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Bovine $H1^0$ Histone Subfractions Contain an Invariant Sequence Which Matches Histone H5 Rather Than H1†

John R. Pehrson and R. David Cole*

ABSTRACT: Histone $H1^0$ differs substantially from H1 in a 37-residue region of the primary structure that is conserved between the usual H1 histones of fish and mammals. Instead, $H1^0$ is homologous to H5 in this region. Histones $H1^0$ and H5 might be classified together in a histone category distinct from the usual H1.

An inverse correlation between levels of $H1^0$ histone and rates of cell division has been established by comparing several mammalian tissues (Panyim & Chalkley, 1969a; Marsh & Fitzgerald, 1973). Recently, we reported that the amount of $H1^0$ in homogenous cell lines increased 3- or 4-fold when cell division was inhibited by high cell density or by serum deprivation (Pehrson & Cole, 1980). Since the synthesis of the major classes of histone is tightly coupled to DNA[1] synthesis (Prescott, 1966), the histone pool of nondividing cells would be expected to remain constant quantitatively and qualitatively. Unlike most histones, however, the accumulation of $H1^0$ in growth-inhibited cell cultures might be accomplished without accompanying DNA synthesis. This situation would be similar to that of histone H5 (Appels & Wells, 1972), which is apparently a variant of H1 unique to mature erythrocytes in birds. In broad terms, histones $H1^0$ and H5 might be considered as related functionally, since the former may suppress replication (Marsh & Fitzgerald, 1973) while the latter seems to suppress transcription (Brasch et al., 1974). This suggested to us that $H1^0$ and H5 might have some structural features in common. In fact, a partial determination of the primary structure, as reported below, has shown bovine $H1^0$ to be strikingly homologous to chicken H5 and rather different from the H1. While this work was in progress, Smith, Walker and Johns reported that a sequence of 17 residues in a particular subfraction of $H1^0$ was quite homologous with the corresponding region in H5 (Smith et al., 1980). Our work not only extends the earlier report by 37 residues but also removes the possibility that microheterogeneity in $H1^0$ includes molecular species very homologous to the usual H1 histones in addition to one homologous to H5.

Smith and Johns recently discovered that, like H1, $H1^0$ is microheterogeneous (Smith & Johns, 1980). Since H5 has been considered to be a variant of H1 (Morris, 1976), it could be postulated that the microheterogeneity in $H1^0$ included molecular species homologous to H1 as well as species homologous to H5. It is conceivable that the particular $H1^0$ subfraction whose sequence was studied by Smith et al. (1980) did not represent the entire population of $H1^0$ molecules. In our studies the sequence determination was made on unfractionated $H1^0$, and since no polymorphism was observed, it is clear that the close homology with H5 is a general characteristic of $H1^0$.

Experimental Procedures

Materials

PMSF, NBS, and Tris-base were obtained from Sigma Chemical Co. Ultra pure urea was from Schwarz/Mann. PCA, hydrochloric acid, trichloroacetic acid, and $NaHSO_3$ were from Malinkrodt, Inc. Acrylamide and bis(acrylamide) were from Bio-Rad Laboratories. Sequenator grade solvents were from Pierce Chemical Co. and Beckman Instruments, Inc.

Methods

Isolation of $H1^0$. Steer kidney cortex was homogenized at 4 °C in 50 mM Tris-base, 50 mM $NaHSO_3$, 5 mM $MgCl_2$, and 250 μm PMSF, pH 7.5, with a Waring blender. The homogenate was centrifuged at 1500*g* for 10 min, the su-

† From the Department of Biochemistry, University of California, Berkeley, California 94720. *Received September 2, 1980.* This work was supported by National Institutes of Health Grants GMS 20338 and NIEHS IT32 ES07075 and by the Agricultural Research Station.

[1] Abbreviations used: DNA, deoxyribonucleic acid; PMSF, phenylmethanesulfonyl fluoride; PCA, perchloric acid; NBS, *N*-bromosuccinimide; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

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Table I: Amino Acid Sequence of the NBS Fragment of Histone H1^0

cycle	residue	means of identification[a]	yield[b] (nmol)	cycle	residue	means of identification[a]	yield[b] (nmol)
1	Lys	a, t	100	21	Gly	g, a	23
2	Val	g, a	100	22	Val	g, a	12
3	Gly	g, a, t	148	23	Leu	g, a	20
4	Glu[c]	a	45	24	Lys	a	16
5	Asn	a, t	98	25	Gln	a, t	13
6	Ala	g, a, t	76	26	Thr	a	6
7	Asp	a, t	66	27	Lys	a	5
8	Ser	g, a	23	28	Gly	a	9
9	Gln	a, t	62	29	Val	a	9
10	Ile	g, a	54	30	Gly	a	9
11	Lys	a, t	26	31	Ala	a	15
12	Leu	g, a, t	56	32	?		
13	Ser	g, a	14	33	Gly	a	7
14	Ile	g, a	31	34	?		
15	Lys	a, t	24	35	Phe	a	5
16	Arg	a		36	Arg	a	
17	Leu	g, a	32	37	Leu	g, a	5
18	Val	g, a, t	21	38	Ala	a	3
19	Thr	g, a	14	39	Lys	a	3
20	Thr	g, a	14				

