# Octamer reconstitution from acid-extracted chicken erythrocyte histones

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Histone octamers have been reconstituted from acid-extracted chicken erythrocyte histones. By the criteria of molecular size on exclusion chromatography as well as sedimentation velocity and conformational properties established by circular dichroism, fluorescence spectroscopy and imido-ester cross-linking, the reconstituted octamers have a structure identical to that of salt-extracted octamers.

*Histone* *Octamer* *Reconstitution*

## 1. INTRODUCTION

We have shown [1] that acid-extracted chicken histones can be reconstituted to a H3–H4 tetramer and a H2A–H2B dimer by transferring a solution of acid-extracted histones from 2 M NaCl at pH 7.4 to 150 mM NaCl. In the course of this reconstitution in addition to tetramers and dimers, an aggregate of H3 and H4 is found. We have assumed that during this procedure a histone octamer may have formed as an intermediate giving rise to tetramer and dimers on lowering the ionic strength. This suggests a simple approach to reconstituting histone octamers from acid-extracted histones. We have therefore investigated the complexes formed by acid-extracted chicken histones in 2 M NaCl.

## 2. METHODS

Nuclei were isolated from washed chicken erythrocytes by digitonin lysis and crude chromatin subsequently prepared by washing the nuclei with 10 mM citrate, 150 mM NaCl (pH 7.4) until the supernatant was free of proteins ($A < 0.1$) [2]. Native histomer octamer for comparison was prepared by extraction of this chromatin with 2 M NaCl preceded by an initial 0.8 M NaCl extraction to remove H1 and H5 [3]. The 2 M NaCl extract was concentrated by ultrafiltration and chromatographed on a Sepharose 6B column in 2 M NaCl, 10 mM Tris (pH 7.4) to remove any excess H2A–H2B dimer. The octamer peak was pooled and reconcentrated. The preparation of acid-extracted total histones and individual histones was as in [4]. Core histones were extracted with 0.25 M HCl from the chromatin pellet after extraction of H1 and H5 with 5% perchloric acid [5]. The extract containing the core histones was dialysed against 0.25 M HCl to remove perchlorate ions before dialysis against water and lyophilisation. SDS–polyacrylamide gel electrophoresis was carried out on 20% slab gels with a 5% stacking gel [6]. Circular dichroism and fluorescence spectroscopy were as in [1]. Cross-linking with dimethylsuberimidate was done as in [7] in 2 M NaCl, 50 mM phosphate (pH 8) [1]. Sedimentation velocity determinations were carried out on a Beckman Model E ultracentrifuge equipped with Schlieren optics by the meniscus depletion method.

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The buffer used was 2 M NaCl, 10 mM Tris (pH 7.4). Centrifugation was at either 56000 or 16000 rev./min. Sample concentration was 3 mg/ml. Further experimental details are given in the figure legends.

## 3. RESULTS AND DISCUSSION

Total acid-extracted histones were dissolved in 2 M NaCl, 10 mM Tris (pH 7.4) and applied to a Sepharose 6B column equilibrated in this same buffer. Three fractions of material were eluted and found to contain H3 and H4 (fraction A), all the core histones (B) and H2A and H2B (C). H1 and H5 eluted in the latter half of fraction B (fig.1a). By comparison salt-extracted octamer, contaminated with some H2A–H2B dimer [3] was found to elute with the same buffer volume as fraction B (fig.1a). An elution profile identical to fig.1a but lacking the H1 and H5 components could be obtained if acid-extracted core histones were used as the starting material. Reconstitution of octamers, however, only occurred from core histones if the perchlorate anion introduced through the H1 and H5 extraction was removed by dialysis against 0.25 N HCl. The material eluting in peak B using core histones as starting material was pooled, concentrated by ultrafiltration and the sedimentation velocity determined by ultracentrifugation on a model E. Salt-extracted octamer from which H2A–H2B dimer had been removed by Sepharose 6B chromatography was used as a comparison. The sedimentation velocities at 16000 rev./min in 2 M NaCl, 10 mM Tris (pH 7.4) were found to be 2.6 S for the reconstituted octamer and 2.7 S for the native octamer (fig.2). No difference could be detected by determining the sedimentation velocities at 56000 rev./min. The sedimentation velocity at 16000 rev./min is in close agreement with that of 2.63 S reported [8] for rat liver octamer in 2 M NaCl, 25 mM phosphate (pH 7) and corresponds to an $s_{20,w}$ of 6.6 S [8]. However, in [8], rat liver octamer broke down to a species with an $s_{20,w}$ of 3.8 S above 28000 rev./min, compared with 3.8 S for a chicken octamer preparation centrifuged at 59000 rev./min in 2 M NaCl, 10 mM cyclohexylaminoethane sulfonic acid (pH 9) in [9]. That salt-extracted and reconstituted octamers in the experiments reported now failed to dissociate on centrifugation at high *g*-force may reflect subtle differences in the structure of the octamer due to the tissue source, the different species, the methods of preparation and the conditions under which these values were determined.

