# THE PROPERTIES OF SALT-EXTRACTED HISTONE H1

S. H. BRAND, N. M. KUMAR and I. O. WALKER
*Department of Biochemistry, South Parks Road, Oxford, OX1 3QU, England*

Received 13 July 1981

## 1. Introduction

The solution properties of the very lysine-rich histones H1 and H5 have been studied with a variety of physical methods [1–4]. In all cases these histones were extracted from chromatin or nuclei by the methods developed by Johns [5] which use strong acids and organic solvents. It is tacitly assumed that the proteins are reversibly denatured during these extraction procedures and that they subsequently regain their original native conformation when placed in aqueous solutions at neutral pH. In the case of the histones this assumption is difficult to test experimentally because there is no sensitive biological assay by which to measure the native character of the protein. One is therefore left with comparing physical parameters of the proteins prepared in different ways. This procedure is not entirely satisfactory because small differences in conformation may not necessarily reflect differences in biological function and vice versa. It would therefore seem prudent, where possible, to avoid extremes of pH and organic solvents during the preparation of the histones since both procedures are known to denature proteins irreversibly.

With this in mind we have examined the physical properties of histone H1 extracted by mild procedures in NaCl at neutral pH. Irreversible changes in the circular dichroism and sedimentation coefficient of H1 occur when it is subjected to acid treatment.

## 2. Experimental

Nuclei were isolated from frozen chicken blood according to [6]. Chromatin was prepared at 4°C by the method in [7]. Histone H1 was prepared by extracting chromatin with 0.5 M NaCl which, under the conditions used here, dissociates H1 and leaves histone H5 and the core histones bound to DNA [8]. The insoluble chromatin was removed by centrifugation at 5000 × *g* for 15 min and the pellet re-extracted 4 times with 0.5 M NaCl. The pooled H1 supernatants were concentrated by ultrafiltration and contaminating chromatin was removed by centrifugation at 110 000 × *g* for 15 h. Gel electrophoresis was done as in [8]. Circular dichroic (CD) spectra were measured at 20°C in a Roussel-Jouan Dicrograph II as in [10]. The results are expressed as mean residue ellipticity $[\theta]$ (degrees . $cm^{-2}$ . $dmol^{-1}$). The mean amino acid residue $M_r$ was taken as 110 and concentrations of H1 were determined from the amino acid analysis. Amino acid analyses were performed on a Durrum D500 analyser after hydrolysing samples for 21 h at 110°C in 6 N HCl, 0.01 M phenol.

Sedimentation coefficients were measured at 20°C in a Beckmann-Spinco analytical ultracentrifuge fitted with an electronic speed control. A photoelectric scanner was used to monitor the boundary at 280 nm using absorption optics. All sedimentation coefficients were corrected to give $s_{20,w}$ using viscosities and densities from the International Critical Tables.

## 3. Results

H1 preparations were pure and undegraded as judged by gel electrophoresis (fig.1 inset). Two bands corresponding to the 2 $M_r$ variants were observed. There were no bands visible in the region corresponding to the core histones or H5. Very faint bands were visible by eye on the gel and on the photograph both above and below the position of H1. These were not detected by the gel scanner and were <3% of the total protein on the gel. All subsequent experiments were carried out on this unfractionated sample. The UV absorption spectrum showed a maximum at

*Published by Elsevier/North-Holland Biomedical Press*
00145793/81/0000–0000/$02.75 

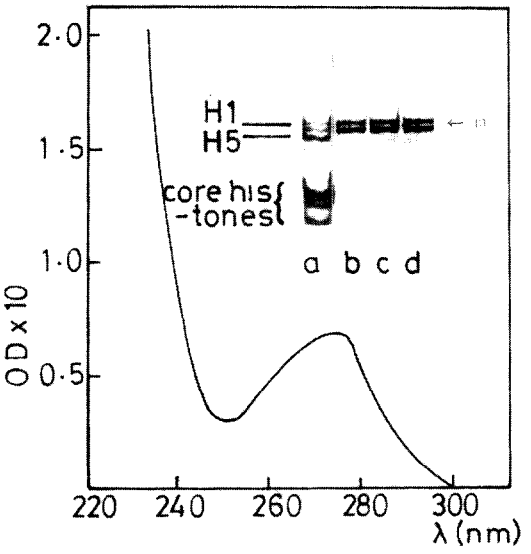


Fig.1. Absorption spectrum of salt-extracted H1 in 0.5 M NaCl, 10 mM Tris (pH 7.0). (Inset) SDS–polyacrylamide gel electrophoresis patterns of chicken erythrocyte H1: (a) chicken erythrocyte chromatin; (b) salt-extracted H1; (c) H1 titrated to pH 2.5, left for 24 h at this pH then titrated to pH 7.0; (d) H1 titrated to pH 11.0, left for 24 h at this pH, then titrated to pH 7.0.

