Structural Studies of Calf Thymus F3 Histone. I. Occurrence of Cysteine, Phosphoserine, and ε-*N*-Acetyllysine in Cyanogen Bromide Peptides†

William F. Marzluff, Jr.,‡ and Kenneth S. McCarty*

ABSTRACT: The F3 histone from calf thymus has been purified. The cyanogen bromide fragments have been resolved and characterized. All the half-cystine in the molecule is on a single 30-residue cyanogen bromide peptide, CNBr-2. All the modified amino acids are present in the large cyanogen bromide peptide, CNBr-1.

Histone F3 is the only histone which contains half-cystine (Phillips, 1965) and hence has the potential of forming aggregates. Histone F3 exists in two forms in calf thymus (Marzluff *et al.*, 1972), one containing a single free sulfhydryl group and the other two free sulfhydryl groups. No aggregates are present, although disulfide interchange occurs readily at neutral pH *in vitro* (Marzluff *et al.*, 1972).

Since histone F3 is rapidly acetylated in many tissues (Wilhelm and McCarty, 1970; Marzluff and McCarty, 1970, 1972a; Desai and Foley, 1971) and calf thymus histone F3 contains more ε-*N*-acetyllysine than other calf thymus histones (Vidali *et al.*, 1968), the present study was designed to characterize some of the modified amino acids occurring in histone F3. Phosphoserine and acetyllysine are found in multiple locations in histone F3, although only a fraction of any given residue is modified. All these modifications are present in the largest (90 residue) cyanogen bromide peptide (CNBr-1).

Methods

Purification of Histone F3. The procedure of Johns (1964) for selective extraction and precipitation of histone F3 was used. The crude F3 was dissolved in 0.1 N acetic acid and lyophilized. The yield was about 400 mg per 100 g of calf thymus. The crude F3 histone was contaminated with small amounts of F1 and F2a1 histones (about 5%), as shown in Figure 1A. Further purification was accomplished by dissolving the crude fraction at a concentration of 10 mg/ml in 6 M Gdn·HCl[1]–0.3 M Tris (pH 8.8) containing 0.01 M dithiothreitol. After 30 min at room temperature, the pH was adjusted to 8.3 and 0.1 volume of 2 M iodoacetate in 4 M Gdn·HCl–0.2 M Tris (pH 8.3) was added rapidly. The pH was maintained between 8.0 and 8.3 with 3 N NaOH. After 30 min, an excess of mercaptoethanol was added, and the solution dialyzed exhaustively against distilled water. The histone was recovered by precipitation with 15% trichloroacetic acid and lyophilized from 0.1 N acetic acid. Electrophoretically pure histone F3 (Figure 1B) was obtained after chromatography on Sephadex G-100 equilibrated with 0.01 N HCl. Less than 5% of the F3 histone was present as high molecular weight aggregates which eluted in the void volume of the column. When cyanogen bromide peptides were prepared, it was unnecessary to separate the F1 histone as this protein does not contain methionine that is subject to cyanogen bromide cleavage and therefore could be readily resolved from the F3 CNBr peptides. It should be noted however, that reduction of the crude F3 histone was incomplete if 0.1 M mercaptoethanol rather than dithiothreitol is used, with the result that some oxidized monomer will be detected after carboxymethylation.

Preparation of [^{14}C]Acetate or $^{32}PO_4$-Labeled Histones. The procedure of Vidali *et al.* (1968) was followed for isolation of calf thymus nuclei. Fresh calf thymus (50 g) was forced through a wire screen into 300 ml of Littlefield's media (Parsons and McCarty, 1968) containing 3 mM $Ca(OAc)_2$. The mixture was homogenized in a Dounce homogenizer by 5 strokes with the A pestle. The nuclei were pelleted by centrifugation for 30 min at 600*g*. They were resuspended in 0.25 M sucrose–3 mM $Ca(OAc)_2$ by homogenization with two strokes of the A pestle and recentrifuged. This washing procedure was repeated four times. The nuclei were then resuspended in 50 ml of 0.25 M sucrose–3 mM $Ca(OAc)_2$ and warmed to 37°.

For acetylation experiments the nuclei were added to a solution containing 20 ml of 0.1 M glucose–3.75 mg of NaCl/ml–4.2 mg of $MgCl_2$/ml (Vidali *et al.*, 1968)–25 ml of 0.1 M $NaHPO_4$ in 0.25 M sucrose (pH 6.8)–10^{-6} M CoASH. [^{14}C]-Acetate (125 μCi, 60 mCi/mole, New England Nuclear) in 4 ml of water was added, and the mixture incubated in a shaking water bath for 30 min at 37°. The nuclei were rapidly chilled and collected by centrifugation. They were washed once in SSC (saline sodium citrate—0.150 M NaCl–0.015 M sodium citrate) and then lysed by homogenizing in 50 ml of SSC, 10 strokes with the B pestle. The chromatin was then washed and the F3 histone purified by the procedure of Johns (1964).

