Primary Structure and Microheterogeneities of Rat Chloroleukemia Histone H_{2A} (Histone ALK, II_{b1}, or F_{2a2})†

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ABSTRACT: Rat chloroleukemia histone H_{2A}, obtained from the F_{2a2} fraction, has been eluted in two peaks from a Biorex 70 column. The amino acid sequence of rat chloroleukemia histone H_{2A} has been determined and compared to that of calf-thymus histone H_{2A}. The structural studies performed on the tryptic peptides from the maleylated histone and on the thermolysin peptides from the native histone clearly demonstrate the existence of three molecular species of histone H_{2A} depending on the nature of the amino acid residue at positions 16 and 99: $H_{2A-\alpha}$ (Ser-16 and Lys-99) accounts for 60% and $H_{2A-\beta I}$ (Thr-16 and Arg-99) and $H_{2A-\beta II}$ (Ser-16 and Arg-99) for 20% each. A threonine residue at position 16 and a lysine residue at position 99 have been found in calf-thymus histone H_{2A}.

In the malignant tumor which results from uncontrolled cell growth and proliferation, the regulatory systems controlling gene activity and cell differentiation may be disturbed. Since the histones probably act as repressors of the gene activity, any mechanism which could decrease their repressor activity has to be investigated. As possible mechanisms, one can imagine a chemical modification by acetylation or phosphorylation or a change in the amino acid sequence.

The rate of phosphorylation of histone H_1 has been shown to be correlated with the growth rate of several tumors (Balhorn et al., 1972). Concerning the eventual structural changes, they have been investigated only in histone H_4 from human leukemic cells (Desai and Foley, 1970), rat Novikoff hepatoma (Wilson et al., 1970), rat chloroleukemia (Sautière et al., 1971), and benzopyrene-induced tumor (A. Martinage, personal communication). No differences have been found between histone H_4 from tumoral source and calf-thymus histone H_4.

The amino acid sequence of histone H_{2A} from rat chloroleukemia has been determined from the study of peptides obtained by hydrolyses with trypsin and thermolysin of native or maleylated protein. Each peptide was compared to the homologous peptide from calf-thymus histone H_{2A} (Sautière et al., 1974). A preliminary account of this work has already been reported (Laine et al., 1973; Laine, 1974).

Materials and Methods

Wistar rats weighing about 250 g were used. Tumors were excised 15 days after subcutaneous inoculation of sarcoma cells. Tumors were frozen on dry ice and kept at −25 °C until use. Chromatin was obtained from pure nuclei prepared by a sucrose method (Sautière et al., 1971). The histone fraction F_{2a2} was selectively extracted from the chromatin with the mixture ethanol–40% guanidinium HCl (3:1, v/v) at pH 7.0 (Johns, 1967).

Highly purified histone H_{2A} was isolated from the fraction F_{2a2} by ion-exchange chromatography on Biorex 70, 200–400 mesh (Bio-Rad) according to a method adapted from Luck et al. (1958). The column (60 × 2.5 cm) was equilibrated with 0.1 M sodium phosphate buffer (pH 6.8) containing 8% guanidinium HCl and eluted with a linear gradient of the latter from 8 to 15% in the same buffer. Further purification of histone H_{2A} was achieved by gel filtration chromatography on Sephadex G 100 (column 200 × 2.5 cm) equilibrated and eluted with 0.01 N HCl containing 0.02% sodium azide.

Polyacrylamide gel electrophoresis was performed in 2.5 M urea at pH 2.7 (Panyim and Chalkley, 1969) using a 17% acrylamide concentration.

Amino acid analyses were performed with a Technicon amino acid analyzer on histone samples hydrolyzed under vacuum at 110 °C for 24 and 72 h in 5.7 N HCl (1 mg of protein/ml of HCl) with 1 drop of 1% phenol to prevent excessive degradation of tyrosine.

Maleylation of the protein was achieved according to the method of Butler et al. (1969). The maleylated histone was hydrolyzed for 4 h at 37 °C and pH 8.0 in 0.1 M ammonium bicarbonate with trypsin treated with L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone (Worthington) using an enzyme:substrate ratio of 1:50 (w/w). Hydrolysis was stopped by lowering the pH at 3.5 with formic acid. Demaleylation of peptides was performed as described by De Lange et al. (1969).

The unmodified protein was hydrolyzed for 45 min at 40 °C and pH 8.0 in 0.1 M ammonium bicarbonate with thermolysin (Merck) using an enzyme:substrate ratio of 1:100 (w/w). Hydrolysis was stopped by lowering the pH to 3.5 with formic acid.

Separation, purification, and sequence determination of the peptides were achieved as described previously (Sautière et al., 1970, 1971).