[a] Means of identification were as follows: g, gas chromatography (Pisano & Bronzert, 1969); a, amino acid analysis (Smithies et al., 1971); t, thin-layer chromatography (Inagami & Murakami, 1972). [b] Yields shown are from amino acid analyses, many of which were checked by gas chromatography. [c] Thin-layer chromatography of the phenylthiohydantoin of residue 4 proved the absence of Gln, but the position for Glu was obscured by artifacts. Amino acid analysis showed Glu unambiguously.

pernatant was discarded, and the crude nuclear pellet was extracted with 0.74 M PCA. The protein was precipitated from the PCA extract by adding trichloroacetic acid to 20% (w/v), and H1^0 was purified from this precipitate by using Bio-Gel P-100 as previously described (Pehrson & Cole, 1980).

NBS Cleavage of H1^0. The purified H1^0 was dissolved in 5% acetic acid at a concentration of 10 mg/mL. NBS was added at a 10-fold molar excess of the tyrosines (Sherod et al., 1974) (assuming two tyrosines per H1^0 molecule), and the reaction was allowed to proceed for 15 min at room temperature.

Molecular Sieve Chromatography of the NBS Peptides of H1^0. The NBS peptide mixture was applied directly to a 2 × 90 cm Bio-Gel P-60 column previously equilibrated in 0.01 N HCl and eluted at 17 mL/h. The appropriate fractions were pooled, lyophilized, and rechromatographed on a 2 × 190 cm Bio-Gel P-100 column. This column was equilibrated and run in 0.01 N HCl at a flow rate of 6 mL/h. Column fractions were monitored by reading their absorbance at 230 nm and by polyacrylamide gel electrophoresis.

Polyacrylamide Gel Electrophoresis. Acetic acid, urea, and polyacrylamide gels were run according to Panyim & Chalkley (1969b). Acrylamide slab gels of 15% were used to monitor column fractions during purification of H1^0. Acrylamide gels of 15% and 20% were used to monitor the column fractions of the NBS peptides.

Sequencing. Edman degradations were performed with a Beckman 890C sequenator and the Beckman *N',N'*-dimethylallylamine peptide program. Acetylated cytochrome *c* (2 mg) was added to the sample during application to prevent losses of the peptide during solvent extractions. Phenylthiohydantoins were analyzed on a Varian 1840 gas chromatograph by using the procedure of Pisano & Bronzert (1969). Some phenylthiohydantoins were identified by thin-layer chromatography; the xylene–2-propanol (70:20 v/v) solvent system of Inagami & Murakami (1972) was used for this purpose. All residues were identified by standard amino acid analysis following hydrolysis of the phenylthiohydantoins to amino acids. The hydrolysis was performed in a desiccator by using HI (Smithies et al., 1971); the desiccator was thoroughly evacuated, then flushed with N_2, evacuated again, and flushed with N_2 2 more times before the final evacuation. It was then autoclaved at 121 °C for 20 h.

Results

Purification of Histone H1^0. Bio-Gel P-100 can be used to resolve H1 and H1^0 (Pehrson & Cole, 1980). When this method was used to purify large amounts of histone (in this case 100–150 mg), it was necessary to run the PCA extraction mixture down the column twice. The first passage gave a large broad peak which was monitored for its H1^0 content by polyacrylamide gel electrophoresis. Fractions containing significant amounts of H1^0 were pooled, lyophilized, and rechromatographed to obtain nearly complete separation of H1 and H1^0 (Pehrson & Cole, 1980). The H1^0 used in our studies was examined on 23 cm long polyacrylamide gels and found to consist of two electrophoretic subfractions as previously described by Smith & Johns (1980). These two subfractions were present in nearly equal amounts.

NBS Cleavage of H1^0 and Purification of NBS Peptide. The purified H1^0 was cleaved at its tyrosine residues with NBS (Sherod et al., 1974). The resulting peptide mixture was chromatographed on Bio-Gel P-60 with the results shown in Figure 1A. The fractions represented by the first peak were examined by polyacrylamide gel electrophoresis and found to contain one major component, a peptide of electrophoretic mobility very similar to that of the COOH-terminal fragment of calf thymus H1 (Sherod et al., 1974). The material of this peak was rechromatographed on Bio-Gel P-100, as shown in Figure 1B, in order to remove contaminating peptides. The fractions of the rechromatographed material that contained a single electrophoretic component (see Figure 1C) were pooled.