For phosphorylation experiments the nuclei were added to a similar solution, except that the phosphate buffer was replaced by 0.1 M Tris (pH 6.8), and the final phosphate concentration was adjusted to 10^{-5} M. In place of CoASH, 10^{-6} M cyclic 3′,5′-AMP was present. Carrier-free $^{32}PO_4$ (5 mCi, New England Nuclear, Corp.) was added, and the incubation continued for 30 min in a shaking water bath. The nuclei were washed twice in 0.25 M sucrose containing 0.1 M $NaHPO_4$, prior to preparation of the chromatin. The F3 histone was prepared as described above.

† From the Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710. *Received July 19, 1972.* This work was supported in part by Grant 5R01-GM12805-07 from National Institutes of Health.

‡ Supported in part by Predoctoral Traineeship GM00233 from U. S. Public Health Service. Present address: Department of Biology, Johns Hopkins University, Baltimore, Md. 21218.

[1] Abbreviations used are: Gdn·HCl, guanidine hydrochloride; CoASH, coenzyme A.

Cyanogen Bromide Digestion. Carboxymethylated F3 histone was dissolved in 70% formic acid and a large excess of CNBr was added (approximately an equal amount by weight). After 18 hr at room temperature, the solution was diluted with distilled water and lyophilized. The peptides were dissolved in 0.1 M acetic acid and relyophilized before separation on Sephadex G-100. In some experiments the oxidized monomer was treated in a similar fashion.

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis was performed exactly as described previously (Marzluff and McCarty, 1970), except that when cyanogen bromide peptides were analyzed, the electrophoresis time was shortened from 12 to 8 hr. Alternatively gel electrophoresis was performed as described by Panyim and Chalkley (1969). Gels (10 × 0.5 cm) were 15% acrylamide cross-linked with 0.1% ethylene diacrylate. Gels were run for 8 hr at 0.9 mA/tube at 170 V, stained, and destained as described earlier (Marzluff and McCarty, 1970).

Amino Acid Analysis. Samples of total F3 histone or the cyanogen bromide peptides for amino acid analysis were hydrolyzed for 20 hr in 6 N HCl at 110° in an evacuated sealed tube. Occasionally a drop of 5% phenol was added to prevent destruction of tyrosine. Norleucine was added to the hydrolysate as an internal standard. The samples were analyzed on a single long column Technicon analyzer. Methyllysines were partially resolved as a shoulder on the lysine peak under these conditions. Performic acid oxidation was performed in a standard manner (Hirs, 1967).

Chemicals. Guanidine hydrochloride was obtained from Heico. Iodoacetic acid was obtained from Eastman and recrystallized from hexane before use. Cyanogen bromide was obtained from Eastman. 5,5′-Dithiobis(2-nitrobenzoic acid)[11] was obtained from Aldrich. Thermolysin was purchased from Calbiochem (grade B). ε-*N*-Acetyllysine was obtained from Sigma and phosphoserine from Calbiochem.

Results

Purification of Calf Thymus F3 Histone. As isolated from calf thymus chromatin, the F3 histone was heterogeneous on electrophoresis at pH 3.2 (Figure 1A). Note arrow. This is in confirmation of our observation of two forms of F3 histones (Marzluff *et al.*, 1972). Small amounts (<5%) of F1 and F2a1 histones frequently contaminated this preparation. After reduction and carboxymethylation, however, the contaminating F1 molecular weight 23,500 and F2a1 molecular weight 11,300+ histones were removed by gel filtration on Sephadex G-100 yielding a carboxymethylated F3 histone as a single electrophoretic component (Figure 1B). Under these conditions less than 10% of the F3 histone was recovered as aggregates in the void volume of the column. The heterogeneity on gel electrophoresis in the crude preparation is due to the presence of an intramolecular disulfide bond in 80% of F3 histone, resulting in a more compact structure and a faster migration rate on electrophoresis than the 20% F3 histone in the reduced form, Figure 1A arrow (Marzluff *et al.*, 1972).

The amino acid composition of calf thymus F3 histone is given in Table I. The number of residues were calculated on the basis of an assumed molecular weight of 16,500.