275 nm and a minimum at 252 nm. The shape of the spectrum indicated that the preparations were essentially free of nucleotide (fig.1). CD spectra of 4 different preparations of H1 in 0.5 M NaCl, 10 mM Tris (pH 7.0) gave $[\theta]_{222\ nm}$ = 7290 ± 150 (standard deviation). Histone H1 (0.1 mg/ml) in 0.5 M NaCl (pH 7.0) was titrated with 0.5 N HCl and the CD spectra recorded as a function of pH. As shown in fig.2, $[\theta]_{222\ nm}$ decreased in a sigmoid fashion over pH 7.0–2.5, from 7290–4600. The protein solution was then neutralized by the addition of 0.5 N NaOH. The value of $[\theta]_{222\ nm}$ increased to 5600 with increasing pH, which was 77% of its initial value at pH 7.0. The same result was obtained if the solution was titrated to pH 2.5, left for 24 h at this pH and then titrated to pH 7.0. Gel electrophoresis of H1 after this treatment showed that degradation was not responsible for the irreversible loss of intensity in $[\theta]_{222\ nm}$ (fig.1 inset). After the initial acid treatment the changes in $[\theta]_{222\ nm}$ were reversible with changes in pH. This implies that the initial loss of CD intensity on titration to acid pH is not due to irreversible aggregation or other irreversible changes of a small fraction of the sample and that the changes are representative of the whole protein sample.

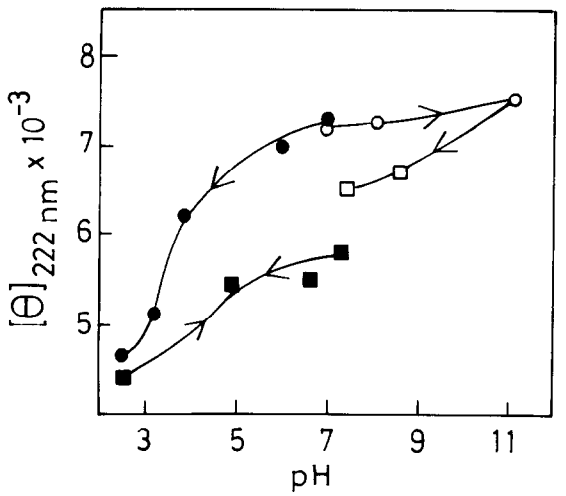


Fig.2. Variation of $[\theta]_{222\ nm}$ with pH; (–●–) forward titration curve to acid pH; (–■–) back titration curve; (–○–) forward titration curve to alkaline pH; (–□–) back titration curve.

H1 in 0.5 M NaCl (pH 7.0) was titrated with 0.5 N NaOH to pH 11.0. There was a 4% increase in $[\theta]_{222\ nm}$. On back titration with 0.5 N HCl to pH 7.2, 7% of the original dichroic intensity at 222 nm had been lost (fig.2). No degradation of H1 occurred after titration (fig.1 insert).

The values of $s_{20,w}$ were determined at 2 mg H1/ml at various NaCl concentrations, in 10 mM Tris–HCl (pH 7.5). In 2 M NaCl $s_{20,w}$ was 2.45 S and remained essentially constant as NaCl was decreased to 0.5 M (fig.3). The value then decreased sharply over 0.2–

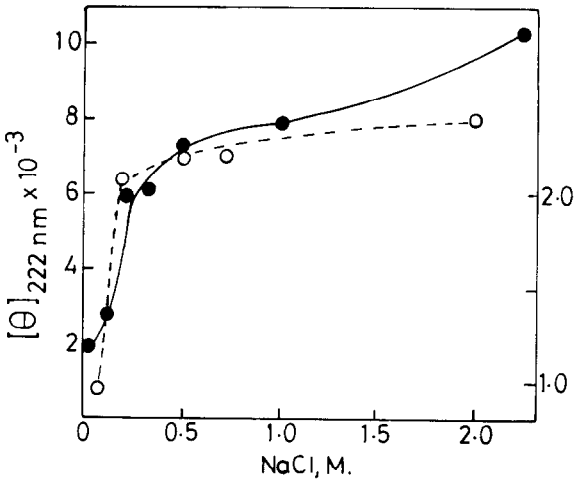


Fig.3. Variation of $[\theta]_{222\ nm}$ and $s_{20,w}$ with [NaCl]: (–●–) $[\theta]_{222\ nm}$; (–○–) $s_{20,w}$.

0.1 M NaCl. When salt extracted H1 in 0.5 M NaCl was titrated to pH 2 with 0.5 N HCl, then readjusted to pH 7.0, $s_{20,w}$ decreased from 2.2 to 1.5 S.

The CD spectrum varied with ionic strength in a manner which was similar to but not identical with the changes in $s_{20,w}$ (fig.3). Values of $[\theta]_{222\ nm}$ decreased slowly over 2.5–0.5 M NaCl but then decreased sharply over 0.2–0.1 M NaCl. These changes were completely reversible over 0.1–0.5 M NaCl provided the alterations in ionic strength were effected by dialysis. Thus the conformation of chicken H1, like mammalian H1, is very dependent on ionic strength [1,3,4]. Large decreases in secondary structure are accompanied by changes in tertiary structure, leading to an unfolding of the molecule as the ionic strength is decreased.

