

REVERSIBILITY OF NUCLEOSOME CONFORMATION PERTURBED BY UREA\*

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**SUMMARY** — Monomer nucleosomes ( $\nu_1$ ) from chicken erythrocyte nuclei were diluted into 9 M urea plus 0.2 mM EDTA (pH 7.0), and urea was removed by dialysis. The  $\nu_1$  thus obtained were fractionated by sucrose gradient ultracentrifugation. Each fraction was examined in 0.2 mM EDTA for reversibility of  $\nu_1$  structure perturbed by urea. At least 30% of the initial amount of  $\nu_1$  exposed to urea was restored to the original structure, as shown by sedimentation velocity, electron microscopy, circular dichroism, thermal melting, and fluorescence of  $\nu_1$  labeled with N-(3-pyrene) maleimide on thiol groups of H3 histone.

As an approach to identification of the conformational states or transitions of nucleosomes which might occur in transcriptionally active chromatin (1-3), extensive studies of the effects of urea or other organic solvents, ionic strength, and pH were made previously in our laboratory (4-7). The disruptive effects of urea on chromatin structure have been observed by means of hydrodynamic (8-11), spectroscopic (12-16), low-angle X-ray scattering (17), thermal denaturation (14, 15, 18), electron microscopic (17, 19, 20), and nuclease digestion (21, 22) studies. It has been suggested that the conformational changes of chromatin induced by 4 or 5 M urea, which is the highest urea concentration employed for most of the studies, are reversed by removal of urea (8, 14).

On the other hand, our recent studies (4) have shown that more extensive denaturation of  $\nu_1$  structure occurs in  $\nu_1$  exposed to 7-10 M urea. In the present studies we have examined the reversibility of nucleosome conformation perturbed by 9 M urea.

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Abbreviations:  $\nu_1$ , monomer nucleosomes; NPM, N-(3-pyrene) maleimide.

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## MATERIALS AND METHODS

Monomer nucleosomes from chicken erythrocytes were obtained by use of methods previously described (23, 24). The KCl-soluble nucleosomes employed were stored frozen at  $-20^{\circ}\text{C}$  in 0.2 mM EDTA, pH 7.0 ( $A_{260} \sim 50$ ), then diluted with the appropriate buffer solutions just before use.

Stock solutions of urea ( $\sim 10\text{ M}$ , ultrapure grade, Schwarz/Mann) were made in 0.2 mM EDTA (pH 7.0). Freshly made solutions exhibited pH 7.0. Each urea solution was measured refractometrically against 0.2 mM EDTA at the same temperature, and urea molarity was calculated from the analytical formula described elsewhere (4).

The removal of urea from nucleosome solutions was done as follows: Dialysis tubing containing 2 ml of  $\nu_1$  (diluted into 9 M urea, 0.2 mM EDTA, pH 7.0) was put in 40 ml of 9 M urea, 0.2 mM EDTA (pH 7.0), 0.1 mM phenylmethylsulfonyl fluoride, and 0.1 mM dithiothreitol. The dialysis against decreasing urea concentrations was done at  $4^{\circ}\text{C}$ , with continual stirring, by a dropwise addition ( $\sim 10\text{ ml/hr}$ ) of 0.2 mM EDTA solution containing 0.1 mM phenylmethylsulfonyl fluoride and 0.1 mM dithiothreitol to the outer solution down to the final urea concentration ( $\sim 2.5\text{ M}$ ), followed by an extensive dialysis against 0.2 mM EDTA. The same procedure was applied to urea-denatured  $\nu_1$  labeled with NPM. The preparation of NPM- $\nu_1$  complexes and their fluorescence and other properties have been presented in our previous papers (5, 6).

Sedimentation experiments of renatured  $\nu_1$  were performed with a Beckman Model E analytical ultracentrifuge equipped with scanner optics. Scanner data were recorded at 265 nm. Sucrose (0.5%) was added to half of each solution to stabilize against mechanical and thermal mixing. Sedimentation coefficients were determined at 30,000 rpm in an An-G rotor. The data were recorded at  $22^{\circ}\text{C}$  and corrected to  $S_{20,w}$  values with standard errors as indicated in Results.