Cyanogen Bromide Digestion. After treatment of carboxymethylated histone F3 with cyanogen bromide for 18 hr, the cleavage products were recovered by lyophilization. They were completely soluble in either 0.01 N HCl or 0.1 N acetic acid. They were resolved into three fractions (I, II, III) by gel

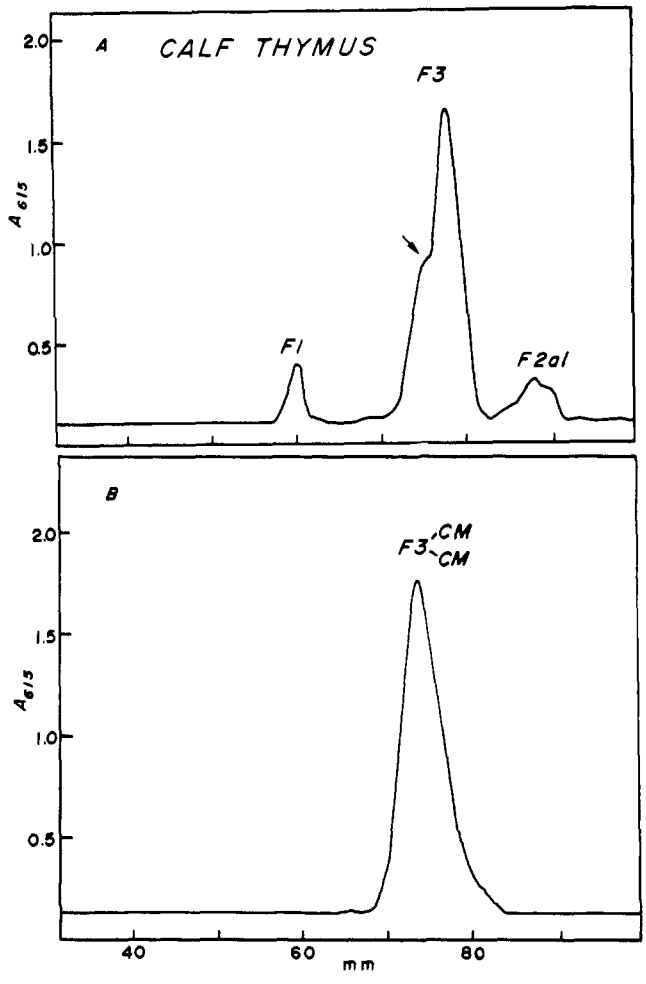

FIGURE 1: A. Electrophoretic analysis of calf thymus histone F3 prepared by the method of Johns. Polyacrylamide gel electrophoresis was performed at pH 3.2 without a spacer gel as described under Methods. B. The calf thymus F3 histone was reduced with 0.01 M dithiothreitol, carboxymethylated with iodoacetate, and purified by gel filtration on Sephadex G-100 as described under Methods. The carboxymethylated F3 histone migrated as a single component when analyzed by gel electrophoresis at pH 3.2.

filtration on Sephadex G-100 equilibrated in 0.01 N HCl (Figure 2A). The low molecular weight peptides, fraction III (CNBr-2, 3), were further resolved by chromatography on Sephadex G-50 (Figure 2B) or by paper chromatography and electrophoresis. Fractions of the CNBr-1A and CNBr-1 peptides from the Sephadex G-100 column each contained a single major component when analyzed by polyacrylamide gel electrophoresis, with less than 15% cross contamination of the two components. Figure 2C represents the coelectrophoresis of CNBr-1A and CNBr I peptides with carboxymethylated F3 histone as a marker. After rechromatography on Sephadex G-100 for CNBr-1A and CNBr-1, or Sephadex G-50 for CNBr-2 and CNBr-3, all the CNBr peptides were resolved into single peptides as shown on paper electrophoresis.

The largest peptide, CNBr-1A, present in fraction I, was distinct from the carboxymethylated F3 histone on gel electrophoresis, as shown in Figure 2C. It should be noted that the carboxymethylated F3 histone migrated at the same electrophoretic rate as the oxidized F3 monomer, and that the CNBr-1 peptide migrated more rapidly than the CNBr-1A. The fact that the amino acid analysis shows that the sum of

TABLE I: Composition of Cyanogen Bromide Peptides.[a]

