A qualitative and quantitative study of subfractions of the histone H1⁰ in various mammalian tissues

Martin R. HARRIS and Bryan J. SMITH

Chester Beatty Laboratories, Institute of Cancer Research, Fulham Road, London SW3 6JB, U.K.

(Received 1 March 1983/Accepted 10 March 1983)

The histone H1⁰ was examined from seven mammalian species. All tissues were shown to contain two subfractions of H1⁰, except for those of rabbit, in which little or no H1⁰ was found. The subfraction composition was compared quantitatively in different mouse and hamster tissues, with the conclusion that this composition is tissue-specific. It is proposed that the wide occurrence of H1⁰, together with the evidence of no more or less than two subfractions wherever it occurs, and the tissue-specific nature of the ratio of subfractions, signify that these two subfractions have specific individual functions.

Heterogeneity amongst histone H1 populations is well known. H1 subfractions exhibit tissue specificity and have been designated as having functional significance (for a review, see Cole, 1977). The nucleated-erythrocyte-specific histone H5 also shows heterogeneity, but in this case, in contrast with H1, the heterogeneity appears not to be significant, since individuals exhibiting only one of the two subtypes appear normal (Greenaway & Murray, 1971). Histone H1⁰ was discovered in 1969 (Panyim & Chalkley, 1969*a*) and has since been found in various mammalian tissues, notably those composed of cells with low rates of proliferation (Panyim & Chalkley, 1969*b*; Balhorn *et al.*, 1972; Marsh & Fitzgerald, 1973). Smith & Johns (1980*a*) found two subfractions of H1⁰ in ox liver, as did later workers studying Chinese-hamster cells and mouse liver (D'Anna *et al.*, 1981; Gjerset *et al.*, 1981). H1⁰ occupies a similar position in chromatin to that of H1 and H5 (Smith & Johns, 1980*b*) and has structural similarities to both of these, especially H5 (Smith *et al.*, 1980*a*; Pehrson & Cole, 1981; Cary *et al.*, 1981; Mura & Stollar, 1981; Allan *et al.*, 1982). Thus the question arises as to whether the subfractions of H1⁰ are of functional significance (like H1) or not, as appears to be the case in H5. In order to test this, the number of subfractions of H1⁰ was examined in several mammalian species and in different strains and organs within species. In addition, the relative amounts of the component subfractions in different mouse and hamster tissues were compared.

Abbreviation used: SDS, sodium dodecyl sulphate.

Experimental

Preparation of protein extracts

Mice and rats were killed by neck dislocation, and hamsters and rabbits by asphyxiation. All organs required were dissected out immediately and stored at -70°C . Bovine and porcine tissues were obtained from local slaughterhouses, transported on solid CO_2 and stored at -70°C . Fractionation of calf brain nuclei was performed as described by Harris *et al.* (1982).

All tissues for part (b) of the Results section were homogenized and extracted four or five times with 0.74 M-HClO₄. Extracted proteins were then precipitated in trichloroacetic acid (final concn. 18%, w/v), redissolved in 0.74 M-HClO₄ and finally precipitated with HCl and acetone (3.5 vol.) (Smith & Johns, 1980*a*).

The survey in part (a) of the Results section includes samples prepared by 0.25 M-HCl extraction of either whole tissue or nuclei, from which proteins were precipitated by the addition of 10 vol. of acetone.

Analysis of protein extracts

Proteins were analysed by electrophoresis on two gel systems: (1) SDS (0.1%, w/v)/polyacrylamide (15%, w/v), pH 6.8, in 0.12 M-glycine: 0.025 M-Tris/0.1% (w/v) SDS buffer; run at 150 V (Laemmli, 1970; these gels were stained in Coomassie Blue R250 (0.1%, w/v); (2) acetic acid/urea (2.5 M)/polyacrylamide (20%, w/v) gels, based on the

Fig. 1. Separation of $H1^0$ subfractions on acid/urea/polyacrylamide gels

Illustrated is a typical acid/urea (2.5 M)/polyacrylamide (20%, w/v) tube gel stained in Procion Navy, showing $H1$ and $H1^0$ from hamster liver. The two subfractions of $H1^0$ are clearly visible as discrete bands.

method of Panyim & Chalkley (1969c). Slab gels were used for part (a) of the Results section, whereas for quantification of $H1^0$ subfractions, very long acid/urea tube gels (45 cm) were used to obtain good resolution between $H1^0a$ and $H1^0b$ (see Fig. 1). These gels were stained overnight in Procion Navy MXRB (0.2%, w/v) (Smith *et al.*, 1980b), and destained electrophoretically. After Coomassie Blue staining minor bands were made visible on some slab gels by use of the silver-staining method of Wray *et al.* (1981). Scanning of tube gels was carried out on a Gilford spectrophotometer at 580 nm.