Nomenclature of Peptides. Tryptic peptides from maleylated protein are designated by Tm- and thermolysin peptides by Th-. Each peptide is numbered according to its position in the amino acid sequence of the histone H_{2A}.

Results

In the early stage of our work, rat chloroleukemia histone H_{2A} was obtained from the Biorex 70 column as a single fraction and was used as such for structural studies. These early studies were performed on tryptic peptides from the maleylated protein and on thermolysin peptides from the

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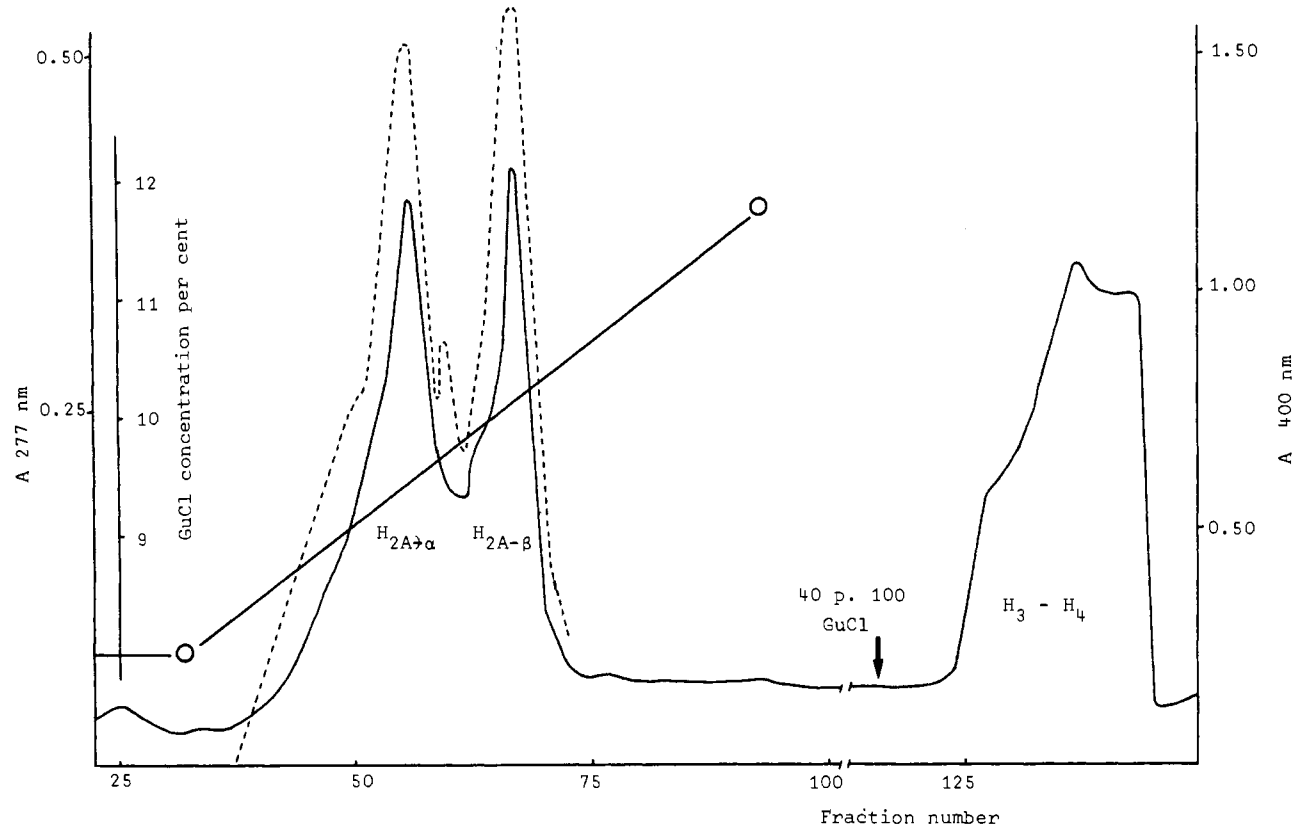

FIGURE 1: Chromatography of the F_{2a2} histone fraction from rat chloroleukemia on a Biorex 70 column. The F_{2a2} histone fraction (300 mg) was dissolved in 3 ml of 0.01 M HCl–6 M urea and kept at 4 °C during 48 h before the chromatography. The histone solution was then diluted with 3 ml of 0.1 M sodium phosphate buffer (pH 6.8) containing 8% guanidinium chloride (GuCl), centrifuged, and applied to the column, equilibrated and eluted as described in the text. The flow rate was 15.5 ml/h and 5.15-ml fractions were collected. Absorbance (—) was measured at 277 nm and turbidity (- - -) at 400 nm (Luck et al., 1958); (O–O) represents the guanidinium chloride gradient. Histones H_3 and H_4 corresponding to the third peak are eluted by 0.1 M sodium phosphate buffer (pH 6.8), containing 40% guanidinium chloride.