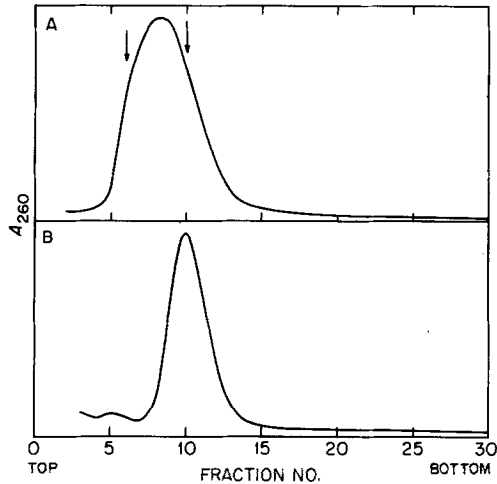
Electron microscopy and thermal melting experiments were performed by the methods described previously (4). Circular dichroism measurements were performed on a Jasco J-40A spectropolarimeter at room temperature. The molar extinction coefficient at 260 nm of DNA ( $\epsilon_p = 6,500$ ) was used in calculations of the molar ellipticity, expressed per mole of nucleotides,  $[\theta]_p$ .

Fluorescence of NPM- $\nu_1$  complexes was measured in a Perkin-Elmer MPF-44 recording spectrofluorometer by the procedure described elsewhere (5, 6). The excitation wavelength was at 340 nm.

## RESULTS

Sucrose gradient sedimentation and sedimentation velocity of urea-treated

nucleosomes — After  $\nu_1$  were mixed with urea to a final concentration of 9 M urea, the urea was dialyzed out from the medium. The  $\nu_1$  thus obtained in 0.2 mM EDTA were run on preparative linear sucrose gradients. In Fig. 1 it is shown that certain fractions of urea-treated  $\nu_1$  sediment where the control native  $\nu_1$  have a sedimentation peak (fraction 10), while a considerable proportion of urea-treated  $\nu_1$  sediments more slowly than control  $\nu_1$ . It was calculated that about 30% of urea-treated  $\nu_1$  sediment above fraction 10.



**Figure 1.** Preparative sucrose gradient ultracentrifugation of urea-treated  $v_1$  and native  $v_1$ . (A) Renatured urea-treated  $v_1$ . The arrows denote the positions for fractions 6 and 10, respectively. (B) Native  $v_1$ . Each sample in 0.2 mM EDTA was loaded on a 5–20% linear sucrose gradient containing 0.2 mM EDTA (pH 7.0). Centrifugation was in an SW 41 rotor (Beckman) at 35,000 rpm and 4°C for 12 hr.

Sedimentation coefficients were obtained for two fractions (10 and 6) from the sucrose gradients.  $S_{20,w}$  for the renatured urea-treated  $v_1$  (fraction 10) was  $9.01 \pm 0.14$ ; the value is  $9.31 \pm 0.11$  for untreated  $v_1$ . This difference in  $S_{20,w}$  between the two particles is probably within the experimental uncertainties. The value for urea-treated  $v_1$  in fraction 6, on the other hand, was  $6.52 \pm 0.06$ , which suggests swelling or unfolding of the particle.

SDS-gel electrophoresis of the sucrose gradient fractions of urea-treated  $v_1$  showed normal amounts of the inner histones (H3, H4, H2A, H2B) in fractions 8–11, whereas, a relative deficiency of H3 and H4 was seen in fractions 6 and 7. Electrophoresis provided no evidence of proteolytic loss of H3 and H4. We attempted to obtain a better yield of normal sedimenting urea-treated particles by use of Spectropor dialysis tubing (Spectrum Medical Industries, Inc., Middle Village Station, New York), which has a smaller pore size (molecular weight cutoff 6,000–8,000); however, the yields were approximately the same. We do not yet understand the basis of the loss of H3 and H4 during the dialysis renaturation step. Adsorption of some of H3 and H4 onto the inside wall of the dialysis tubing remains a possibility.