Amino Acid	Calf F3	CNBr-1A	CNBr-1	CNBr-2	CNBr-3
Lysine	13.6 (14)	13.0 (13)	11.2 (11)	2.1 (1)	1.1 (1)
Histidine	2.3 (2)	2.2 (2)	1.8 (1)	0.5 (1)	0
Arginine	16.8 (17)	13.1 (13)	13.3 (11)	1.2 (1)	3.7 (4)
Aspartic acid	5.7 (6)	5.5 (5)	3.7 (4)	1.9 (2)	1.0 (1)
Threonine	8.1 (8)	7.6 (8)	7.7 (7)	1.8 (2)	0
Serine	5.7 (6)	6.2 (6)	5.4 (5)	0.4 (0)	0
Glutamic acid	14.3 (14)	12.1 (12)	8.9 (9)	3.9 (4)	2.3 (2)
Proline	6.1 (6)	5.1 (5)	5.6 (4)	0.1 (0)	0.9 (1)
Glycine	9.0 (9)	8.2 (8)	6.8 (6)	1.4 (1)	1.2 (1)
Alanine	17.8 (18)	16.3 (16)	11.2 (11)	4.8 (5)	2.1 (2)
Valine	6.7 (7)	7.0 (7)	5.8 (6)	1.8 (2)	0
Methionine	1.6 (2)	0.2 (0)	0	0	0
Isoleucine	5.9 (6)	4.3 (4)	4.0 (3)	1.8 (2)	1.9 (2)
Leucine	11.7 (12)	11.4 (11)	7.8 (7)	3.6 (4)	1.1 (1)
Tyrosine	2.9 (3)	2.8 (3)	2.4 (2)	0.8 (1)	0
Phenylalanine	3.2 (3)	2.8 (3)	2.9 (2)	0.8 (1)	0
Homoserine	1.6 (2)	1.2 (2)	0.9 (1)	0.5 (1)	0
Carboxymethylcysteine	1.6 (2)	1.2 (2)	0	1.1 (2)	0
Cysteic acid	1.8	Nd	Nd	Nd	Nd
Methionine sulfone	1.8	Nd	Nd	Nd	Nd
Total residues	(135)	(120)	(90)	(30)	(15)

[a] The amino acid composition of calf thymus F3 histone and the cyanogen bromide peptides. Results are expressed as residues per 16,500 g for the total F3 histone. Duplicate samples of the calf thymus histone and cyanogen bromide peptides were analyzed after hydrolysis for both 24 and 72 hr. The values for serine and threonine were corrected for losses during hydrolysis. Homoserine was determined after treatment of the sample with the dilute 0.1 N NH_4OH. The assumed number of residues is given in parentheses. The values for lysine include 0.5–1 residue of methyllysine present in the calf thymus F3 histone and in the calf thymus F3 histone cyanogen bromide peptide CNBr-1 and CNBr-1A. Cysteic acid and methionine sulfone were determined after performic acid oxidation. A molecular weight of 16,435 was calculated for F3 histone from the assumed composition. A molecular weight of 11,615 was calculated for peptide CNBr-1.

the residues of CNBr-1 and CNBr-2 equals that of CNBr-1A, and that the same acetylated and phosphorylated thermolysin peptides were detected in peptide CNBr-1A (Marzluff and McCarty, 1972b) as in the whole F3 histone and peptide CNBr-1, indicated that peptide CNBr-1A was a partial digestion product composed of CNBr-1 and CNBr-2. The low yield of this peptide (20–30%) also supported the interpretation that the CNBr-1A peptide was a partial digestion product.

Fraction II (Figure 2A) contained a single major component, peptide CNBr-1. On rechromatography on Sephadex G-100, peptide CNBr-1 was obtained electrophoretically pure. The CNBr-1 peptide was obtained in a 75% yield.

The cyanogen bromide peptides CNBr-2 and CNBr-3 were partially resolved by chromatography on Sephadex G-50 (Figure 2B) and were then purified by rechromatography on Sephadex G-50 and Sephadex G-25.

The amino acid compositions of the cyanogen bromide peptides are given in Table I. CNBr-1, -2, and -3 accounted for all the amino acids in the protein. The absence of homoserine in the CNBr-3 peptide and its presence in both CNBr-1 and CNBr-2 peptides indicated that the CNBr-3 peptide was the carboxy terminal. The amino acid composition of peptide CNBr-1A was consistent with its being composed of CNBr-1 and CNBr-2. CNBr-1A contained slightly more than one residue of homoserine per molecule and only traces of methionine. Traces of methyllysine (about one residue per molecule) were found in peptides CNBr-1 and -1A but none was found in either CNBr-2 or CNBr-3.

Occurrence of Half-Cystine in the Cyanogen Bromide Peptides. Chemical analysis showed that carboxymethylcysteine was present in both peptides CNBr-1A and CNBr-2, although the yields were somewhat less than the expected two residues per molecule, Table I. No carboxymethylcysteine was found in either CNBr-1 or CNBr-3.

To further define the location of the half-cystine residues, F3 histone was reduced and carboxymethylated with [^{14}C]-iodoacetate. The histone was digested with cyanogen bromide, and the peptides were separated on Sephadex G-100. The radioactivity was associated only with peptides CNBr-1A and CNBr-2 (Figure 3). The CNBr-2 and CNBr-3 peptides were resolved as in Figure 2B to confirm that all of the radioactivity as [^{14}C]carboxymethylcysteine was in the CNBr-2 peptide. The yield of peptide CNBr-1A was 30% and 30% of the radioactivity was associated with this peptide. The rest of the radioactivity was associated with peptide CNBr-2. Thus both peptides, CNBr-1A and CNBr-2, contained the same amount of half-cystine per molecule.

