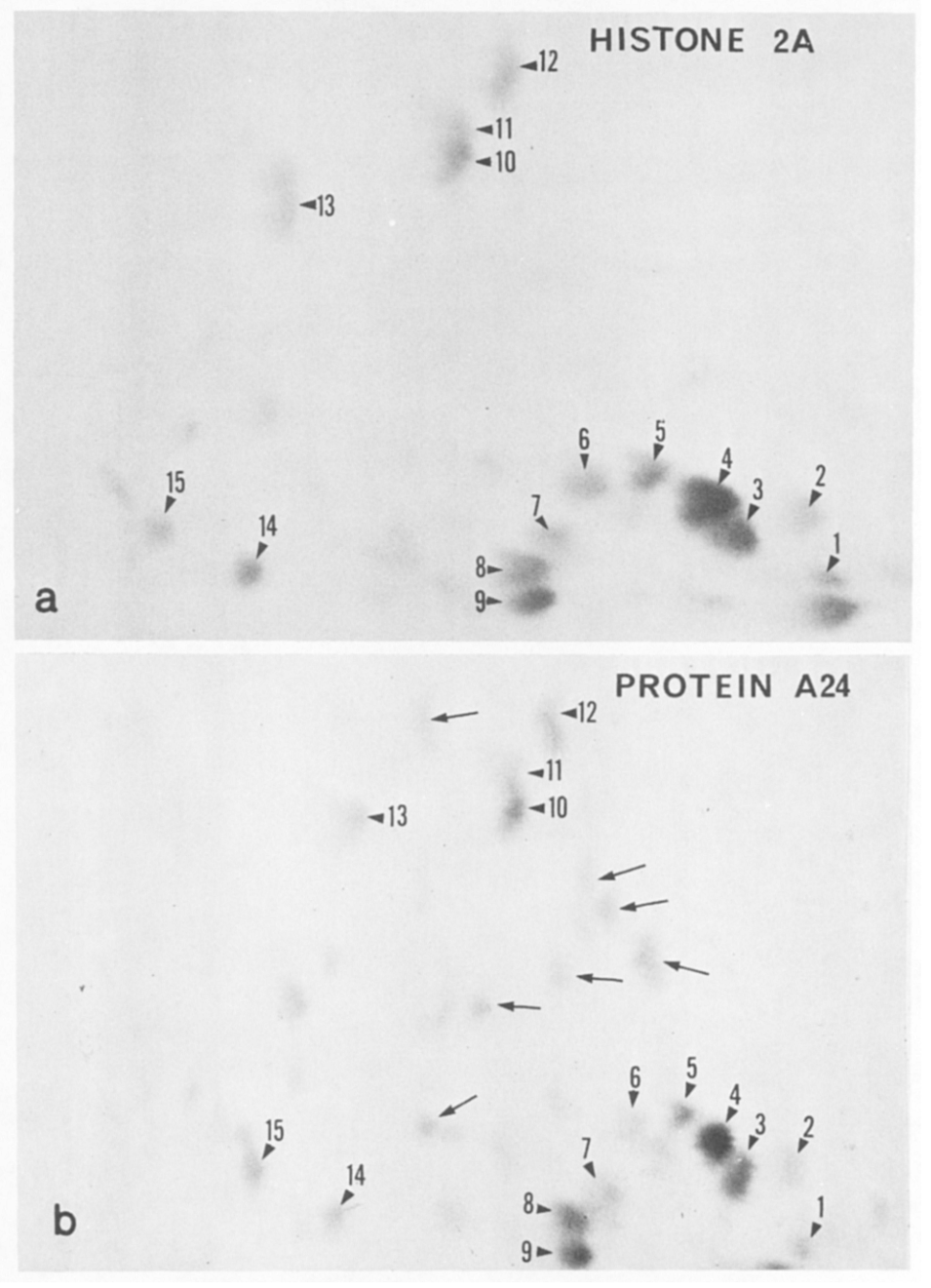

Figure 2 - Tryptic peptide maps of heavy loads (500 μg each) of (a) histone 2A and (b) protein A24 to demonstrate peptides difficultly visualized at low loads. The digestions and chromatography were performed at different times. The peptides unique to protein A24 (in b) are indicated by arrows.