native protein. When we finally discovered in the enzymatic hydrolysates of the protein that there were at least two molecular species of histone H_{2A}, we tried to separate them by using a shallower gradient of guanidinium chloride. Indeed, Kinkade and Cole (1966) have shown that the resolution of lysine rich histones on columns of Amberlite IRC-50 was influenced by the slope of the guanidinium chloride elution gradient. The chromatography of histone fraction F_{2a2} on Biorex 70 resin produced two sharp peaks, $H_{2A-\alpha}$ and $H_{2A-\beta}$, satisfactorily resolved as shown by Figure 1. The relative percentages of $H_{2A-\alpha}$ and $H_{2A-\beta}$ were estimated as 58 and 42%, respectively.

After a further purification by chromatography on Sephadex G 100, these two subfractions appeared homogeneous in polyacrylamide gel electrophoresis at pH 2.7 and each of them had an electrophoretic migration identical with that of calf histone H_{2A}.

The amino acid compositions of rat chloroleukemia histones $H_{2A-\alpha}$ and $H_{2A-\beta}$ are presented in Table I. Both proteins have a similar composition closely related to that of calf-thymus histone H_{2A}. However, differences can be noticed in threonine and serine values as well as in lysine and arginine values. The differences appear more significantly in threonine/serine ratios and lysine/arginine ratios.

Histones $H_{2A-\alpha}$ and $H_{2A-\beta}$ were hydrolyzed by thermolysin at pH 8.0 and 40 °C for 45 min. Each hydrolysate was fractionated on Chromobeads P column as previously described (Sautière et al., 1970, 1971).

Tryptic Peptides of the Maleylated Histone. The insolu-

Table I: Amino Acid Compositions of Rat Chloroleukemia Histones $H_{2A-\alpha}$ and $H_{2A-\beta}$.[a]

	$H_{2A-\alpha}$		$H_{2A-\beta}$		Calf-Thymus H_{2A}	
Amino Acid	Calcd	Found	Calcd	Found	Calcd	Found
Asp	7.86	8	8.12	8	8.50	8
Thr[b]	4.59	4	4.91	5–4[d]	5.00	5
Ser[b]	5.18	5	4.74	4–5[d]	4.07	4
Glu	12.20	12	12.51	12	12.18	12
Pro	4.90	5	5.53	5	5.32	5
Gly	14.19	14	14.15	14	14.02	14
Ala	16.92	17	16.68	17	16.27	17
Val	7.87	8	8.04	8	7.99	8
Ile[c]	5.39	6	5.53	6	5.65	6
Leu	15.13	16	15.74	16	15.75	16
Tyr	2.99	3	3.07	3	2.95	3
Phe	1.07	1	1.10	1	1.16	1
Lys	13.31	14	13.11	13	13.59	14
His	3.81	4	4.07	4	3.91	4
Arg	11.82	12	12.70	13	11.98	12
No. of residues	129		129		129	
Lysine/arginine	1.13		1.03		1.13	
Threonine/serine	0.89		1.04		1.23	

[a] The results are expressed in numbers of residues per mole of protein. The amino acid composition of calf-thymus histone H_{2A} (Sautière et al., 1974) is given for comparison. [b] Values for threonine and serine were obtained by linear extrapolation to zero hydrolysis time. [c] Seventy-two hour hydrolysis values. [d] Values found respectively in $H_{2A-\beta I}$ and $H_{2A-\beta II}$.