Treatment of proteins with CNBr was as described by Walker *et al.* (1976).

Results

(a) Survey of the number of $H1^0$ subfractions in different tissues

Tissues from seven mammalian species were examined; these are listed in Table 1. $H1^0$ was found in all these tissues with the exception of rabbit lung and possibly also rabbit liver and kidney. The identity of the protein was confirmed by its solubility in 0.74 M-HClO₄ or 0.25 M-HCl, by co-electrophoresis with bovine liver $H1^0$ on two polyacrylamide-gel

systems (SDS and acid/urea) and finally by its ability to produce peptides on CNBr treatment similar to those generated from bovine $H1^0$ (Smith & Johns, 1980a).

Examination of SDS/polyacrylamide gels containing the rabbit tissue extracts showed only the slightest trace of a band in rabbit liver or kidney which co-electrophoresed with ox liver $H1^0$, and no perceptible band in rabbit lung. The reason for this anomaly of rabbit tissue is unknown. Levels of $H1^0$ accumulate with the age of the animal (Medvedev *et al.*, 1977, 1978), although the protein has been found in young rats and in calves (Bregnard *et al.*, 1979; Varrichio, 1977; Piha & Valkonen, 1979). The rabbits from which the liver and kidney tissues were taken were 18 months old and sexually mature, so that $H1^0$ levels would not be expected to be very low merely because of age. The results may simply reflect a varying requirement for $H1^0$ between species; such a variance is certainly found for the related histone H5 (Miki & Neelin, 1977). A technical explanation of the apparent lack of $H1^0$ in rabbit was considered: namely that minor differences may exist in the composition of rabbit $H1^0$ that either alter its solubility characteristics such that it is not extracted with 0.74 M-HClO₄ or else change its electrophoretic behaviour, causing it to run coincidentally with $H1$. HCl (0.25 M) extracts of rabbit kidney and liver (see the Experimental section) and ethanol (80%, v/v) extracts of rabbit liver by the method of Johns (1964) successfully extracted all core histones (and $H1$ in the case of HCl) but revealed no band in the $H1^0$ position on SDS/polyacrylamide gels. Since treatment of the HClO₄ extract with CNBr revealed no typical peptide, it does not seem possible that the ' $H1^0$ ' species in rabbit runs coincidentally with $H1$, unless the former contains no methionine residue. Such an absence would make it different from all other $H1^0$ species studied here and elsewhere (H5 also typically contains methionine).

(b) Quantification of $H1^0a$ and $H1^0b$

Several tissues were selected from those in Table 1 for a more detailed study of the ratios of the two $H1^0$ subtypes [henceforth called ' $H1^0a$ ' and ' $H1^0b$ ' (Smith & Johns, 1980a)]. This was achieved by analysis of tissue extracts on very long acid/urea tube gels (see Fig. 1). The effects of variability due to preparative and electrophoretic artefacts were decreased by repetition and concurrent statistical tests (analysis of variance). No significant variation was found between different gels of a given sample run together or between batches of gels of a given sample run on different days. Neither was there any significant difference in results from different preparations of the same batch of tissue.

Table 1. *A list of tissues examined for H1⁰ content*

All except the rabbit tissues contained two subtypes of H1⁰ with electrophoretic mobilities, on acid/urea(2.5 M)/polyacrylamide (20%, w/v) gels similar to those of the subtypes in ox liver. Abbreviations used: Unk, unknown; wk(s), week(s); mths, months.

