Urea perturbation and the reversibility of nucleohistone conformation

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ABSTRACT

Urea effect on conformation and thermal stabilities in nucleohistone and NaCl-treated partially dehistonized nucleohistones has been studied by circular dichroism (CD) and thermal denaturation. Urea imposes a CD change at 278nm of DNA base pairs in native and NaCl-treated nucleohistones which can be decomposed into two parts: a decrease in $\Delta\epsilon$ for histone-free base pairs and an increase for histone-bound base pairs. The reduction by urea of $\Delta\epsilon$ of bound histones is approximately proportional to the increase of $220\Delta\epsilon$ of histone-bound base pairs. Urea also lowers the melting temperatures of base pairs both free and bound by histones. The presence of urea indeed destroys the secondary structure of bound histones, causing changes in the conformation and thermal stabilities of histone-bound base pairs in nucleohistone. Such a urea perturbation on nucleohistone conformation is reversible.

INTRODUCTION

Native chromatin contains DNA, histones, nonhistone proteins and RNA, of which DNA and histones are the two major components. The existence of a supercoiled structure for the chromatin has been demonstrated by X-ray diffraction (1). Two different thermal stabilities of base pairs bound by histones (2-4), and conformational change of DNA in chromatin have also been demonstrated (5-8).

In protein it is generally believed that the native structure is thermodynamically the most stable form. If a protein structure is partially disrupted or even destroyed, it can go back to the most stable form if appropriate conditions for renaturation be provided (9). It has been generally accepted that the same is true for DNA (10,11). In considering the same question with respect to chromatin we asked ourselves whether the

bound histones and histone-bound DNA base pairs would possess the most stable conformation. To answer this question we looked for a method which would destroy, either fully or partially, the structure of bound histones and enable us to examine whether or not the native structure of chromatin could be restored. Both thermal denaturation and circular dichroism (CD) were chosen as the analytical tools. Thermal denaturation measures thermal stability of DNA base pairs whether free or bound by histones and indicates the proportional amount of base pairs in each category. CD, on the other hand, measures the conformation of bound histones and histone-bound base pairs. With these two methods it is possible to follow the structural changes in chromatin caused by some disturbing factor and then examine the restoration of chromatin structure when this factor is removed. Urea has been widely used as a denaturing reagent in proteins (9), and for this reason urea was chosen for use with chromatin since any destruction of histone structure by urea is also expected to lead to an alteration of the structure of histone-bound base pairs.

It is reported here that the main effect of urea on nucleohistone is the destruction of helical structure in bound histones, which causes a change in the structure and thermal stabilities of histone-bound base pairs. All these structural disturbances in nucleohistone by urea prove to be temporary as the native structure can be restored when urea is removed. This confirms the belief that nucleohistone in its native form indeed possesses the most stable structure with respect to both histones and DNA.

MATERIALS AND METHODS

Calf thymus chromatin was prepared according to the method of Shih and Bonner (12). The soluble and the NaCl-treated, partially dehistonized nucleohistones were prepared from chromatin as previously described (2). They were dialyzed against $2.5 \times 10^{-4}M$ EDTA, pH8.0 (EDTA buffer) for

studies.

Calf thymus DNA was purchased from Sigma Chemical Co. and was purified by phenol extraction. The molar extinction coefficient of $6,500\text{M}^{-1}\text{cm}^{-1}$ in nucleotide was used for both nucleohistones and DNA at 260nm.

Ultra pure urea was purchased from Schwarz/Mann. An appropriate amount of 8.0M urea in EDTA buffer was added to nucleohistone. The final urea concentration was recorded.

The absorbance and thermal denaturation measurements of DNA and nucleohistones in the absence or the presence of urea were obtained using a Gilford Spectrophotometer Model 2400-S. h is the percent increase in hyperchromicity referred to the absorbance at 260nm, A_{260} , at room temperature. dh/dT is the derivative of the melting curve.

The CD spectra of the samples were taken on a Durrum-Jasco Spectropolarimeter Model J-20 at room temperature. The CD results are reported as $\Delta\epsilon = \epsilon_l - \epsilon_r$, where ϵ_l and ϵ_r are respectively molar extinction coefficients for the left- and the right-handed circularly polarized light. The units of $\Delta\epsilon$ are $\text{M}^{-1}\text{cm}^{-1}$ in terms of nucleotide.

RESULTS

Thermal Denaturation of Nucleohistone in Urea

The derivative melting curves of native and NaCl-treated partially dehistonized nucleohistones from calf thymus in EDTA buffer can be distinguished as three melting bands and a shoulder. Melting band I at 47° corresponds to the melting of free base pairs, bands III at 72° and IV at 82° to those bound by histones and a shoulder near 57° (II) to those bound by nonhistone proteins or small gaps between histone-bound regions (4). For nucleohistone, the addition of urea to the medium in general lowers the melting temperatures of these melting bands, in the order of I, II > III >

IV. This is in agreement with the report of Ansevin et al., (3). In addition, a new melting band V at 95 to 100° becomes apparent at higher urea concentration. At 5.0M urea this new melting band has an area equal to 7 to 10% of total melting area (Fig. 1a).