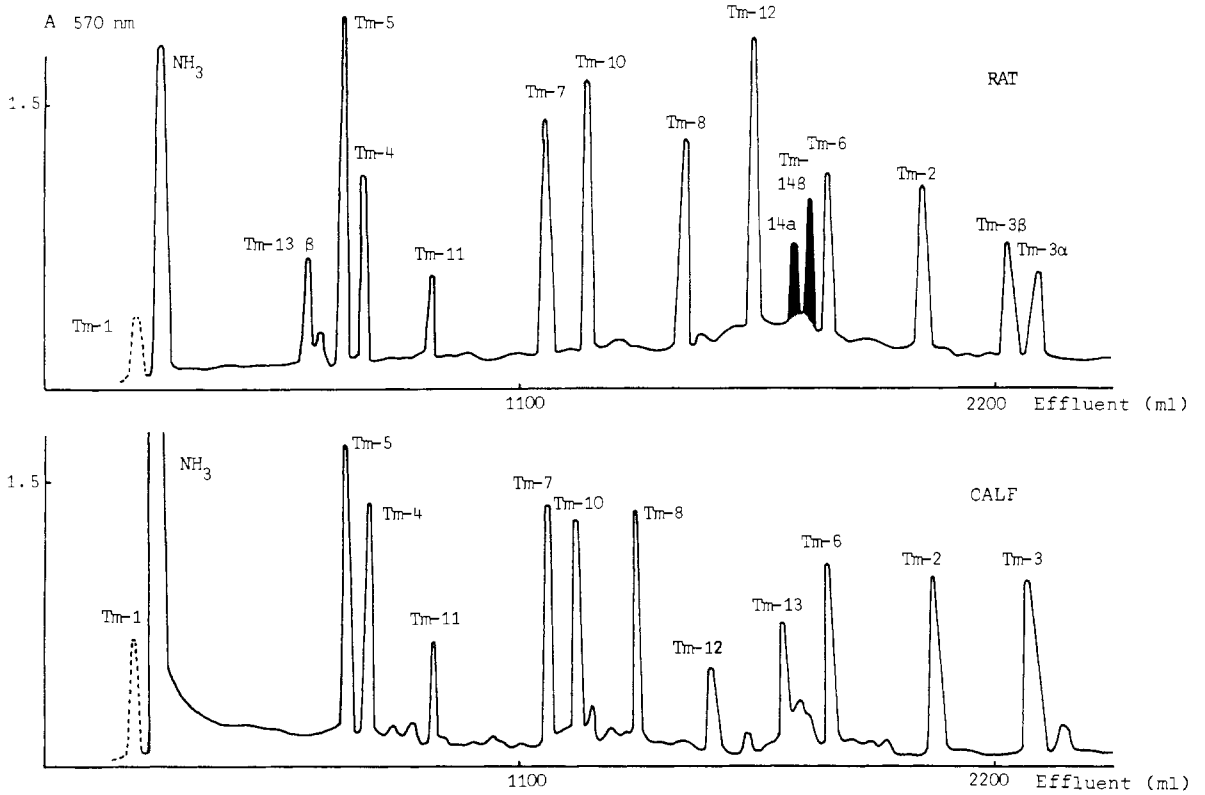

FIGURE 2: Elution diagram of soluble tryptic peptides from maleylated histone H_{2A} fractionated on Chromobeads P column. Peptides were eluted with pyridine–formate and pyridine–acetate buffers used in a gradient of ionic strength and pH. The fraction represented by a dotted line corresponds to a ninhydrin-negative reaction. The hatched fractions correspond to a Sakaguchi-negative reaction. Peptides, obtained pure in each fraction, were designated by the symbol Tm and numbered according to their position in the amino acid sequence of the protein. The elution diagram of soluble tryptic peptides from calf-histone H_{2A} is given for comparison.

ble material which precipitated during the demaleylation of the tryptic hydrolysate was removed by centrifugation. It corresponds to two tryptic insoluble cores as evidenced by previous structural studies on calf histone H_{2A} (Sautière et al., 1974).

The elution diagram of the soluble tryptic peptides is shown in Figure 2. The peptides were obtained directly in pure form. Their amino acid sequence is given in Table II.

All peptides previously identified in the tryptic hydrolysate of the maleylated histone H_{2A} from calf thymus are present in the tryptic hydrolysate of the maleylated histone H_{2A} from rat chloroleukemia together with three new peptides, Tm-3α, Tm-13β, and Tm-14β. The amino acid composition of these peptides is given in Table III, together with that of overlapping thermolysin peptides.

Peptide Tm-3α (residues 12–17) contains a serine residue in position 16 which replaces a threonine residue as found in peptide Tm-3β from rat chloroleukemia and in the homologous peptide from calf thymus.

Peptides Tm-13β and Tm-14β result from the cleavage of an arginyl bond in position 99. In calf-thymus histone H_{2A}, the lysyl bond found in that position is resistant to cleavage by trypsin, because of the maleylation. The peptide Tm-14βa comes from peptide Tm-14β likely by partial deamidation of a residue of asparagine or glutamine.

The peptides Tm-9 (residues 43–71) and Tm-13α (residues 89–129) were not isolated. They correspond to highly hydrophobic sequences of the peptidic chain and become insoluble at pH 3.5 during the demaleylation.

Thermolysin Peptides. The short-time thermolysin hydrolysis of the histone H_{2A} yielded 27 peptides which were separated by ion-exchange chromatography (Figure 3). The amino acid sequence of these peptides is presented in Table IV. For most of them the sequence was deduced from the structural studies on the tryptic peptides of the maleylated histone as well as from comparative studies with thermolysin peptides of the calf-thymus histone H_{2A}. The only thermolysin peptides to be completely sequenced were those corresponding to the two hydrophobic cores of the protein and those where microheterogeneity appears.

Because of incomplete cleavage by thermolysin, three peptides (Th-7, Th-11, and Th-15) are accompanied by one or several derivatives (indicated as Th-7a, Th-7b, etc.).