Occurrence of ε-N-Acetyllysine in Histone F3 Cyanogen Bromide Peptides. When calf thymus nuclei were isolated and incubated with [^{14}C]acetate, [^{14}C]acetyl groups were incorporated into the arginine-rich F3 and F2al histones. In agreement with Vidali *et al.* (1968) all the radioactivity was present as ε-*N*-acetyllysine in the F3 histone. When the [^{14}C]acetate-labeled, carboxymethylated histone F3 was digested with cyanogen bromide, all the radioactivity was associated with peptides CNBr-1 and CNBr-1A (Figure 4). It is important to

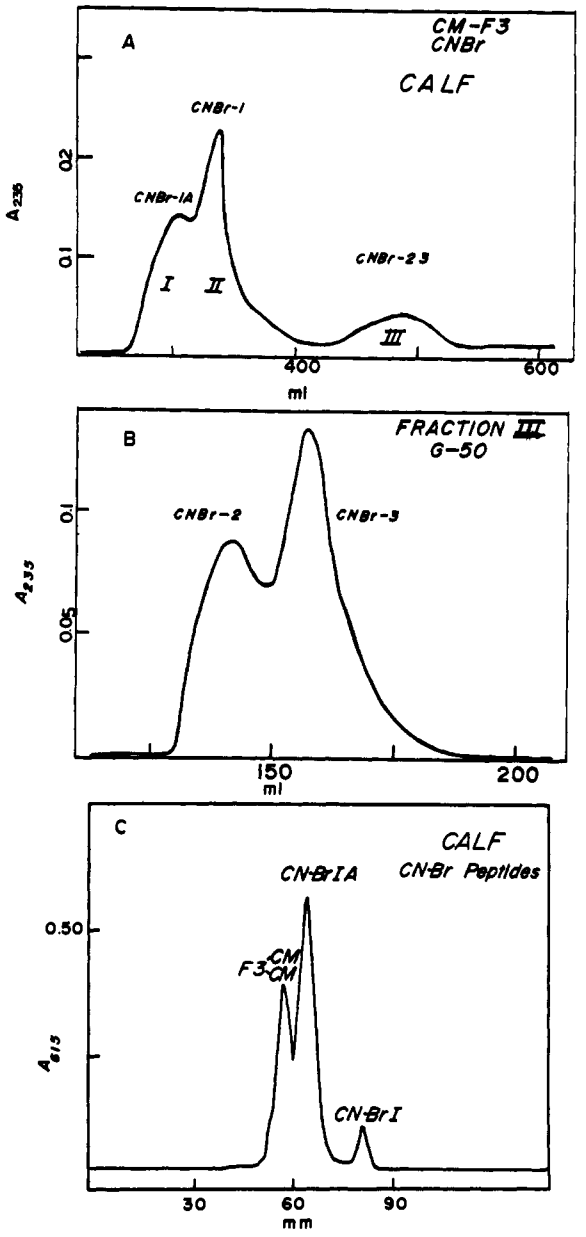

FIGURE 2: Fractionation of calf thymus F3 histone cyanogen bromide peptides. Calf thymus F3 histone (2 μmoles) was dissolved in 70% formic acid and digested with cyanogen bromide. A. Fractionation of the peptides by gel filtration on Sephadex G-100 equilibrated with 0.01 N HCl. The column was 3.5 × 100 cm and the flow was 16 ml/hr. Fractions (4 ml) were collected and pooled as indicated in the figure. B. The low molecular weight peptides of fraction III, CNBr-2 and CNBr-3, were further purified by chromatography on Sephadex G-50, equilibrated with 0.1 N acetic acid. The column was 2.4 × 100 cm, and the effluent flow rate was 12 ml/hr. Fractions (3 ml) were collected and pooled as indicated. As judged by paper chromatography, pure peptides CNBr-2 and CNBr-3 were obtained from the leading and trailing edges of the two peaks. C. The electrophoretic analysis of CNBr-1A. As shown peptide CNBr-1A had the same electrophoretic mobility as carboxymethylated F3 histone when analyzed by polyacrylamide gel electrophoresis at pH 4.3 for 8 hr as described under Methods.

note that when these peptides were analyzed by polyacrylamide gel electrophoresis (Figure 4B,C) the radioactivity migrated slightly slower than the protein, indicating that only a small proportion of the molecules were acetylated. A similar result has been found previously for the mouse mammary gland histones (Marzluff and McCarty, 1970). That the specific activities of the two peptides were the same is consistent with the fact that the CNBr-1A peptide represents a partial digestion product. A large proportion (about 50%) of the radioactivity migrated slower on electrophoresis, however, than one would expect for a reduction of single positive charge in peptide CNBr-1 (Figure 4C); note the double partially resolved radioactive peak, which is consistent with the possibil-

