Variation in Primary Structure at a Phosphorylation Site in Lysine-rich Histones

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SUMMARY

A lysine-rich histone fraction (Fraction 3) from rabbit thymus has a diminished capacity to accept phosphate transferred by a liver histone kinase preparation. Certain lysine-rich histone components from other species are also not readily phosphorylated. Examination of tryptic peptides of individual rabbit thymus lysine-rich histone fractions shows that Fraction 4, a readily phosphorylated fraction, contains serine in a location previously shown to be a major site of lysine-rich histone phosphorylation, while Fraction 3 contains alanine in this position. Fraction 3 and Fraction 4 of rabbit thymus lysine-rich histone therefore differ in primary structure by virtue of an alanine-serine replacement which determines the presence or absence of a major site of enzymatic phosphorylation.

The sequence of the first 72 amino acid residues of a lysine-rich histone fraction (Fraction 3) from rabbit thymus has been determined by Hall and Cole (1). In this region of the histone, a sequence occurs which closely resembles a phosphopeptide isolated from enzymatically phosphorylated calf thymus lysine-rich histone (2). The phosphopeptide contains a serine residue which is a major site of lysine-rich histone phosphorylation in vitro and in vivo (2, 3). However, the sequence in Fraction 3 of rabbit thymus lysine-rich histone differs from the isolated phosphopeptide in that an alanine replaces the phosphorylated serine residue. This finding led us to examine the phosphorylation of individual components of lysine-rich histones, and also to determine the amino acid sequence of the corresponding region in one other fraction of rabbit thymus lysine-rich histone. The results, reported in this communication, show a difference in the primary structure of two components of rabbit thymus lysine-rich histone. They also show that the ability of lysine-rich histone components to undergo phosphorylation by preparations of liver histone kinase depends to a great extent on the presence of a serine residue at a particular location in the histone molecule.

The total complement of lysine-rich histones was prepared from rabbit thymus as described by Bustin and Cole (4) and from rat and calf thymus by Method 1 of Johns (5). Several preparations from rabbit thymus were also made by the Johns method. Enzymatic phosphorylation of lysine-rich histones by a partially purified histone kinase from calf liver (6) was carried out as previously described (7), except that reaction mixtures contained 2.5 μM cyclic adenosine 3',5'-monophosphate and β, γ-labeled ATP with a specific activity of 2.5 to 7.5 × 10⁶ cpm per amole of β, γ-phosphate. Incubation was continued for 2 hours at 37°C, at which time phosphate transfer to the histone was near completion. The phosphorylated histone was extracted from the incubation mixtures with 5% trichloroacetic acid, and isolated by repeated precipitation with 25% trichloroacetic acid and washing with acidified ethanol-ether (7). The dry histone was dissolved in water and a small amount of insoluble material was removed by centrifugation.

The enzymatically phosphorylated lysine-rich histones were fractionated by the system of ion exchange chromatography described previously (4, 8). The patterns of resolved lysine-rich histone components (Fig. 1) are similar to those observed earlier (4, 8, 9) and are numbered accordingly. The fractions designated H consist of nonhistone contaminants and partially degraded histone. Fig. 1A shows that the phosphorylation of rabbit thymus lysine-rich histone occurs predominantly in Fractions 1, 2, and 4, whereas Fraction 3 incorporates little phosphate. A number of other lysine-rich histone fractions are also not readily phosphorylated by the enzyme, e.g. Fraction 0 from rabbit thymus (Fig. 1A) and rat thymus Fractions 1 and 2 (Fig. 1B). Also, Fraction 3 from calf thymus incorporates a relatively low amount of phosphate (Fig. 1C). Since the latter fraction contains two components (8), the reduced incorporation suggests that only one of these is phosphorylated by the enzyme. Preferential phosphorylation of different electrophoretic fractions of rat thymus lysine-rich histone in isolated thymus nuclei has also been observed (10).