At the beginning of our work, peptides Th-3α and Th-3β-I were obtained together from histone H_{2A} and eluted in fractions 24 and 23, respectively. Later when the molecular subspecies α and β of histone H_{2A} were characterized and separately hydrolyzed by thermolysin, one peptide with serine at position 16 was found in histone $H_{2A-\alpha}$, whereas two peptides, one with serine at position 16 and one with threonine at the same position, were obtained with similar yields from the histone $H_{2A-\beta}$.

Discussion

The complete amino acid sequence of the rat chloroleukemia histone H_{2A} was deduced from the study of tryptic peptides from maleylated protein and of thermolysin peptides from native protein (Table V). The thermolysin peptides of the two hydrophobic regions of the protein, for which no overlapping tryptic peptides were available, were ordered by comparison with the amino acid sequence of the calf homologous histone.

From the amino acid sequence determination, evidence appears that three molecular species of histone H_{2A} exist in

Table II: Amino Acid Sequences of Soluble Tryptic Peptides from Maleylated Histone H_{2A}.[a,b]

Tm-1	(1–3)[c]	*N*-Acetyl-Ser-Gly-Arg
Tm-2	(4–11)	Gly-Lys-Gln-Gly-Gly-Lys-Ala-Arg
Tm-3-α	(12–17)	Ala-Lys-Ala-Lys-Ser-Arg
Tm-3-β	(12–17)	Ala-Lys-Ala-Lys-Thr-Arg
Tm-4	(18–20)	Ser-Ser-Arg
Tm-5	(21–29)	Ala-Gly-Leu-Gln-Phe-Pro-Val-Gly-Arg
Tm-6	(30–32)	Val-His-Arg
Tm-7	(33–35)	Leu-Leu-Arg
Tm-8	(36–42)	Lys-Gly-Asn-Tyr-Ala-Glu-Arg
Tm-10	(72–77)	Asp-Asn-Lys-Lys-Thr-Arg
Tm-11	(78–81)	Ile-Ile-Pro-Arg
Tm-12	(82–88)	His-Leu-Gln-Leu-Ala-Ile-Arg
Tm-13-β	(89–99)	Asn-Asp-Glu-Glu-Leu-Asn-Lys-Leu-Leu-Gly-Arg
Tm-14-β	(100–129)	Val-Thr-(Ile,Ala,Gln,Gly,Gly,Val,Leu,Pro, Asn,Ile,Gln,Ala,Val,Leu,Leu,Pro,Lys-Lys-Thr-Glu-Ser-His-His-Lys-Ala-Lys-Gly-Lys

[a] Methods used for determination of the sequence of peptides are indicated as follows: dansylation and Edman degradation, — and → above the amino acid residue, respectively; hydrazinolysis and carboxypeptidases A and B hydrolysis, — and ← below the amino acid residue, respectively; T and Th indicate cleavage by trypsin and thermolysin, respectively. [b] For each tryptic peptide except for peptide Tm-14-β, the arginine residue was assigned to the COOH terminus from the specificity of the trypsin limited to the arginyl bonds in a maleylated protein. The amino acid residue immediately before the arginine residue was generally placed by difference. [c] Residues.

Table III: Amino Acid Composition of Peptides which Indicate Unequivocally the Presence of Microheterogeneities at Positions 16 and 99 of Rat Chloroleukemia Histone H_{2A}.[a]

A. Tryptic Peptides from Maleylated Histone H_{2A}

Tm-3-α	$Ser_{1.09(1)}$, $Ala_{1.72(2)}$, $Lys_{1.79(2)}$, $Arg_{1.00(1)}$
Tm-3-β	$Thr_{1.04(1)}$, $Ala_{1.75(2)}$, $Lys_{1.89(2)}$, $Arg_{1.00(1)}$
Tm-13-β	$Asp_{3.20(3)}$, $Glu_{1.78(2)}$, $Gly_{0.95(1)}$, $Leu_{2.61(3)}$, $Lys_{0.93(1)}$, $Arg_{1.00(1)}$
Tm-14-β	$Asp_{1.08(1)}$, $Thr_{1.66(2)}$, $Ser_{1.06(1)}$, $Glu_{2.91(3)}$, $Pro_{2.01(2)}$, $Gly_{2.92(3)}$, $Ala_{3.10(3)}$, $Val_{2.69(3)}$, $Ile_{1.79(2)}$, $Leu_{2.98(3)}$, $Lys_{5.00(5)}$, $His_{1.89(2)}$

B. Thermolysin Peptides from Histone H_{2A}

Th-3-α	$Ser_{2.80(3)}$, $Gly_{1.20(1)}$, $Ala_{2.00(2)}$, $Lys_{1.11(1)}$, $Arg_{2.00(2)}$
Th-3-β	$Thr_{1.00(1)}$, $Ser_{2.04(2)}$, $Gly_{1.44(1)}$, $Ala_{2.00(2)}$, $Lys_{0.97(1)}$, $Arg_{1.70(2)}$
Th-16-α	$Thr_{0.86(1)}$, $Gly_{1.05(1)}$, $Val_{0.89(1)}$, $Leu_{1.00(1)}$, $Lys_{1.00(1)}$
Th-16-β	$Thr_{0.94(1)}$, $Gly_{1.34(1)}$, $Val_{1.00(1)}$, $Leu_{0.83(1)}$, $Arg_{1.06(1)}$

[a] Number in parentheses is the nearest integer.