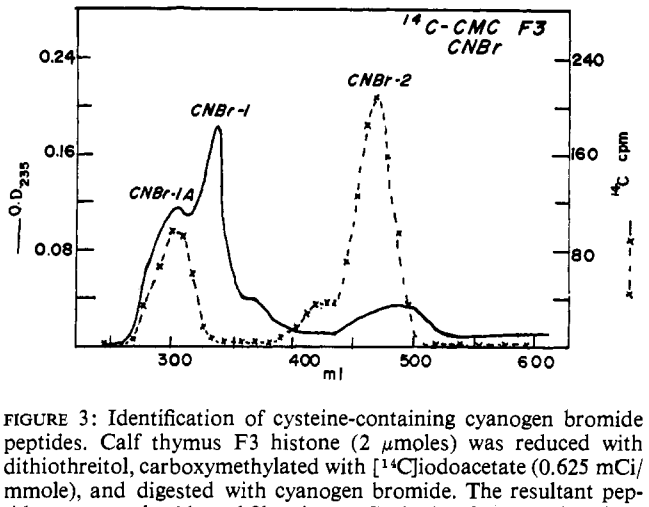

FIGURE 3: Identification of cysteine-containing cyanogen bromide peptides. Calf thymus F3 histone (2 μmoles) was reduced with dithiothreitol, carboxymethylated with [^{14}C]iodoacetate (0.625 mCi/mmole), and digested with cyanogen bromide. The resultant peptides were resolved by gel filtration on Sephadex G-100 as described in the legend to Figure 2A. Radioactivity was determined as described in the Methods.

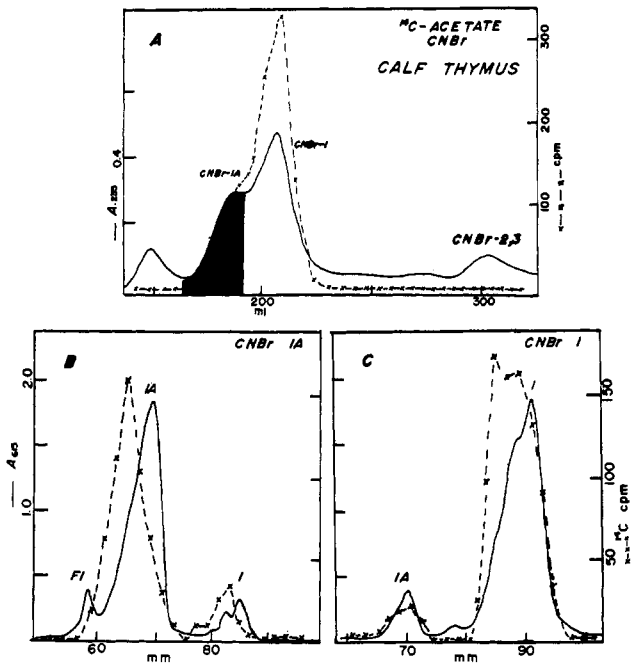

FIGURE 4: The resolution of ^{14}C-acetylated calf thymus F3 histone cyanogen bromide peptides. A. ^{14}C-Acetylated calf thymus F3 histone (1 mole, specific activity of 10,000 cpm/mg) was prepared as described under Methods and digested with cyanogen bromide. The peptides were resolved by chromatography on Sephadex G-75 equilibrated with 0.01 N HCl. The column was 2.4 × 160 cm, and the flow was 12 ml/hr. Each fraction was analyzed by polyacrylamide gel electrophoresis for 8 hr at a pH of 4.3. B. The electrophoresis of fraction I containing peptide CNBr-1A with histone F1 as a marker protein. C. Electrophoresis of fraction II containing peptide CNBr-1. Radioactivity was determined in these fractions as described in the Methods.

