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Effects of pH on the stability of chromatin core particles

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ABSTRACT

Chromatin core particles near physiological ionic strength undergo a reversible transition induced by changes in pH near neutrality. While sedimentation studies indicate no significant effect on size or shape, changes in tyrosine fluorescence anisotropy and in circular dichroism suggest a somewhat looser structure at high pH. Further support of this suggestion is given by high salt dissociation experiments; at pH 8 core particles begin to show changes at lower salt concentration than at pH 6. The pH transition appears unaffected by the presence of Mg^{2+} but can be blocked by crosslinking of the histones. A possible relationship is suggested between this transition and increases in intracellular pH which correlate with enhancement in several aspects of cellular activity including DNA replication.

INTRODUCTION

Dramatic changes in several cellular functions are accompanied by changes in intracellular pH (see reference 1 for a review of various aspects of this subject). For example, pH increases in the range of 0.1 to 0.5 units accompany increases in such diverse functions as glycolysis, initiation of protein synthesis in egg development, and DNA synthesis. In the case of DNA synthesis, an increase in pH is correlated with the S phase of the cell cycle.[1-4] This effect could, of course, be ascribed to pH induced variations in the activity of enzymes directly or indirectly involved in replication. However, it also seems reasonable to suggest that alterations in chromatin structure which facilitate replication might be induced by a change in pH.

Studies of the effect of pH on chromatin or on its subunits (nucleosomes, core particles) are relatively rare. Labhart, et al.[5] recently reported electron microscopic observations on soluble chromatin at pH 7 and 9. They found that soluble chromatin stripped of histone H1 was indistinguishable at these two pH values but H1-containing chromatin was distinctly less stable at the higher pH. Early work on core particles indicated only

one distinct structural transition which occurred at very low ionic strength near pH 5.[6,7]

We recently reported on effects of pH on the low-salt transition of core particles.[8] The low-salt transition shifts to higher salt with an increase in pH. This suggests that core particles at higher pH have decreased stability toward the electrostatic repulsion presumed to be responsible for the low-salt transition. We also noted that these changes appeared to be coordinated with a pH induced transition detected by a small change in the fluorescence properties of histone tyrosines. This pH transition was observed at near physiological ionic strength and was centered near pH 7, suggesting that it might be important in vivo. Further studies of this transition and its relationship to pH effects on salt induced changes in core particles are reported here.

METHODS

Core particles were prepared from chicken erythrocytes as described previously.[9] The concentration in all studies was between 0.5 and 1.0 μM (A_{260} = 1 to 2). Other details are included in the figure captions.

Fluorescenace measurements were made on a computer controlled fluorescence anisotropy spectrometer using the conditions described previously.[8] The samples were excited at 279 nm and emission observed at 325 nm. Sedimentation coefficients were determined on a Beckman Model E ultracentrifuge operated at 40,000 rpm. Circular dichroism was determined with a JASCO J-41A spectrophotometer and is reported as Δε per mole DNA phosphate. Individual values of Δε at the peak (284 nm) were determined by 5 min. of averaging using a bandwidth of 4 nm. Temperature was maintained at 20° for all measurements.

RESULTS AND DISCUSSION

The effect of pH at 0.10 M ionic strength on the fluorescence properties of core particles is illustrated in Figure 1. The anisotropy (r) decreases with increasing pH in a manner which clearly suggests the titration of one or more groups with an apparent pK_a near 7. An increase in fluorescence intentity (I) from pH 5 to 8 may also reflect this titration; however, above pH 8, I drops rather steeply with increasing pH. This latter change reflects ionization of some of the tyrosine residues to form the much less fluorescent tyrosinate ion. (The pK_a of the tyrosine hydroxyl in a peptide is about 10.) The data in Figure 1 were obtained about three hours after the samples were made up. Aliquots from the same samples were

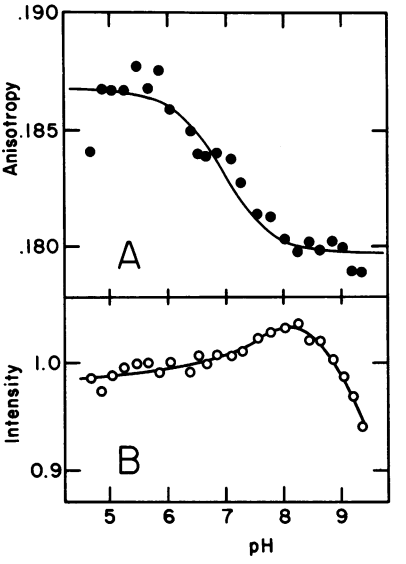

Figure 1. Effect of pH on core particle tyrosine fluorescence anisotropy (A) and intensity (B) at 0.10 M ionic strength. Core particles were diluted into 0.10 M NaCl to a final concentration of 0.5 μM (A_{260} = 1.0). The starting pH was 6.8. Adjustment to lower pH was done by addition of 0.1 M Tris hydrochloride. A solution of 0.1 M NaOH was added slowly to adjust the pH to higher values. Aliquots were removed and kept at 20° for about 3 hours before measurement of the fluorescence. The pH was then determined directly on each sample. Results after storage for 24 hours at 20° were very simlar to those shown here. The solid curve drawn through the data in A represent a least squares best fit to the results, using the model described in the text. The pK was 6.97.

checked about 24 hours later with similar results. These and other data indicated that there is no significant dependence of the titration curve on times over 1 hour.