Urea effect on melting properties of 0.6M NaCl-treated nucleohistone is similar to that of native nucleohistone with the appearance of melting band V at 95 to 100° at higher urea concentration. For 1.6M NaCl-treated nucleohistone, adding urea also reduces the melting temperatures of the melting bands in the same order as that for native nucleohistone (Fig. 1b).

Fig. 2 summarizes the urea dependence of melting temperatures and areas of the melting bands of native nucleohistone. As shown in Fig. 2a, the temperatures for both melting bands III and IV are decreased at higher urea concentrations. Such a dependence is greater for band III than IV. Since melting bands III, IV and V are distinguishable, their melting areas and that of melting bands I + II can be estimated at each urea concentration. As shown in Fig. 2b, within experimental error, there is no change in the area of melting bands I + II, and III. On the other hand, a decrease of melting band IV is accompanied by an increase of melting band V. The constant fraction of melting area of bands I + II at various urea concentrations implies that the presence of urea does not change the fraction of DNA base pairs free of histone binding. In other words, no full or partial dissociation of histones from DNA could be detected in 5.0M urea.

Results similar to those of Fig. 2 have also been observed for 0.6M NaCl-treated nucleohistone at various urea concentrations.

Fig. 3 summarizes the urea dependence of melting temperatures and areas of the melting bands of 1.6M NaCl-treated nucleohistone. They are similar to those in Fig. 2 for native nucleohistone. Since melting band I of free base pairs is distinguishable in this partially dehistonized nucleohistone,

Fig. 1. Derivative melting profiles (a) and 1.6M NaCl-treated, partially dehistonized nucleohistone (b). Controls in EDTA buffer (—) in 5.0M urea (----) and dialyzed back to EDTA buffer from 5.0M urea (- · -). Melting bands assigned are described in the text

Fig. 2. Urea dependence of melting temperature (a) and fraction of area (b) of each melting band in native nucleohistone.

Fig. 3. Urea dependence of melting temperature (a) and fraction of area (b) of each melting band in 1.6M NaCl-treated nucleohistone. Also included is the melting temperature of pure DNA.

Fig. 4. CD spectra of native nucleohistone. Control in EDTA buffer (—), in 5.0M urea (----) and dialyzed back to EDTA buffer from 5.0M urea (- Δ -).

the dependence of $T_{m,I}$ on urea can be measured accurately. It is seen from Fig. 3a that the dependence of $T_{m,I}$ on urea is exactly the same as that of T_m of pure DNA. This is another example of a localized effect of urea on base pairs rather than an overall effect of urea on the supercoiled structure of the whole chromatin as to be discussed later. Fig. 3b further shows that the fraction of melting area of bands I + II, III or IV is constant irrespective of urea concentration.

CD of Nucleohistone in Urea

CD spectra of native nucleohistone in the absence and the presence of urea (5.0M) are shown in Fig. 4. In agreement with previous reports (13, 14), urea increases the positive CD band at 275nm and reduces the negative band at 220nm. The reduction of the negative band can be interpreted as due to a destruction of α -helix of bound histones by urea. The interpretation of the urea effect on the positive CD band is not quite as obvious. Previously it was explained as a consequence of destruction by urea of the supercoiled structure of native nucleohistone (13,14). Another possibility will be presented below with experimental results.

Our interpretation of the urea effect on the CD of nucleohistone (Fig. 4) is that urea destroys some α -helical structure of bound histones; this reduces the conformational effect of histone binding to base pairs and therefore yields a greater positive CD band. The destruction of the supercoiled structure of nucleohistone by urea (13,14), is a result and not a cause of the above events. In order to determine whether or not this view is correct, the urea effect on the CD of NaCl-treated, partially dehis-tonized nucleohistones was also studied.

The urea effect on CD spectrum of 0.6M NaCl-treated nucleohistone (not presented here) is very similar to that of native nucleohistone shown in Fig. 4, with an increase of the positive band and a decrease of the nega-

tive band. The urea effect on the CD spectrum of 1.6M NaCl-treated nucleohistone (Fig. 5) is quite different from that exhibited by native and 0.6M NaCl-treated nucleohistone. Although 5.0M urea reduces the negative CD at 220nm, indicating destruction of α -helix of the remaining histones, enhancement of the positive CD band shown with native nucleohistone is absent. Instead, this CD band with 1.6M NaCl-treated nucleohistone is reduced by urea.