Phosphorylation of individual fractions of lysine-rich histones, previously isolated by preparative scale ion exchange chromatography (4), was also studied. In these experiments, a very large excess of enzyme was used (a total of 0.2 unit per mg of histone as compared to approximately 0.02 unit per mg for the phosphorylation of unfractionated histones), and the time course of phosphorylation was followed in order to determine the completeness of the reaction. As shown in Fig. 2, the phosphate acceptor capacity of rabbit thymus Fraction 3 is much reduced compared to Fraction 4 and to unfractionated rabbit thymus lysine-rich histone. In similar experiments, the capacity of rat
Fig. 1. Ion exchange chromatography of enzymatically phosphorylated lysine-rich histones derived from various tissues. A, rabbit thymus. Three milligrams of 32P-labeled phosphorylated histone were applied to a column (0.9 X 46 cm) of Amberlite IRC-50 (Bio-Rex 70, 200 to 325 mesh range, Bio-Rad, Richmond, California) and the column was eluted with a linear gradient of 7 to 14% guanidine hydrochloride in 0.1 M sodium phosphate, pH 6.8 (total gradient volume, 460 ml) at a flow rate of 1.8 ml per hour. Fractions were collected at 30-min intervals. Histone protein thymus Fractions 1 and 2 was found to be 20 nmoles of phosphate per mg of histone as compared to 46 nmoles per mg for rat thymus Fraction 4.

The reduced phosphate acceptor capacity of rabbit thymus Fraction 3 is consistent with the finding noted above (1), that this histone contains alanine in place of the serine identified as a major phosphorylation site in calf thymus lysine-rich histone. To test whether a readily phosphorylated rabbit thymus lysine-rich histone fraction contains serine in this position, the amino acid sequence in the corresponding region of Fraction 4 was determined. A sample of this histone fraction was cleaved with N-bromosuccinimide as described by Bustin and Cole (11). The NH2-terminal fragment, N*, was isolated by chromatography on Sephadex G-100 (11) and digested with trypsin (previously treated with L-1-p-toluenesulfonylamiido-2-phenylethyl chloromethyl ketone) in a pH-stat at pH 8.0. Digestion was maintained for 8 hours with two equal additions of trypsin (1:100, enzyme to substrate by weight) at 0 and 2 hours. The digest was chromatographed on a column (2.0 X 190 cm) of Sephadex G-25 (fine beads) at a flow rate of 30 ml per hour, eluting with 0.02 N HCl. A peptide which eluted at 350 ml was separated was monitored by absorbancy at 218 mp, and the readings corrected for absorption due to guanidine hydrochloride. 32P was measured in a low background G-M counter. B, rat thymus. Phosphorylated histone (4.5 mg) was chromatographed as above at a flow rate of 2.2 ml per hour. C, calf thymus. Phosphorylated histone (0.6 mg) plus 2.5 mg of non-phosphorylated carrier histone was chromatographed as above on a column (0.9 X 28 cm) at a flow rate of 1.8 ml per hour. The total volume of the gradient was 250 ml.

Fig. 2. Extent of phosphorylation of rabbit thymus lysine-rich histone fractions in the presence of excess histone kinase. The reaction mixtures (0.25 ml) contained 50 mM Tris-HCl, pH 7.5, 5 mM MgCl2, 1 mM dithiothreitol, 10 mM cyclic adenosine 3',5'-monophosphate, 0.25 mg of lysine-rich histone, 0.04 units of liver histone kinase (5), and 0.5 mM ATP, specific activity 0.5 to 1 X 106 cpm per pmole of β,γ-phosphate. The mixtures were incubated at 37° and aliquots of 25 µl were taken at the indicated times for determination of acid-insoluble 32P (5). At 120 min, 0.193 unit of fresh enzyme was added from a minor contaminant by high voltage paper electrophoresis at pH 6.4 and found to have a composition shown below. For comparison, the composition of the analogous peptide from rabbit thymus Fraction 3, isolated by a similar procedure, is also shown.
From Fraction 3: Lys 1.3 (1), Thr 0.8 (1), Ser 1.0 (1), Glu 0.9 (1),
Pro 1.0 (2), Gly 1.1 (1), Ala 2.1 (2), Val 0.9 (1),
Ile 0.8 (1), Leu 1.0 (1)

From Fraction 4: Lys 1.1 (1), Thr 0.9 (1), Ser 1.8 (2), Gly 1.1 (1),
Pro 1.0 (2), Gly 1.0 (1), Ala 1.2 (1), Val 1.0 (1),
Ile 0.8 (1), Leu 0.8 (1)

The compositions of the peptides differ only in the number of serine and alanine residues present.