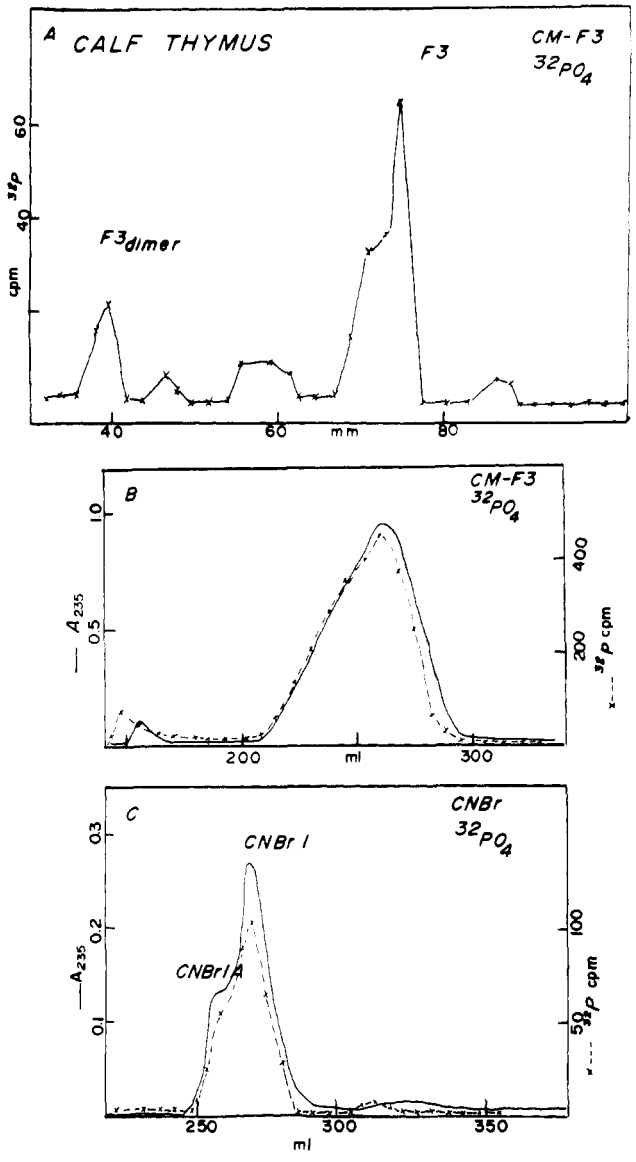

FIGURE 5: The association of $^{32}PO_4$ with calf thymus histone F3. The F3 histone was purified from nuclei which had been incubated for 30 min with $^{32}PO_4$. A. The histone (specific activity of 3000 cpm/mg) was analyzed by polyacrylamide gel electrophoresis for 8 hr at a pH of 4.3. Since the specific activity of the protein was low, a relatively large amount of protein (50 μg) was applied to the gel. The gel was sliced into 2-mm fractions which were dried on filter paper and counted in 5 ml of a toluene base scintillation fluid. The positions of the dimer and monomer forms of the F3 histone are indicated. The radioactivity was clearly associated with the F3 histone. B. The histone F3 was reduced with 0.01 M dithiothreitol and carboxymethylated with iodoacetate. It was further purified by chromatography on Sephadex G-100, and all of the radioactivity was associated with the F3 histone under these conditions; 2 μmoles of the histone was applied to the column. C. Resolution of $^{32}PO_4$-labeled calf thymus F3 histone cyanogen bromide peptides. An aliquot of 0.75 μmole of ^{32}P-labeled F3 histone was digested with cyanogen bromide, and the peptides were then resolved by gel filtration on G-100 as in B. No radioactivity was detected in cyanogen bromide peptides 2 or 3.

ity that some of the molecules were modified in more than one lysine residue.

Occurrence of Phosphoserine in the F3 Histone. The occurrence of ϵ-*N*-acetyllysine in calf thymus histone F3 had been reported earlier (Vidali *et al.*, 1968). When isolated nuclei were incubated with $^{32}PO_4$ and the F3 histone was purified, substantial amounts of $^{32}PO_4$ were associated with the F3 histone as detected by polyacrylamide gel electrophoresis (Figure 5A) and Sephadex gel filtration (Figure 5B). Carboxymethylated F3 histone labeled with $^{32}PO_4$ was hydrolyzed for 8 hr in 6 N HCl, and the products were analyzed by electrophoresis at pH 1.9 (Kabat, 1971). Only two radioactive spots were present, one identical with phosphoserine and one with orthophosphate.

Occurrence of Phosphoserine in F3 Histone Cyanogen Bromide Peptides. Carboxymethylated F3 histone was labeled with $^{32}PO_4$ and then digested with cyanogen bromide. These peptides were resolved by gel filtration on Sephadex G-100. More than 90% of the radioactivity was present in peptides CNBr-1A and CNBr-1 when resolved as shown in Figure 5C. In addition all the modified amino acids (methyllysine, phosphoserine, and ϵ-*N*-acetyllysine) detected by these techniques have also been shown to be present in the CNBr-1 peptide. This large peptide consists of about 90 residues and is almost equal in size to the intact F2a1 histone. However, it was considerably more basic than F2a1 histone as judged by its greater migration rate on polyacrylamide gel electrophoresis.

Discussion

The F3 histone isolated from calf thymus represents a basic protein with an asymmetic distribution of its basic amino acid residues confined to the amino terminal portion of the molecule.

Only three peptides are obtained on digestion with cyanogen bromide since there are only two methionine residues in the molecule. The largest peptide is composed of 90 amino acid residues and represents the basic portion of the molecule that is most likely involved in DNA binding or association. The intermediate peptide was composed of 30 amino acid residues and contained all the half-cystine. The smallest peptide composed of 15 amino acid residues represents the carboxyl amino acid terminal, which was completely devoid of aromatic amino acids. Although the CNBr-3 peptide had one lysine residue, it is not acetylated. The CNBr-2 peptide has one lysine residue which was not acetylated. The single serine residue in this peptide was not phosphorylated.