Species	Strain	Tissue	Age	Sex
Cow		Liver	Adult	♂
		Thymus	Immature	Unk
		Brain neuronal nuclei	Immature	Unk
		Brain glial nuclei	Immature	Unk
		Testis	Adult	♂
Mouse	C3H Avy	Liver	30–40 wks	♂ + ♀
	CBA/T6T6	Liver	37–85 wks	♂ + ♀
	C3H Avy	Kidney	30–40 wks	♂ + ♀
	CBA/T6T6	Kidney	37–85 wks	♂ + ♀
	CBA/CA	Liver	7 wk; 12 wk	♂
			26 wk; 40 wk	♀
	CBA/CA	Submaxillary gland	8 wk	Unk
	CBA/CA	Submaxillary gland stimulated with isoproterenol	5 wk	Unk
	Balb/c	Submaxillary gland	7–13 wk	♂
	3T3/TK3	Cultured fibroblasts		
Hamster	Chester Beatty Creams Syrian	Exponential phase		
		Stationary phase		
		Liver	34 wks	♂ + ♀
		Liver	34 wks	♂ + ♀
		Kidney	34 wks	♂ + ♀
Pig	Syrian	Kidney	34 wks	♂ + ♀
		Liver	Adult	Unk
		Thymus	Adult	Unk
Rat	Wistar	Liver	8–11 mths	♂ + ♀
		Kidney	8–11 mths	♂ + ♀
		Heart	8–11 mths	♂ + ♀
		Testis	8–11 mths	♂ + ♀
				♀
Human	HeLa	Cervical carcinoma		♀
Rabbit	New Zealand White	Lung	17 wks	Unk
	New Zealand White × Dutch Lop	Liver	78 wks	♂
	New Zealand White × Dutch Lop	Kidney	78 wks	♂

Two strains of mice (C3H/Avy and CBA/T6T6) were studied, but no difference was found between corresponding tissues in the two strains. Hence the data for the two strains were combined to give an overall value for H1⁰a/H1⁰b in each mouse organ.

Table 2 shows that in both the mice and the hamsters examined, the value for H1⁰a/H1⁰b is higher in the kidney than in their corresponding liver. This difference is highly significant ($P \leq 0.005$) in both cases. Although the values for hamster liver and kidney are in each case higher than for the corresponding mouse organ, the difference between liver and kidney in each species is consistent [(kidney a/b)/(liver a/b) ≈ 1.3]. The differences between mouse kidney and hamster kidney were not significant at $P < 0.05$). Mouse brain was also examined, the H1⁰a/H1⁰b ratio falling between that of liver and that of kidney. The difference between brain and liver was significant to $P = 0.05$, but that

Table 2. *Ratio of H1⁰ subtypes (a:b) in different mouse and hamster tissues as calculated from scans of 45 cm acid-urea (2.5 M)/polyacrylamide (20%, w/v) tube gels* Each gel was scanned at least twice, these values being averaged and then compared with those from other gels.

Species	Strain	Tissue	No. of gels	H1 ⁰ a/H1 ⁰ b (mean $\pm$ s.d.)
Mouse	C3H/Avy and CBA/T6T6	Liver	15	1.19 $\pm$ 0.203
		Kidney	12	1.51 $\pm$ 0.303
		Brain	9	1.39 $\pm$ 0.142
Hamster	Chester Beatty Cream and Syrian	Liver	12	1.29 $\pm$ 0.175
		Kidney	9	1.67 $\pm$ 0.124

between brain and kidney was not: a larger volume of data would be necessary to prove or disprove this small difference.

Discussion

H1⁰ was found in nearly all tissues examined, including those which might be expected to be dividing relatively rapidly, such as calf and pig thymus [the presence of H1⁰ in calf thymus in very small amounts is mentioned also by Smith & Johns (1980b)]. Moreover, in every tissue in which the protein was detected, two subfractions were present with electrophoretic mobilities identical with those originally found in ox liver by Smith & Johns (1980a). Rykowski *et al.* (1981) have shown that, at least in the case of yeast histone H2B variants, ubiquity does not necessarily mean indispensability. However, the widespread occurrence of neither more nor less than two subfractions of H1⁰ argues strongly that both are functionally significant. Homozygous inbred strains of mice are used in the present work, so the implication is that the haploid genome contains two types of H1⁰ gene. An alternative explanation, that H1^a and H1^b are merely modified forms of the same protein, seems unlikely. The two forms of H1⁰ run coincidentally on SDS/polyacrylamide gels, suggesting that the difference between them is one of charge not size. At least two common possible post-synthetic modifications (phosphorylation and ADP-ribosylation) have been excluded for this protein (D'Anna *et al.*, 1980; Smith & Johns, 1980a). Thus it seems likely that the difference between the subfractions is one of primary sequence, as is the case in H5 (Greenaway & Murray, 1971; Krieg *et al.*, 1982).

The tissues examined here contained two subfractions of H1⁰ regardless of their state of proliferation [cf. exponential-phase cultured cells with stationary-phase ones and also normal submaxillary glands with those stimulated to enter the cell cycle by administration of isoproterenol (Baserga, 1966)]. Thus H1⁰ would seem *not* to execute its suggested function of suppression of DNA synthesis by simple qualitative changes in its subfractions. However, the consistent difference in the ratio H1^a/H1^b between liver and kidney in mouse and hamster suggests that H1⁰ may function in whatever role by *quantitative* changes in its subfractions.