Direct mixing in 150 mM NaCl (pH 7) of H3 and H4, purified via exclusion chromatography in

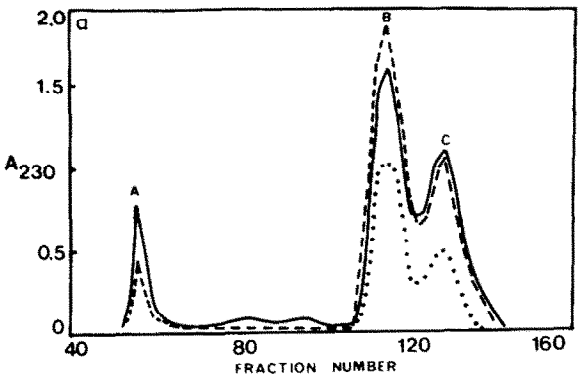


Fig.1. (a) Sepharose 6B chromatography of the products of histone reconstitution: buffer, 2 M NaCl, 10 mM Tris (pH 7.4); column size, 1 m × 2.5 cm; (——) acid-extracted or core histones were dissolved in 2 M NaCl, 10 mM Tris (pH 7.4) and applied to the column; (– – –) acid-extracted or core histones were dissolved in 8 M urea, dialysed against 2 M NaCl, 10 mM Tris (pH 7.4) and applied to the column; (···) for calibration a 2 M NaCl, 10 mM Tris (pH 7.4) extract of 0.8 M NaCl-washed chromatin [2] was applied to the column; SDS–polyacrylamide gel (fig.1b) shows protein eluted using core histones as starting material.

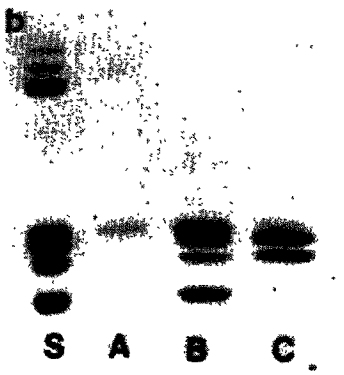


Fig.1. (b) SDS–polyacrylamide gel of protein eluted from the Sepharose 6B column (fig.1a). Lanes S, A, B, C denote acid-extracted histone standard, and the material eluted in peaks A, B, C, respectively.

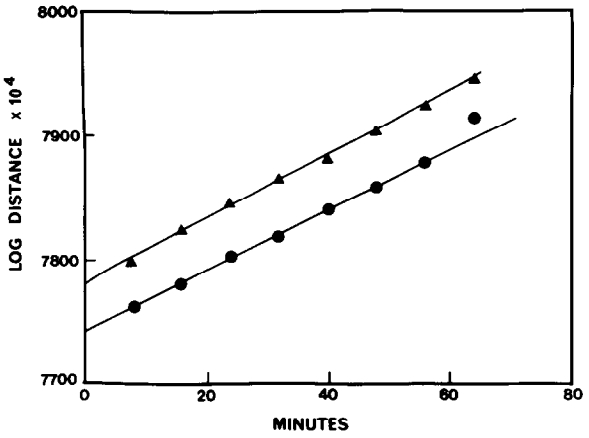


Fig.2. Sedimentation velocity determinations of salt-extracted (▲—▲) and acid-reconstituted (●—●) octamers; rotor speed, 56000 rev./min; no difference could be detected by carrying out the experiments using a rotor speed of 16000 rev./min.

dilute HCl, resulted in the production of an aggregate [1]. A tetramer can, however, be produced by direct mixing provided urea is present initially [10]. Urea presumably unlocks the unnatural structures and allows correct orientation and bonding to occur. We therefore attempted to reduce the amount of H3–H4 aggregate formed during octamer reconstitution by dissolving the acid-extracted total or core histones in 8 M urea and subsequent dialysing against 2 M NaCl, 10 mM Tris (pH 7.4). The yield of octamer appeared to increase at the expense of H3–H4 aggregate formation (fig.1a). This effect of urea was even more striking when histones individually purified by Biogel P60 chromatography [4] were used for reconstitution. No octamer was formed if individually purified freeze-dried histones were used for reconstitution without prior urea treatment (fig.3a); octamer formation however, occurred after urea pretreatment (fig.3b). The optimum yield from individually purified histones was obtained (fig.3c) by omitting the freeze-drying step and instead concentrating by ultrafiltration the histones eluted from the P60 column. To these solutions, solid urea was added to 8 M. Aliquots were mixed in equimolar proportions and subsequently dialysed against 2 M NaCl, 10 mM Tris (pH 7.4). By replacing the ultrafiltration-concentrated histones one-at-a-time by freeze-dried material it was found that lyophilisation of H2A,