Each peptide was digested with leucine amino peptidase (kindly supplied by Dr. F. H. Carpenter, University of California, Berkeley) for 3 hours at 40°C in 0.05 M Tris, 0.01 M MgCl₂,

pH 9.0. Digestion of the peptide from Fraction 3 released 1.45 moles of alanine per mole of peptide and no other amino acids. Digestion of the peptide from Fraction 4 released 1.0 residues of alanine and 0.3 residue of serine. These peptides are derived from the same sequence in the histone, since each contains the only isoleucine residue present in the N₂ fragment. The partial sequence of amino acids in this region of Fraction 4 of rabbit thymus lysine-rich histone is therefore:

Ala-Ser-(Thr, Ser, Glu, Pro, Gly, Val, Ile, Leu)₁-Lys

As shown by the above data, the corresponding sequence in Fraction 3 is:

Ala-Ala-(Thr, Ser, Glu, Pro, Gly, Val, Ile, Leu)₁-Lys

Fraction 3 and Fraction 4 therefore differ in primary structure by virtue of an alanine-serine replacement in the 2nd residue of this sequence, which is residue 37 in Fraction 3 of rabbit thymus lysine-rich histone (1). The partial sequence found for this region of Fraction 4 is identical with that previously determined for a phosphopeptide isolated from enzymatically phosphorylated calf thymus lysine-rich histone (2). The alanine-serine replacement occurs at the position in this sequence shown to be a major phosphorylation site in lysine-rich histones, and appears to be the basis for the differences in phosphate acceptor capacities of rabbit thymus F3 and 4. This finding also suggests that the differences observed in the phosphorylation of other lysine-rich histone fractions are due to a similar amino acid replacement. Support for these conclusions was obtained by examination of the phosphopeptides present in tryptic digests of phosphorylated lysine-rich histone fractions. The major phosphopeptide in digests of rabbit thymus F3 and 4 and rat thymus Fraction 4 was identical in electrophoretic and chromatographic behavior with the phosphopeptide previously obtained from calf thymus lysine-rich histone, while most of the phosphate in digests of rabbit thymus Fraction 3 and rat thymus Fractions 1 and 2 was present in other phosphopeptides.

The difference in primary structure between Fraction 3 and Fraction 4 of rabbit thymus lysine-rich histones shows that these histones are distinct molecular species, and not simply phosphorylated or acetylated forms of a single species which differ in chromatographic mobility. In fact phosphorylation has little effect on the chromatographic behavior of lysine-rich histones, as shown by the near coincidence of the protein and radioactivity peak in Fig. 1C, in which a small amount of phosphorylated histone was co-chromatographed with a large amount of non-phosphorylated carrier histone. In addition, the finding that this variation in primary structure determines the presence or absence of a major site of enzymatic phosphorylation indicates that functional differences exist between these species of lysine-rich histone.

REFERENCES


SUMMARY

Oligodeoxyribonucleotides of defined structure have been linked covalently to cellulose. Columns containing the oligonucleotide-celluloses preferentially retain complementary oligodeoxyribo- and oligoribonucleotides. The oligonucleotides can be eluted, conveniently, with a linear temperature gradient. The data indicate that probably the entire oligonucleotide attached to the cellulose is capable of hydrogen bonding with its complementary sequence, and that the resolution obtained with these columns is such that oligonucleotides differing in length by 1 nucleotide residue may be resolved. Preliminary experiments suggest that these oligonucleotide-celluloses are capable of selectively removing a complementary sequence of nucleic acid from a mixture of nucleic acids.

Although methods are available (1) for the isolation of various classes of eucaryotic nucleic acids, techniques for the isolation of mRNA and, in particular, specific mRNAs are poorly developed.
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