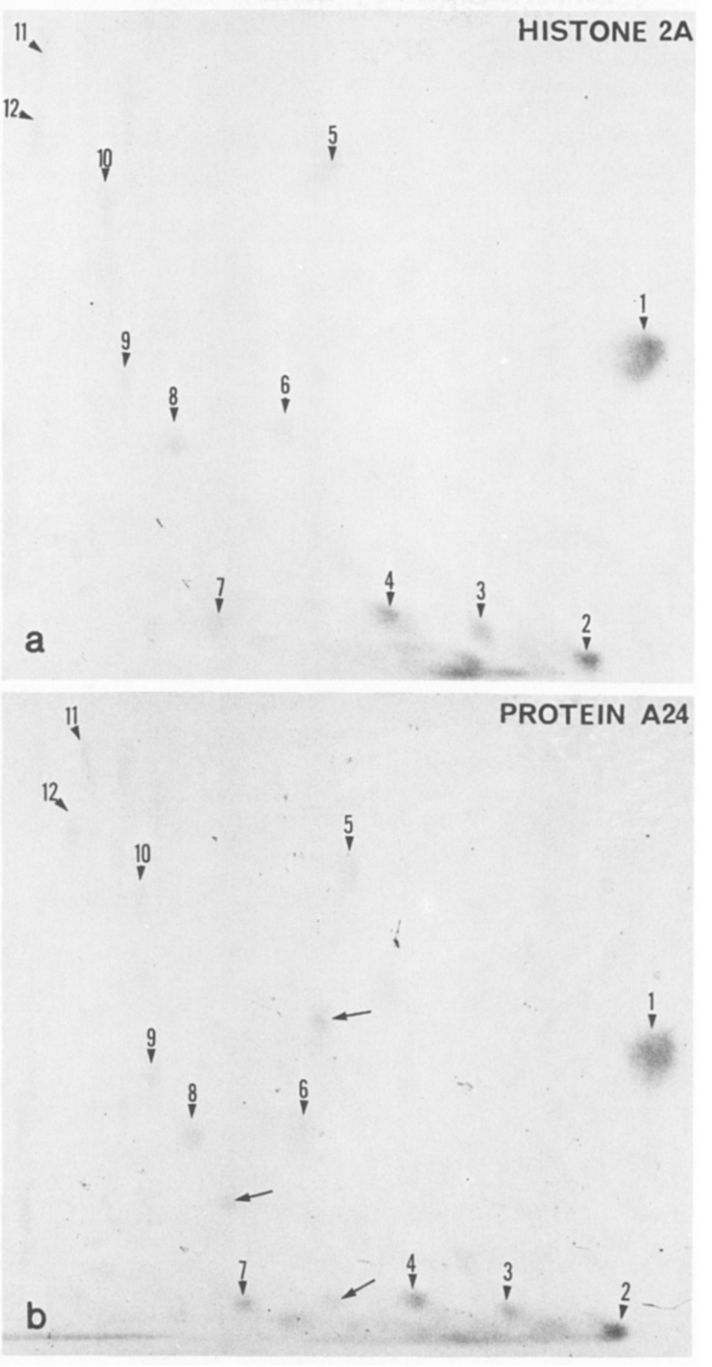

Figure 3 - Chymotryptic peptide maps (250 μg each) of (a) histone 2A and (b) protein A24. The major common peptides are indicated with pointers and numbers and the peptides definitely unique to protein A24 are indicated by unnumbered arrows.

and chymotryptic peptides of protein A24 and histone 2A indicate that they have common amino acid sequences. Protein A24 has a single methionine residue at its NH_2-terminus and histone 2A contains no methionine. Protein A24 also has a molecular weight greater than histone 2A and it produced additional peptides not produced by histone 2A upon both tryptic and chymotryptic digestion. Therefore, it is possible that histone 2A is on the C-terminal side of protein A24.

The evidence that histone 2A may be a part of the structure of protein A24 suggests the possibility that protein A24 may be a "prohistone" for histone 2A. Precursors have been found for many peptides and proteins including insulin (15,16), albumin (17,18), collagen (19), immunoglobulin (20,21), phage proteins (22) and viral proteins (23).

On the other hand, a protein with similar migration characteristics had a high rate of amino acid incorporation during early rat liver regeneration (25). A high synthetic rate for protein A24 when the rate of histone 2A synthesis is low is not consistent with a simple precursor-product relationship. Furthermore, protein A24 has been found tightly bound to chromatin (4,5) in constant amounts in several tissues (5,25). Therefore, protein A24 may be an independent protein entity with a bifunctional structure that contains much, if not all, of the amino acid sequence of histone 2A as well as additional amino acid sequences that make it a nonhistone chromosomal protein.

Goodwin and Johns (26) and Smith and Stocken (27) reported nonhistone proteins with lysine contents similar to histone 1. It is possible that there may be other nonhistone proteins with remarkable structural similarities to other histones.

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