Table IV: Amino Acid Sequences of Thermolysin Peptides from Histone H_{2A}.[a]

Th-1	(1–11)[b]	*N*-Acetyl-Ser-Gly-Arg-Gly-Lys-Gln-Gly-Gly-Lys-Ala-Arg
Th-2	(12–13)	Ala-Lys
Th-3-α and Th-3-β-II	(14–22)	Ala-Lys-Ser-Arg-Ser-Ser-Arg-Ala-Gly
Th-3-β-I	(14–22)	Ala-Lys-Thr-Arg-Ser-Ser-Arg-Ala-Gly
Th-4	(23–26)	Leu-Gln-Phe-Pro
Th-5	(27–29)	Val-Gly-Arg
Th-6	(30–33)	Val-His-Arg-Leu
Th-7	(34–50)	Leu-Arg-Lys-Gly-Asn-Tyr-Ala-Glu-Arg-Val-Gly-Ala-Gly-Ala-Pro-Val-Tyr
Th-7a	(34–39)	Leu-Arg-Lys-Gly-Asn-Tyr
Th-7b	(40–50)	Ala-Glu-Arg-Val-Gly-Ala-Gly-Ala-Pro-Val-Tyr
Th-8 and Th-14	(51–52) (85–86)	Leu-Ala
Th-8a	(51–53)	Leu-Ala-Ala
Th-9	(53–57)	Ala-Val-Leu-Glu-Tyr
Th-10	(58–64)	Leu-Thr-Ala-Glu-Ile-Leu-Glu
Th-11	(65–77)	Leu-Ala-Gly-Asn-Ala-Ala-Arg-Asp-Asn-Lys-Lys-Thr-Arg
Th-11a	(65–75)	Leu-Ala-Gly-Asn-Ala-Ala-Arg-Asp-Asn-Lys-Lys
Th-11b	(76–77)	Thr-Arg
Th-12	(78–82)	Ile-Ile-Pro-Arg-His
Th-13	(83–84)	Leu-Gln
Th-15	(87–96)	Ile-Arg-Asn-Asp-Glu-Glu-Leu-Asn-Lys-Leu
Th-15a	(87–95)	Ile-Arg-Asn-Asp-Glu-Glu-Leu-Asn-Lys
Th-16-α	(97–101)	Leu-Gly-Lys-Val-Thr
Th-16-β	(97–101)	Leu-Gly-Arg-Val-Thr
Th-17	(102–110)	Ile-Ala-Gln-Gly-Gly-Val-Leu-Pro-Asn
Th-18	(111–113)	Ile-Gln-Ala
Th-19	(114–125)	Val-Leu-Leu-Pro-Lys-Lys-Thr-Glu-Ser-His-His-Lys
Th-20	(126–129)	Ala-Lys-Gly-Lys

[a] Symbol Th means cleaved by thermolysin. The meaning of the signs —, →, and ← is presented in footnote *a* of Table II. [b] Residues.

the rat chloroleukemia. One molecular species, termed $H_{2A-\alpha}$, accounting for about 60% of histone H_{2A}, has a serine residue at position 16 and a lysine residue at position 99.

The two other molecular species termed $H_{2A-\beta I}$ and $H_{2A-\beta II}$, each accounting for about 20% of histone H_{2A}, have an arginine residue at position 99 and differ from each other by the nature of the amino acid residue at position 16. At that position a threonine residue is found in $H_{2A-\beta I}$ whereas a serine residue is found in $H_{2A-\beta II}$. Limited microheterogeneities have also been observed in other histones.

Two histones H_5 have been identified in chicken erythrocyte (Greenaway and Murray, 1971): one with a glutamine residue and the other with an arginine residue in position 15.

Two molecular species of histone H_3 exist in pea embryo (Patthy et al., 1973) as well as in calf-thymus (Patthy and Smith, 1975). In pea histone H_3 a serine residue or an alanine residue is found at position 96, whereas in calf-histone H_3 a serine residue or a cysteine residue is identified at position 96.

The substitutions observed in rat chloroleukemia histone H_{2A} are rather conservative and should not affect the general conformation of the protein. The threonine–serine replacement at position 16 requires a single transversion of the first or of the second base of the DNA TG (X) → AG (X) or TC (A or G). The lysine–arginine replacement at position 99 requires a single transition of the second base of the DNA TT (T or C) → TC (T or C).