At 220nm, the CD of nucleohistone and NaCl-treated, partially dehistonized nucleohistones is mainly contributed by bound histones. In order to compare the CD effects of urea at various concentrations, Fig. 6 shows $\Delta\epsilon_{220}$ for bound histones in native, 0.6M and 1.6M NaCl-treated nucleohistones and for DNA. For native and partially dehistonized nucleohistones, $\Delta\epsilon_{220}$ becomes less negative at higher concentration of urea. The reduction is approximately proportional to the urea concentration. For pure DNA, the CD at 220nm does not change in the presence of urea but the major CD band at 275nm is reduced (Fig. 7).

Fig. 7 also shows the change in $\Delta\epsilon_{278}$ of native and partially dehistonized nucleohistones in urea. For native and 0.6M NaCl-treated nucleohistones, there is no change in $\Delta\epsilon_{278}$ at less than 2.0M urea; at concentrations greater than 2.0M an increase is observed at this wavelength. Urea effect on $\Delta\epsilon_{278}$ is quite different in 1.6M NaCl-treated nucleohistone. There is a decrease of $\Delta\epsilon_{278}$ up to 3.0M urea, beyond which the trend is reversed.

Since a nucleohistone molecule can generally be classified into two fractions, histone-free and histone-bound base pairs, urea can conceivably have different conformational effects on these two classes of base pairs. Possibly the results just presented (Fig. 7) reflect these differences, because the fraction of histone bound base pairs is 78% for native nucleo-

Fig. 5. CD spectra of 1.6M NaCl-treated nucleohistone. Control in EDTA buffer (—), in 5.0M urea (----) and dialyzed back to EDTA buffer from 5.0M urea (-Δ-).

Fig. 6. Urea dependence of $\Delta\epsilon_{220}$ of nucleohistones and DNA. Native nucleohistone (●), 0.60M NaCl-treated (▲) and 1.6M NaCl-treated nucleohistone (◐), and DNA (▼).

Fig. 7. Urea dependence of $\Delta\epsilon_{278}$ of nucleohistones and DNA. Native nucleohistone (●), 0.60M NaCl-treated (▲) and 1.6M NaCl-treated nucleohistone (◐) and DNA (▼). Calculated CD at 278nm ($F\Delta\epsilon_B$) of histone-bound base pairs from equation (1) for native nucleohistone (○), 0.60M NaCl-treated (△) and 1.6M NaCl-treated nucleohistone (◑).

histone, 60% for 0.6M NaCl-treated and 34% for 1.6M NaCl-treated nucleohistone, as determined by thermal denaturation.

In order to analyze the different CD effects on histone-free and -bound base pairs, it is assumed that the measured CD ($\Delta\epsilon_m$) at each urea concentration, is contributed by the CD of histone-free ($\Delta\epsilon_o$) and histone-bound base pairs ($\Delta\epsilon_b$) at each urea concentration. The following equation is used.

$$\Delta\epsilon_m = (1-F) \Delta\epsilon_o + F \Delta\epsilon_b \quad (1)$$

where F is the fraction of base pairs bound by histones in each nucleohistone. In other words, 1-F is the fraction of base pairs not bound by histones. This can be determined by thermal denaturation, through use of the equation

$$1 - F = \frac{A_{T_{m,I}} + A_{T_{m,II}}}{A_T} \quad (2)$$

where $A_{T_{m,I}} + A_{T_{m,II}}$ is the sum of melting area under melting bands I and II and A_T the total area under all melting bands (4).

According to Fig. 3, for nucleohistone and NaCl-treated nucleohistones, $(A_{T_{m,I}} + A_{T_{m,II}})/A_T$ is independent of urea concentration. It is determined that 1 - F is 78% for nucleohistone, 60% for 0.6M NaCl-treated nucleohistone and 34% for 1.6M NaCl-treated nucleohistone. At each urea concentration $\Delta\epsilon_o$ of pure DNA is used which is taken from the straight line in Fig. 7. From equation (1), $F \Delta\epsilon_b$ from these nucleohistones at each urea concentration is calculated. The results are shown in Fig. 7 and indicate a consistent increase of $\Delta\epsilon_{278}$ at higher urea concentration for histone-bound base pairs from native and partially dehistonized nucleohistones. In other words, as far as the CD is concerned, urea effect on nucleohistone can indeed be decomposed into two parts, the effect on histone-free and histone-bound base pairs. If urea effect on $\Delta\epsilon_{220}$ (Fig. 6) and on $F \Delta\epsilon_b$

at 278nm (Fig. 7) of each nucleohistone is compared, it is seen that a decrease of A_{220} of histones yields an increase of A_{278} for histone-bound base pairs. In other words, the destruction of secondary structure of bound histones makes these bound base pairs less tilted, as they are in the absence of urea.