Only the large cyanogen bromide peptide contains amino acid modifications induced by acetylation, phosphorylation, or methylation and represents the most probable candidate for binding to the DNA.

Further analysis of the large cyanogen bromide peptide CNBr-1 has been analyzed by thermolysin digestion, and these results are presented in an accompanying paper (Marzluff and McCarty, 1972b).

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Structural Studies of Calf Thymus F3 Histone. II. Occurrence of Phosphoserine and ε-*N*-Acetyllysine in Thermolysin Peptides†

William F. Marzluff, Jr.,‡ and Kenneth S. McCarty*

ABSTRACT: Thermolysin digestion of a large cyanogen bromide peptide (CNBr-1) from calf thymus F3 histone yields three peptides which contain ε-*N*-acetyllysine and two peptides which contain phosphoserine, one of which is also acetylated. Only a small proportion of the purified peptides were modified with either acetate (<5–33%) or phosphate (20–22%). One peptide was obtained with 22% in the phosphorylated form, 33% in the acetylated form, and 45% in the unmodified form. There was no evidence of doubly modified peptides. These experiments establish that extensive microheterogeneity exists in F3 histones in that region of the molecule which contained few aliphatic amino acids.

The present study is designed to partially characterize some of the modified amino acids in the F3 histone from calf thymus. Only the cyanogen bromide peptides containing the modified amino acids have been examined.

Methods

Labeling of F3 histone with [^{14}C]acetate and [^{32}P]phosphate, its purification, analysis by acrylamide gel and paper electrophoresis, chromatography, amino acid analysis, and preparation of cyanogen bromide peptides, CNBr-1 and CNBr-1A, have been described previously, Marzluff and McCarty (1972a).

Thermolysin Digestion. Samples of either carboxymethylated F3 histone or the fractionated cyanogen bromide peptides were dissolved in deionized water (2–4 mg/ml). The pH was adjusted to 8.0 with 0.1 N NaOH, and 0.1 volume of 25 mM $CaCl_2$ was added. Thermolysin was added as a freshly prepared solution (2 mg/ml in 25 mM $CaCl_2$) at a final concentration of 2:100 by weight. The pH was maintained between 7.5 and 8.0 for 3.5 hr, after which the pH was adjusted to 4 with 30% acetic acid, and the solution was lyophilized.

Paper Chromatography and Electrophoresis. Paper chromatography was performed as described by DeLange *et al.* (1969a). Solvent systems A–D were butanol–acetic acid–water: A, 200:30:75; B, 100:60:75; C, 90:60:75; D, 70:60:75. Solvent system E was pyridine–butanol–acetic acid–water in proportions 15:10:3:12. Electrophoresis at pH 1.9 was performed in 8.7% acetic acid–2.5% formic acid. Electrophoresis was at 1000 V for 100 min.

Full size (43 × 57 cm) Whatman No. 3M paper was used. The papers were scalloped at the bottom edge to insure an even solvent flow. In preparative runs the sample was applied across the width of the paper. An edge of the sample was cut and sprayed with ninhydrin for identification and cut into 1-cm pieces for scintillation counting. Efficiency for ^{14}C under these conditions was about 40%. The strips containing the peptides were cut out, hung in the opposite direction in a chromatography tank, and eluted with 30% acetic acid for 12–18 hr. A volume of 0.5–1.0 ml was collected in a test tube, diluted, and lyophilized. The yield of this procedure varied from 50 to 70% as judged by recovery of the radioactivity.

Results

Digestion of Cyanogen Bromide Peptides from Calf Thymus F3 Histones. Carboxymethylated F3 histone labeled with either [^{14}C]acetate or inorganic $^{32}PO_4$ was digested with thermolysin, and the peptides were resolved by gel filtration on Sephadex G-25. Ten per cent of alternate fractions were assayed for radioactivity, and the radioactive fractions pooled as shown (Figure 1A,B). The same radioactive peptides were obtained whether one digested the whole molecule or isolated peptides CNBr-1 or CNBr-1A with no evidence of label in CNBr-2 or CNBr-3 (Marzluff and McCarty, 1972b). The radioactive profiles of the acetylated peptides differ in size distribution from those peptides that are phosphorylated. For example, the thermolysin peptide fractions from 128–139 ml, designated as fraction I (for analysis see Figure 2), were phosphorylated (Figure 1B) but not acetylated (Figure 1A), whereas the thermolysin peptide fractions from 158–169 ml, designated as fraction IV, were acetylated but not phosphorylated.

† From the Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27706. *Received July 19, 1971.* This work was supported in part by Grant 5R01-GM12805-07 from National Institutes of Health.
‡ Supported in part by Predoctoral Traineeship GM00233 from U. S. Public Health Service. Present address: Department of Biology, Johns Hopkins University, Baltimore, Md.