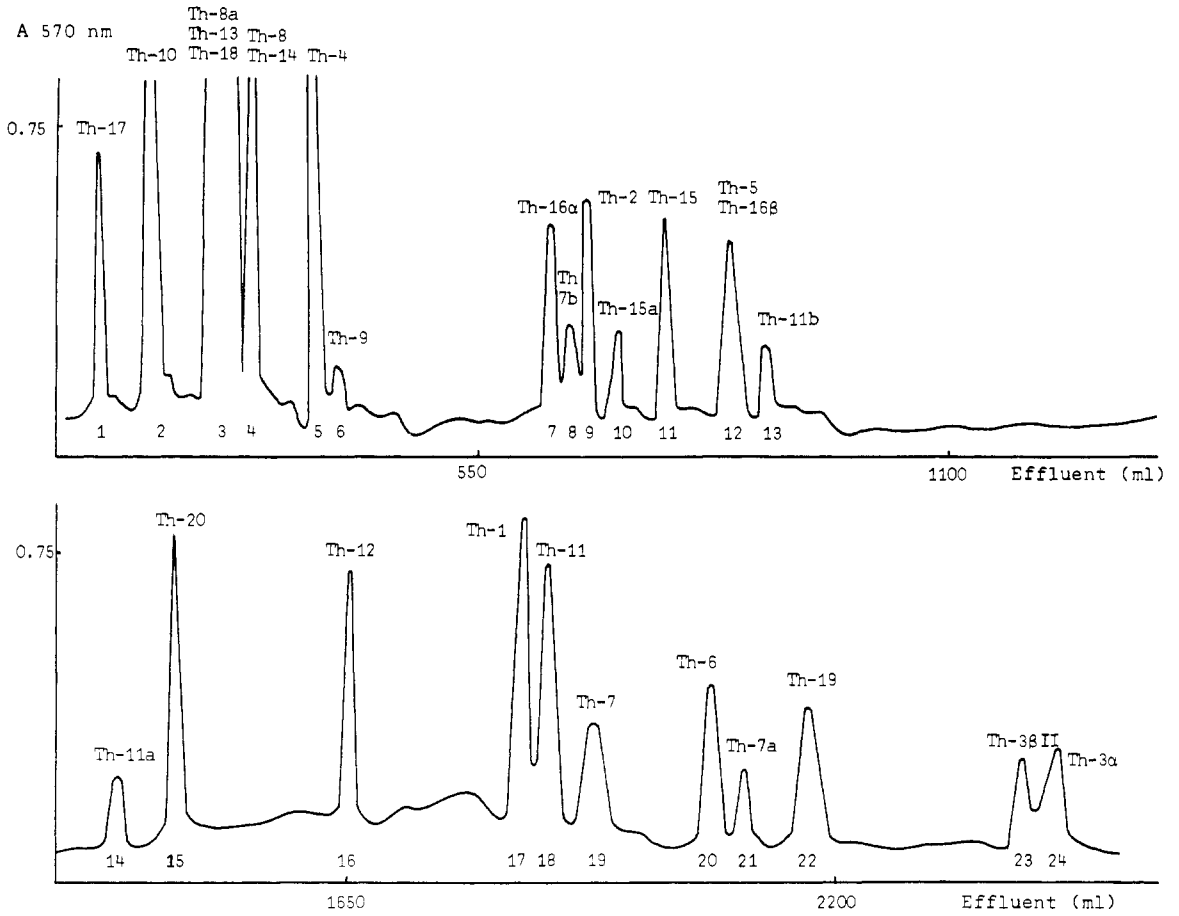

FIGURE 3: Elution diagram of thermolysin peptides from histone H_{2A} fractionated on Chromobeads P column. Peptides were eluted with pyridine–formate and pyridine–acetate buffers used in a gradient of ionic strength and pH. Elution of peptides was monitored by a ninhydrin reaction. Peptides were designated by the symbol Th and numbered according to their position in the amino acid sequence of the protein.

Table V: Complete Amino Acid Sequence of Histone H_{2A} from Rat Chloroleukemia.