Although it is not clear what the subfractions' roles are, it does appear that they do have separate individual functions, and in this way H1⁰ is like H1, but differs from H5.

We thank Dr. E. W. Johns for his support. This work was funded by Cancer Research Campaign/Medical Research Council grant no. 7601207.

References

- Allan, J., Smith, B. J., Dunn, B. & Bustin, M. (1982) *J. Biol. Chem.* **257**, 10533–10535
- Balhorn, R., Chalkley, R. & Granner, D. (1972) *Biochemistry* **11**, 1094–1098
- Baserga, R. (1966) *Life Sci.* **5**, 2033–2039
- Bregnard, A., Ruch, F., Lutz, H. & Kuenzle, C. C. (1979) *Histochemistry* **61**, 271–279
- Cary, P. D., Hines, M. L., Bradbury, E. M., Smith, B. J. & Johns, E. W. (1981) *Eur. J. Biochem.* **120**, 371–377
- Cole, R. D. (1977) *The Molecular Biology of the Mammalian Genetic Apparatus* (Ts'o, P., ed.), Elsevier/North-Holland Biomedical Press, Amsterdam
- D'Anna, J. A., Gurley, L. R., Becker, R. R., Barham, S. S., Tobey, R. A. & Walters, R. A. (1980) *Biochemistry* **19**, 4331–4341
- D'Anna, J. A., Gurley, L. R. & Becker, R. R. (1981) *Biochemistry* **20**, 4501–4505
- Gjerset, R., Ibarrando, F., Saragosti, S. & Eisen, H. (1981) *Biochem. Biophys. Res. Commun.* **99**, 349–357
- Greenaway, P. J. & Murray, K. (1971) *Nature (London)* **229**, 233–238
- Harris, M. R., Harborne, N., Smith, B. J. & Allan, J. (1982) *Biochem. Biophys. Res. Commun.* **109**, 78–82
- Johns, E. W. (1964) *Biochem. J.* **92**, 55–59
- Krieg, P. A., Robins, A. J., Colman, A. & Wells, J. R. E. (1982) *Nucleic Acids Res.* **10**, 6777–6785
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Marsh, W. H. & Fitzgerald, P. J. (1973) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 2119–2125
- Medvedev, Zh. A., Medvedeva, M. N. & Huschtscha, L. I. (1977) *Gerontology* **23**, 334–341
- Medvedev, Zh. A., Medvedeva, M. N. & Robson, L. (1978) *Gerontology* **24**, 286–292
- Miki, B. L. A. & Neelin, J. M. (1977) *Can. J. Biochem.* **55**, 1220–1227
- Mura, C. V. & Stollar, B. D. (1981) *J. Biol. Chem.* **256**, 9767–9769
- Panyim, S. & Chalkley, R. (1969a) *Biochem. Biophys. Res. Commun.* **37**, 1042–1049
- Panyim, S. & Chalkley, R. (1969b) *Biochemistry* **8**, 3972–3979
- Panyim, S. & Chalkley, R. (1969c) *Arch. Biochem. Biophys.* **130**, 337–346
- Pehrson, J. R. & Cole, R. D. (1981) *Biochemistry* **20**, 2298–2301
- Piha, R. S. & Valkonen, K. H. (1979) *FEBS Lett.* **108**, 326–330
- Rykowski, M. C., Wallis, J. W., Choe, J. & Grunstein, M. (1981) *Cell* **25**, 477–487
- Smith, B. J. & Johns, E. W. (1980a) *FEBS Lett.* **110**, 25–29
- Smith, B. J. & Johns, E. W. (1980b) *Nucleic Acids Res.* **8**, 6069–6079
- Smith, B. J., Walker, J. M. & Johns, E. W. (1980a) *FEBS Lett.* **112**, 42–44
- Smith, B. J., Toogood, C. I. A. & Johns, E. W. (1980b) *J. Chromatogr.* **200**, 200–205
- Varrichio, F. (1977) *Arch. Biochem. Biophys.* **179**, 715–717
- Walker, J. M., Goodwin, G. H. & Johns, E. W. (1976) *Eur. J. Biochem.* **62**, 461–469
- Wray, W., Bouliskas, T., Wray, V. P. & Hancock, R. (1981) *Anal. Biochem.* **118**, 197–203