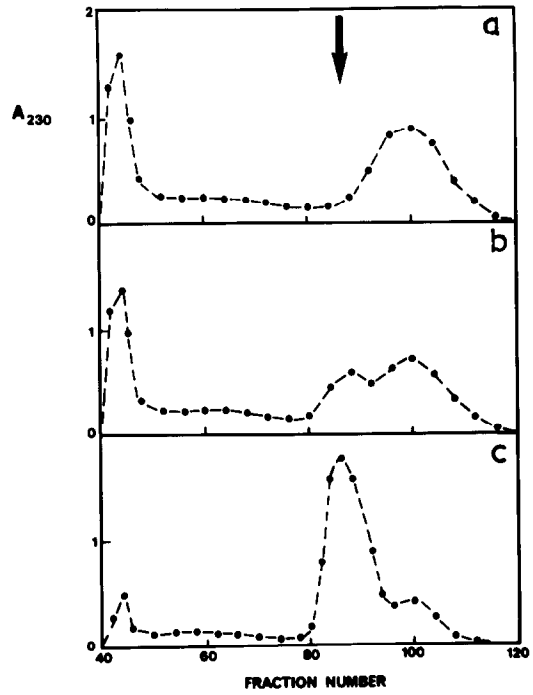


Fig.3. Octamer reconstitution from individual acid-purified histones: (a) equimolar quantities of individually freeze-dried histones were dissolved in 2 M NaCl, 10 mM Tris (pH 7.4) and applied to the column; (b) equimolar quantities of individual freeze-dried histones were dissolved in 8 M urea, dialysed against 2 M NaCl, 10 mM Tris (pH 7.4) and applied to column; (c) solid urea was added to 8 M to equimolar quantities of individual ultrafiltration-concentrated histones; the solution was dialysed against 2 M NaCl, 10 mM Tris (pH 7.4) and applied to the column. The 3 peaks eluted represent aggregate, octamer and dimers, respectively; (⟶) octamer elution volume.

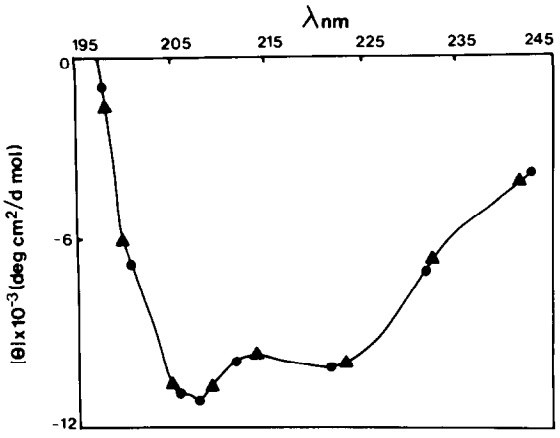


Fig.4. Circular dichroism spectra of acid-reconstituted (●—●) and salt-extracted (▲—▲) histone octamers.

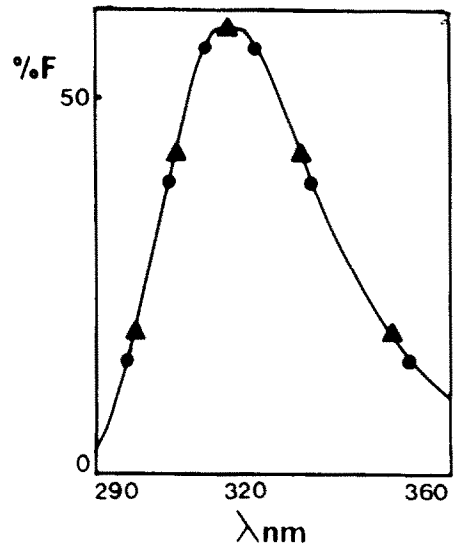


Fig.5. Fluorescence spectra of acid-reconstituted (●—●) and salt-extracted (▲—▲) histone octamers; excitation wavelength, 269 nm.

H2B or H4 had no adverse effect on octamer reconstitution. However, if lyophilised H3 were used an elution profile similar to fig.3b was obtained.

The circular dichroism and tyrosine fluorescence spectra and the behaviour on cross-linking with bifunctional imido-esters are suitable criteria for determining whether reconstituted histone complexes are of the same molecular structure as the salt-extracted complexes [1]. No differences could be detected between the reconstituted and salt-extracted octamers by measurement of their CD spectra (fig.4), fluorescence spectra (fig.5) and their behaviour on cross-linking (fig.6). We conclude that histone octamers reconstituted as described from acid-extracted histones have a structure identical to that of salt-extracted histone octamers.


Fig.6. Dimethyl suberimidate cross-linking of acid-reconstituted and salt-extracted histone octamers. The reaction was stopped with 50 mM glycine at the stated times. After dialysis overnight against 50 mM glycine, an equal volume of twice concentrated sample application buffer [5] was added: lane 1, salt-extracted octamer, 20 min; lane 2, salt-extracted octamer, 40 min; lane 3, acid-reconstituted octamer, 20 min; lane 4, acid-extracted octamer, 40 min; lane 5, total acid-extracted chicken histone standard.

## ACKNOWLEDGEMENT

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