**Electron microscopy** — Figure 2 presents the electron microscopy of control and urea-treated  $v_1$ . The  $v_1$  exposed to 9 M urea resemble short rods (Fig. 2D). Similar observations on the disruptive effects of urea on the ultrastructure of  $v_1$  have been

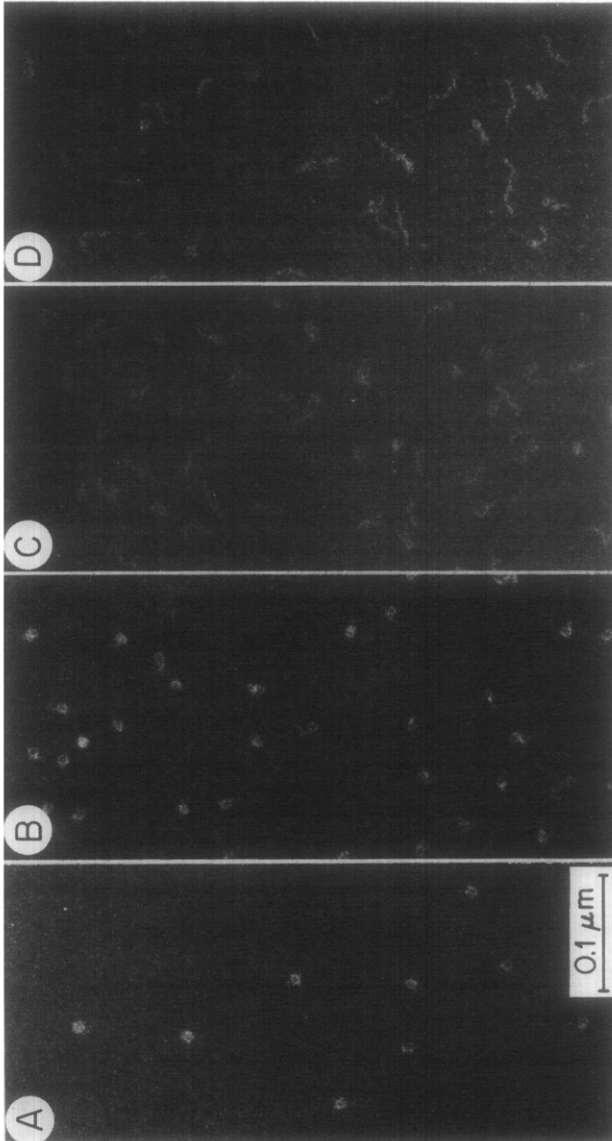


Figure 2. Dark-field electron microscopy of urea-treated  $v_1$ . (A) Native  $v_1$ , (B) renatured urea-treated  $v_1$  (fraction 10), (C) renatured urea-treated  $v_1$  (fraction 6), (D)  $v_1$  in 9 M urea. All samples contained 0.2 mM EDTA (pH 7.0).

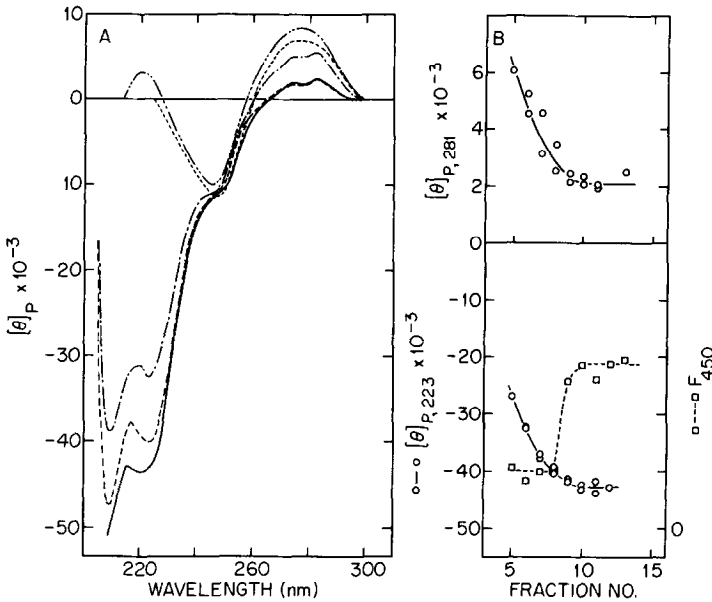


Figure 3. Circular dichroism of urea-treated  $v_1$ . (A) Chicken DNA (— — —), native  $v_1$  (— — —), renatured urea-treated  $v_1$  (fraction 10) (— — —), renatured urea-treated  $v_1$  (fraction 6) (— — —),  $v_1$  in 9 M urea (-----).  $[\theta]_P$ : molar ellipticity per mole of nucleotides. All samples contained 0.2 mM EDTA. (B) — o —, Ellipticity of renatured urea-treated  $v_1$  as a function of the fraction number from sucrose gradient ultracentrifugation (see Fig. 1); — □ —, the relative fluorescence intensity at 450 nm of  $v_1$  labeled with NPM as a function of fraction number. Excitation wavelength was at 340 nm.

previously reported (4, 20). However, Fig. 2B shows that after urea is dialyzed out, fraction 10 appears to consist of normal  $v_1$ , whereas fraction 6 consisted of partially unfolded  $v_1$  (Fig. 2C).