The peptide purified from the NBS cleavage products was subjected to automated Edman degradation, and individual residues were identified by direct application of the phenylthiohydantoins to gas chromatography or thin-layer chromatography or by standard amino acid analysis following hydrolysis to amino acids. The results of the sequence analysis are shown in Table I. A total of 37 residues were identified in 39 cycles of automatic degradation. In Figure 2 the H1^0 sequences determined by Smith et al.(1980) and by us are

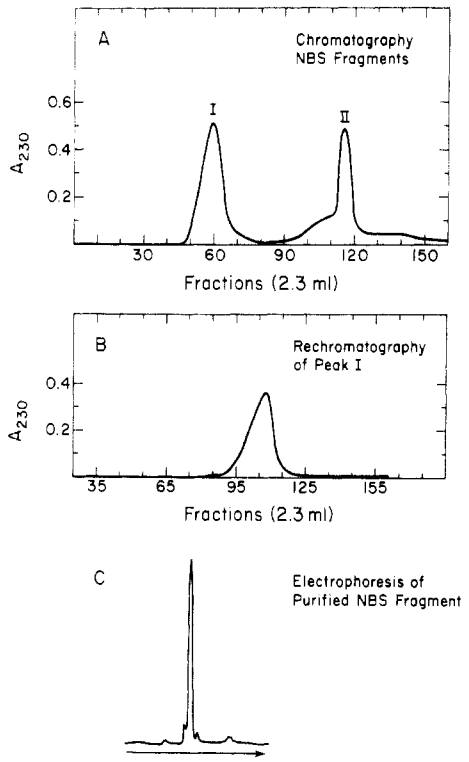

FIGURE 1: Purification of NBS peptide of $H1^0$. (A) Chromatography of the NBS peptides of $H1^0$ on Bio-Gel P-60. (B) Rechromatography of the material contained in peak I on Bio-Gel P-100. (C) Scan of a polyacrylamide electrophoretogram of the purified NBS peptide.

compared to related sequences of H1 and H5 histones. These same data are treated numerically in Table II. Although no difference was counted between Asp and Asn or between Glu and Gln, all other differences were counted, even for such homologous pairs as Val and Ile. The count thus exaggerated differences and underestimated similarities, but it was felt that this was much less apt to be misleading than a counting system that was subject to such ambiguities as the potential homology of Val and Thr or a replacement of a negatively charged side chain by a positive one.

Discussion

Histone $H1^0$ was identified originally as a member of the histone 1 class that normally exists as a family of closely related molecular species (Kinkade & Cole, 1966). Although the quantitative recipe of H1 subfractions varies according to the state of differentiation (Bustin & Cole, 1968; Kinkade, 1969) or embryonic development (Ruderman & Gross, 1974), it is not clear that the subfractions are distinct functionally. However, unlike the case with the usual H1 subfractions, the levels of $H1^0$ in tissues have been associated (by correlation) with a particular physiological process, the inhibition of cell division. If $H1^0$ truly represented other H1 subfractions, then the inverse correlation between its occurrence and mitotic rate would support the notion that H1 subfractions differ among themselves functionally. The data just presented indicate that $H1^0$, although it might still be considered a variant of H1, does not represent the usual subfraction.

Table II: Numerical Treatment of Data in Figure 1

	difference (%)					
comparison	$H1^0$	$H5_{chicken}$	$H5_{goose}$	$H1_{ox}$	$H1_{trout}$	$H1_{ps}$[a]
$H1^0$						
$H5_{chicken}$	20					
$H5_{goose}$	20	10				
$H1_{ox}$	52	56	58			
$H1_{trout}$	54	60	60	13		
$H1_{ps}$[a]	59	54	54	45	48	
$H1_{pa}$[a]	60	59	60	66	64	54

[a] $H1_{ps}$ is H1 from *Psammechinus miliaris*; $H1_{pa}$ is H1 from *Parechinus angulosus*.

Our data and the recent report of Smith et al. (1980) reveal that the amino acid sequence in the central region of $H1^0$ is not very homologous to the corresponding region of H1. By combination of the 17 residues identified in the previous work (Smith et al., 1980) with the 37 residues found in the present study, a major portion of the primary structure has been determined for the central part of the molecule. It must be pointed out that the central nonpolar region of H1 seems to be the most significant part of the molecule for which to make comparisons between variants of H1. Between mammals and fish, as well as among subfractions within one species, the central region (approximately residues 40–115) of H1 is highly conserved in primary structure, suggesting that its amino acid sequence is essential for some biological function that could be thought of as characteristic of H1 subfractions generally. Since this sequence was not found in $H1^0$, this histone probably ought not to be considered a subfraction of H1.