Conformational Changes on Nucleohistone by Urea are Reversible

As mentioned above, urea destroys some of the secondary structure of bound histones and also changes the conformation of histone-bound base pairs. Urea also changes thermal stabilities of histone-free and -bound base pairs. If the original conformation of bound histones and histone-bound base pairs are indeed the most stable ones in nature, it should be possible to achieve a return to the original conformations by removal of the disturbant, urea for example, from the medium. In order to see whether this could be done, native, 0.6M NaCl- and 1.6M NaCl-treated nucleohistones were mixed with urea to a final concentration of 1.0M, 3.0M and 5.0M respectively. Urea was then dialyzed out from the medium and both thermal denaturation and CD of these samples were measured. Some results using 5.0M urea are shown in Fig. 1 on thermal denaturation and Figs. 4 and 5 on CD. For both native and 1.6M NaCl-treated nucleohistones, the removal of 5.0M urea from the medium restores all melting bands to the original locations, with the exception of melting band III which remains about 2 to 3 degrees lower than the controls which received no urea treatment. Fig. 4 shows the reversals of the CD band of bound histones at 220nm and histone-bound base pairs at 275nm. The reversibility is not complete as indicated by a slightly smaller CD at 220nm for the samples having been exposed to urea as compared to the controls. It is possible that such a nonperfect reversibility in CD at 220nm (Fig. 4) is related to a slight lower $T_{m,III}$ after urea treatment as shown in Fig. 1. Though the reversibility may not be complete, the results

in Figs. 1 and 4 strongly indicate that, in terms of the conformation and thermal stability, the original structures of native and NaCl-treated partially dehistonized nucleohistones can be mostly restored after urea perturbation. Results similar to those just presented also support the same conclusion for 0.6M NaCl-treated nucleohistone. Further, as expected, the CD and melting curves of these nucleohistones treated by lower concentrations of urea, 3.0 and 1.0M for example, but dialyzed back to EDTA buffer without urea, are closer to their controls than those treated by 5.0M urea. All these results support the view that, at the level of histone molecules and histone-bound base pairs, nucleohistone, indeed, possesses the most stable conformation.

DISCUSSION

Because of the existence of supercoiled structure in native nucleohistone (1) interest was generated regarding the roles of histones in maintaining this structure (15,16). Since its observance, this structure has been interpreted as a possible cause for CD changes from B form DNA found in nucleohistone (5,6). Destruction of this supercoiled structure has also been used to interpret the CD changes in nucleohistone caused by urea (13,14). Here we present a view that the conformational effect on DNA in nucleohistone is a direct result of histone binding and is a localized event. A similar view has been proposed which is supported by experiments showing that thermal stabilities in nucleohistone result from a localized stabilization of base pairs by histone molecules (2,17). In other words, both CD and thermal denaturation properties of nucleohistone result from direct binding of histones to DNA, irrespective of supercoiled structure.

One of the major objectives of this study is to disturb the conformations of both histones and DNA in nucleohistone and to examine whether these conformations, once disturbed, can go back to their former state. It

has been shown here that the conformations and thermal denaturation properties of native and NaCl-treated, partially dehistonized nucleohistones can be reversed after they have been significantly disturbed by urea up to 5.0M. More importantly, it has been demonstrated that both CD and thermal denaturation measure the conformation of bound histones and the conformation and thermal stabilities of histone-bound base pairs. Because of this, it is legitimate to conclude that the conformations of bound histones and histone-bound base pairs in nucleohistone are in their most stable form. This conclusion can also be applied to NaCl-treated, partially dehistonized nucleohistones, though up to 60% of histones have been removed from nucleohistone by 1.6M NaCl. If the conformation of bound histones and histone-bound base pairs can be restored, it is consequently expected that the supercoiled structure of nucleohistone can also reform after urea treatment. As a matter of fact, using intrinsic viscosity to probe the supercoiled structure of native nucleohistone, Bartley and Chalkley (18) reported the structure to be reversible after the treatment of 4.0M urea.

It was shown by electrophoresis that urea did not fully dissociate histones from DNA (13,14). The constant melting area of bands I + II are in agreement with this conclusion. The melting results imply further that dissociation of parts of histone molecules from DNA by urea does not occur either. If it did, we would expect more melting of histone-free fractions, which as shown in Figs. 2 and 3, is not the case.

The appearance of melting band V at 95 to 100° for native and 0.6M NaCl-treated nucleohistones is not well understood. Such a stabilization on some of histone-bound base pairs by urea is contrary to destabilization affected by urea on histone-free and on the majority of histone-bound base pairs. It is possible that parts of histones and histone-bound base pairs in nucleohistone respond to urea differently from the others.

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