Circular dichroism — The circular dichroism spectra of urea-treated  $v_1$  are shown in Fig. 3A. As has been reported previously (4), in 9 M urea the dominant  $\alpha$ -helix contribution ( $<240$  nm) is completely lost and the suppressed spectrum (260–300 nm) becomes like that of naked DNA. The renatured urea-treated  $v_1$  (fraction 10) yield identical circular dichroism spectra at 260–300 nm, within the experimental uncertainties of our measurements, and exhibit slightly higher  $\alpha$ -helix content (i.e., molar ellipticities of urea-treated  $v_1$  as a function of fraction number of the sucrose gradient sedimentation. The ellipticities decreased with increasing fraction number; at 281 and 223 nm plateau values are reached by fraction 10 and are similar to the values of control  $v_1$ . This fact indicates the recovery of both the DNA structure and the secondary structure of histones after treatment with 9 M urea and subsequent dialysis.

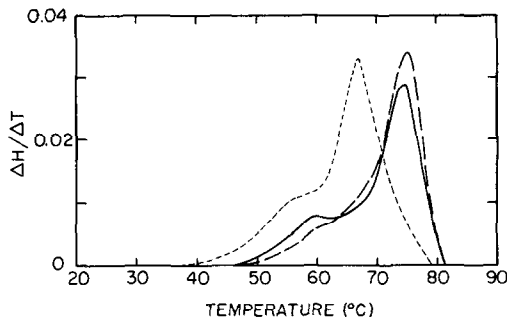


Figure 4. Thermal denaturation at 260 nm of urea-treated  $v_1$ . Derivative melting profiles are shown. All samples contained 0.2 mM EDTA (pH 7.0). Native  $v_1$  (—), urea-treated  $v_1$  (fraction 10) (— — —), urea-treated  $v_1$  (fraction 6) (— · — · —).

Fluorescence of NPM- $v_1$  complexes — To investigate the tertiary or quaternary structure of urea-treated  $v_1$ , we exposed NPM- $v_1$  complexes to 9 M urea, then removed the urea by dialysis. The fractionation profile by sucrose gradient ultracentrifugation of the urea-treated NPM- $v_1$  complexes was very similar to that of the urea-treated  $v_1$  (Fig. 1A). As shown in Fig. 3B, the NPM- $v_1$  complexes above fraction 9 exhibit excimer fluorescence, in contrast to the complexes below fraction 8 which do not exhibit any fluorescence characteristic of the NPM excimer. This observation suggests that the  $v_1$  particles above fraction 9 maintain their tertiary and/or quaternary structure after urea treatment (5, 6).

Thermal denaturation — Native and renatured urea-treated  $v_1$  were thermally denatured in 0.2 mM EDTA (pH 7.0). Figure 4 shows the derivative plots of the thermal denaturations. Native  $v_1$  melt with a wide premelt centered at 59°C and a main transition at ~75°C, very similar to the published data in which 0.25 mM EDTA was used as solvent (25). Melting of the urea-treated  $v_1$  (fraction 10) is slightly different from that of native  $v_1$ ; the main transition is at 75°C, but there is slightly higher hyperchromicity and also some difference in the premelt region. The urea-treated  $v_1$  (fraction 6) exhibit a main transition at 67°C, considerably reduced compared with native  $v_1$ .

## DISCUSSION

The present study demonstrates that at least 30% of the initial amount of  $v_1$  exposed to 9 M urea restore their native compact structure after urea is removed by dialysis. Several studies have demonstrated that the histones remain associated with DNA in chromatin treated with up to 5 M urea, at low ionic strength (8, 12, 14, 26). In our previous studies (4) we have shown that treatment of  $v_1$  with at least 8 M urea plus 0.2 mM EDTA does not dissociate histones from DNA. It is not yet clear why we obtain less than com-

plete recovery of native-like  $v_1$ . Nonetheless, the procedures described here will permit investigators to chemically modify urea-unfolded  $v_1$  and obtain significant amounts of renatured modified particles.

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