## 4. Discussion

Chicken erythrocyte histone H1 extracted in the presence of salt at neutral pH undergoes similar pH and ionic strength-dependent conformational changes, as monitored by CD and sedimentation coefficient, to other H1 histones isolated by acid extraction [1,2,4]. Chicken H1 isolated under the mild conditions described here appears to have a higher absolute intensity at $[\theta]_{222\ nm}$ relative to calf thymus H1 [1,3,4]. This may reflect an intrinsic difference in structure between chicken and calf H1. Chicken H1 undergoes an irreversible decrease in CD absorption of ~20% after exposure to acid. The absolute intensity that remains then compares well with that estimated for acid-extracted calf thymus H1 [1,3,4]. The loss of structure, as revealed by CD, appears to be due to titration of a group or groups with p$K$ 4.0, possibly glutamic or aspartic acid side chains. There is a smaller, irreversible loss of structure when the protein is titrated to pH 11.0. It was not possible to increase the pH further because at higher pH the protein became insoluble and did not completely dissolve on lowering the pH.

There is a greater loss of CD intensity as the ionic strength is decreased at constant pH (7.0) but unlike the loss incurred at acid pH this change is reversible. Decreasing the ionic strength also results in a decrease in sedimentation coefficient which may be interpreted as reflecting a gross unfolding of the molecule at the level of tertiary structure. The sedimentation coefficient of salt-extracted chicken erythrocyte H1 is significantly greater than values reported for acid extracted calf thymus H1 at high ionic strength. For example, in 1 M NaCl $s_{20,w}$ = 1.2 S for calf thymus H1 [12], compared to a value of 2.2 S obtained here under comparable conditions for chicken H1. However, the value for chicken H1 decreases to 1.5 S after acid treatment which then compares rather better with the value obtained for acid-extracted calf thymus H1. Since both H1 have very similar $M_r$-values this difference in sedimentation properties may reflect a real difference in conformation between chicken and calf H1 at high salt or simply a difference resulting from the method of preparation. If the latter explanation is correct, the salt-extracted H1 has a smaller frictional coefficient and is thus less hydrated, more compact or both, compared to acid-extracted H1. The frictional coefficient is irreversibly increased on acid-denaturation. Another possibility is that the increased sedimentation coefficient reflects a self-aggregation of salt-extracted H1 at high salt, a property not associated with acid-extracted H1 [11]. Again, the self-aggregation process is lost on acid denaturation.

In conclusion, there are irreversible changes in secondary, tertiary and possibly quaternary structure when salt-extracted chicken H1 is subjected to mild acid treatment. These observations, taken together with [13], which showed that there is a difference in the antigenic properties between salt and acid-extracted calf thymus H1, suggest that salt extraction at neutral pH may be a better method for preserving the native structure of the very lysine-rich histones intact.

## Acknowledgement

The authors wish to thank Mrs A. Watson for excellent technical assistance.

## References

[1] Bradbury, E. M., Cary, P. D., Chapman, G. E., Crane Robinson, C., Danby, E. and Rattle, H. W. E. (1975) Eur. J. Biochem. 52, 605–613.
[2] Puigdomenech, P., Cabré, O., Palau, J., Bradbury, E. M. and Crane Robinson, C. (1975) Eur. J. Biochem. 59, 237–243.
[3] Smerdon, M. J. and Isenberg, I. (1976) Biochemistry 15, 4233–4242.
[4] D'Anna, J. A., Strniste, G. F. and Gurley, L. R. (1979) Biochemistry 18, 943–951.

[5] Johns, E. W. (1974) Biochem. J. 92, 55–59.
[6] Shaw, B. R., Herman, T. M., Kovacic, R. T., Beaudrean, G. S. and Van Holde, K. E. (1976) Proc. Natl. Acad. Sci. USA 73, 502–505.
[7] Zubay, G. and Doty, P. (1959) J. Mol. Biol. 1, 1–20.
[8] Kumar, N. M. and Walker, I. O. (1980) Nucleic Acids Res. 8, 3535–3551.
[9] Thomas, J. O. and Kornberg, R. D. (1978) Proc. Natl. Acad. Sci. USA 72, 2626–2630.
[10] Henson, P. and Walker, I. O. (1970) Eur. J. Biochem. 16, 524–531.
[11] Haydon, A. J. and Peacocke, A. R. (1968) Biochem. J. 110, 243–250.
[12] Hartman, P. G., Chapman, G. E., Moss, T. and Bradbury, E. M. (1977) Eur. J. Biochem. 77, 45–51.
[13] Mihalakis, M., Miller, O. J. and Erlanger, B. F. (1976) Science 192, 469–470.