N-Acetyl-Ser-Gly-Arg-Gly-Lys-Gln-Gly-Gly-Lys-Ala-Arg-Ala-Lys-Ala-
10
Lys-$\frac{\text{Ser}}{\text{Thr}}$-Arg-Ser-Ser-Arg-Ala-Gly-Leu-Gln-Phe-Pro-Val-Gly-Arg-Val-
16 20 30
His-Arg-Leu-Leu-Arg-Lys-Gly-Asn-Tyr-Ala-Glu-Arg-Val-Gly-Ala-Gly-
40
Ala-Pro-Val-Tyr-Leu-Ala-Ala-Val-Leu-Glu-Tyr-Leu-Thr-Ala-Glu-Ile-
50 60
Leu-Glu-Leu-Ala-Gly-Asn-Ala-Ala-Arg-Asp-Asn-Lys-Lys-Thr-Arg-Ile-
70
Ile-Pro-Arg-His-Leu-Gln-Leu-Ala-Ile-Arg-Asn-Asp-Glu-Glu-Leu-Asn-
80 90
Lys-Leu-Leu-Gly-$\frac{\text{Lys}}{\text{Arg}}$-Val-Thr-Ile-Ala-Gln-Gly-Gly-Val-Leu-Pro-Asn-
99 110
Ile-Gln-Ala-Val-Leu-Leu-Pro-Lys-Lys-Thr-Glu-Ser-His-His-Lys-Ala-
120
Lys-Gly-Lys(OH)
129

Positions 16 and 99 seem to be privileged substitutions sites in histone H_{2A}, since a serine residue was found at position 16 in sea urchin histone H_{2A} (Sautière et al., 1975) and a glycine residue was found at position 99 by comparison with the calf-histone H_{2A} sequence, in trout histone H_{2A} (Bailey and Dixon, 1973). The structural studies performed on the histone H_{2A} from four different animal species (calf, rat, trout, and sea urchin) indicate that this histone is less conservative than histones H_3 and H_4 and confirm the observations of Panyim et al. (1971) based on the electrophoretic behavior of the histones from different vertebrate species.

On the other hand, the substitution of a threonine residue by a serine residue at position 16 of the amino acid sequence of the histone H_{2A} from rat chloroleukemia introduces a potential site for phosphorylation. Indeed, histone phosphorylation has been shown to be positively correlated with rate of cell replication of tumor growth (Balhorn et al., 1972). Although a recent paper (Shlyapnikov et al., 1975) indicates that in calf-histone H_{2A} the serine residue at position 19 is preferentially phosphorylated by a protein kinase from pig brain, it may be of interest to investigate the sites of phosphorylation of the rat chloroleukemia histone $H_{2A-\alpha}$. Moreover, we must determine if the substitutions Thr → Ser (at residue 16) and Lys → Arg (at residue 99) observed in the rat chloroleukemia histone H_{2A} depend on the tumoral nature of the tissue or on the animal species.

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Physical Studies of the Nonhistone Chromosomal Proteins HMG-1 and HMG-2†

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ABSTRACT: The nonhistone chromosomal proteins, HMG-1 and HMG-2, have a folded conformation, with a high α-helical content, over a wide pH range. At high and low pH values, the molecules unfold. Both molecules contain cysteine and tryptophan. The tryptophans appear to be buried in the folded form. HMG-1 shows aggregation at pH 5.7, as does HMG-2 at pH 9.0. The folded form is insensitive to high concentrations of salt, suggesting that charge-charge interaction plays no role in stabilizing the tertiary structure.

Early work on nonhistones was hampered by their insolubility. However, it was found (Goodwin et al., 1973) that differential Cl_3CCOOH precipitation could separate the nonhistones extracted from chromatin with 0.35 M NaCl into low and high molecular weight fractions. The low molecular weight fraction is freely soluble. Chromatography of this fraction, named the high mobility group, or HMG[1] proteins, on a carboxymethyl-cellulose column at pH 9.0 (Goodwin and Johns, 1973) yields two pure fractions called HMG-1 and HMG-2. These are the same proteins found as contaminants in F1 isolated by perchloric acid extraction (Johns 1964a, 1964b).

HMG-1 and HMG-2 are present in about 10^5 to 10^6 copies per nucleus (Johns et al., 1975; Walker et al., submitted) making it unlikely that they are specific gene activators. HMG-1 has been shown to complex with F1 and bind nonspecifically to DNA (Shooter et al., 1974; Goodwin et al., 1975). HMG-1 stimulates chromatin template activity (Johns et al., 1975).

HMG-1 and HMG-2 contain approximately 25% basic and 30% acidic residues which appear to be asymmetrically distributed. Preliminary results on the sequencing of the N-terminal residues indicate that HMG-1 and HMG-2 are similar, but contain some amino acid substitutions (Johns et al., 1975)

In this work we present a number of fundamental properties of HMG-1 and HMG-2. We report that they each contain cysteine and tryptophan. We give molar extinction coefficients for each protein. We describe a number of physical properties of HMG-1 and HMG-2, and present new data on the electrophoretic mobility of these proteins, and describe the results of CD fluorescence anisotropy, and turbidity measurements. We show that HMG-1 is an acidic and HMG-2 a basic protein. Among other things, we show that these proteins can exist in a highly folded conformation which has a high α-helical content. The conformation is very insensitive to high salt concentration. We show that

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[1] Abbreviation used: HMG, high mobility group.