The notion that $H1^0$ ought to be classified separately from the usual subfractions of H1 gathers further strength from another finding in the present work. That is the observation that the amino acid sequence for the 37 residues identified was

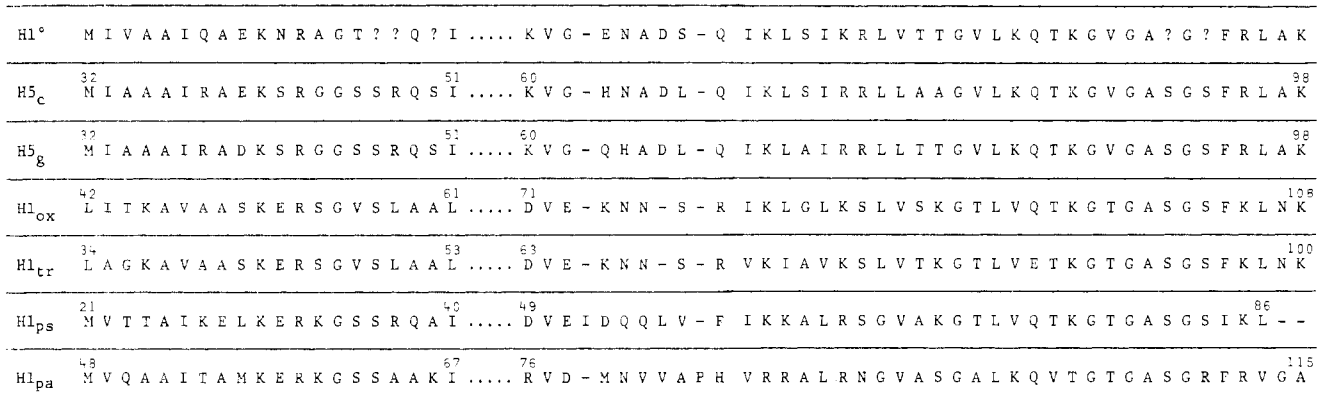

FIGURE 2: Comparison of $H1^0$ sequences to related H1 and H5 sequences. For $H1^0$, the first 17 residues were determined by Smith et al. (1980); the remaining 37 were identified in the present work. The sequences used for comparison were as follows: $H5_c$, chicken erythrocyte H5 (Sautiere et al., 1976; Yaguchi et al., 1979); $H5_g$, goose erythrocyte H5 (Yaguchi et al., 1979); $H1_{ox}$, ox thymus H1 (L. Liao, M. W. Hsiang, and R. D. Cole, unpublished experiments); $H1_{tr}$, trout testis H1 (Macleod et al., 1977); $H1_{ps}$, H1 from *Psammechinus miliaris* (Schaffner, 1978); $H1_{pa}$, H1 from *Parechinus angulosus* sperm (Strickland et al., 1980).

invariant among the subfractions of $H1^0$ known to be present. The conservation of sequence points to a function of $H1^0$ that requires this particular primary structure. Therefore, not only does the sequence differ between H1 and $H1^0$ but also the difference probably matters functionally; each histone seems to have conserved its own distinct structure to preserve its own distinct function.

The third argument favoring the classification of $H1^0$ outside the H1 family of subfractions is that $H1^0$ and H5 are as closely related to each other as they are by sequence in this diagnostic part of the molecule. In structural terms, two categories can be proposed—one containing $H1^0$ and H5 and the other containing H1. The amino acid sequences of ox $H1^0$ and chicken H5 are as nearly matched (80% identities) to each other as ox H1 is to trout H1 (87% identities). In fact, despite the wide evolutionary distance between ox and chicken, the ox $H1^0$ and chicken H5 are much more alike than are ox $H1^0$ and an ox H1 (48% identities). This observation strongly implies a function that is common to histones $H1^0$ and H5 and distinct from that of H1. It must be recognized that the amino acid sequences known for two sea urchin H1 histones do not fit our scheme for the segregation of $H1^0$ and H5 from H1, but the sea urchin sequences are so dissimilar from each other and from those known for other H1 histones that their relationship to histones in higher organisms will probably remain obscure for awhile. In any case, the evolutionary range between fish, fowl, and mammals seems sufficiently broad to validate the notion that the amino acid sequence of the central region of higher histones H1, $H1^0$, and H5 is diagnostic of some functional relatedness. In this regard it is interesting to note that $H1^0$ and H5 are both thought to restrict, directly or indirectly, the accessibility of the DNA template to transcription or replication.

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