The effect on the pH transition of histone crosslinking by dimethylsuberimidate is shown in Figure 2. The transition is still evident but much reduced in magnitude. A control, treated identically in the absence of crosslinker, showed a transition comparable in position, magnitude and signal-to-noise to that in Figure 1A. Since dimethylsuberimidate crosslinks only the protein component of core particles, this result suggests that changes in protein structure or of protein-protein interactions are involved in the pH transition. However, the extensive derivatization of protein amine groups resulting from the crosslinking might in itself account for the altered dependence of core particle anisotropy on pH.

Reversibility of the pH transition in the pH range 5 to 9 was checked

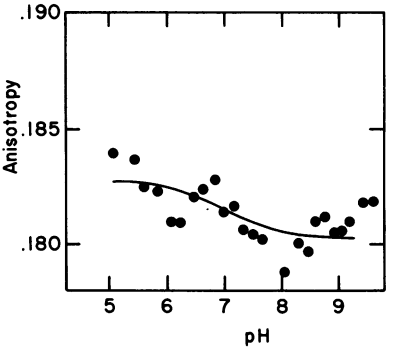

Figure 2. Effect of dimethylsuberimidate crosslinking on the pH transition. Core particles were crosslinked at a concentration of 5 μM (A_{260} = 10) in 0.1 M sodium borate, pH 10, containing 5 mg/ml dimethylsuberimidate (SIGMA). After one hour at room temperature, the solution was dialyzed at 5° against 0.01 M sodium phosphate, pH 7. Electrophoresis of the histones in 10% SDS-polyacrylamide gels (8) demonstrated that extensive crosslinking had occurred. Preparation of samples for fluorescence measurements was done as described in the caption to Figure 1.

by titration of samples which had been first adjusted to either pH 5 or 9. The results for the two procedures were indistinguishable, thus indicating that the states of the core particles responsible for the observed changes are in equilibrium. The simplest model of the transition would assume two states:

$$A \underset{}{\overset{K}{\rightleftharpoons}} C + H^{+}. \qquad (1)$$

In this model, A and C may represent the core particle as a whole or equivalent halves of the particle so long as the halves undergo the transition independently. Using this model, and least squares iterative fitting as applied in reference 8, a best fit to the data in Figure 1 was found with pK = 6.97. This result was very similar for other sets of data. In Figure 1, the solid curve represents the least squares fit.

The effect of the transition on the circular dichroism (CD) and on the sedimentation coefficient of core particles was also examined. Results are shown in Figure 3. CD at 284 nm, which arises primarily from the DNA, shows a distinct increase with pH. The sigmoidal character of the curve suggests that the transition reflected by the histone tyrosine anisotropy changes also has an effect on the DNA. However, the lack of plateauing by $\Delta\varepsilon_{284}$ at high or low pH suggests a second effect which results in a more or less continuous increase in $\Delta\varepsilon_{284}$ with pH, onto which the effects of the pH

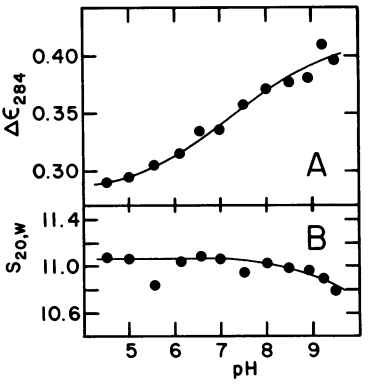

Figure 3. Effect of pH on circular dichroism at 284 nm (A) and on the sedimentation coefficient (B) of core particles. The samples contained 0.7 μM core particles (A_{260} = 1.4), 0.1 M NaCl and 0.01 M buffer. Below pH 7 the buffer was sodium cacodylate. Above 7 Tris was used. Tyrosine fluorescence anisotropy measurements on the same samples resulted in a curve very similar to Figure 1.

transition are superimposed. The CD below 260 nm, which is dominated by the histones, did not show measureable changes over the pH range examined.

The sedimentation coefficient (S) was virtually independent of the pH (Figure 3B). This is in contrast to transitions induced by changing salt concentration, both at very low salt[9] and at moderate salt[10], which induce changes in $S_{20,w}$ as large as two units. Thus, the pH transition causes comparatively little change in the core particle size and shape.

The effect of divalent cation was checked by comparing titration results for samples containing 0.1 M NaCl and either 1 mM Mg^{2+} or 1 mM EDTA. Again no differences were noted. Higher concentrations of Mg^{2+} (up to 10 mM) were checked by observing the effect of [Mg^{2+}] on core particle anisotropy at pH 5.6 and 8.8, again with no significant effect.

The effect of pH at very low salt on core particles has been described in reference 8. At sufficiently low ionic strength a transition to the low salt form of the particle is observed accompanied by a decrease in $S_{20,w}$ from 11 to 9 or lower,[9] a large increase in CD near 280 nm (unpublished results) and a decrease in tyrosine anisotropy ($\underline{r}$).[8,9] These changes all indicate an opening up or unfolding of the core particle at low salt. For the pH transition the observed changes in $\underline{r}$ and $\Delta\epsilon_{284}$ are about four-fold smaller in magnitude than those for the low-salt transition. Considering that no significant change in $S_{20,w}$ is seen, increased pH apparently only induces a loosening rather than a significant opening of the structure.

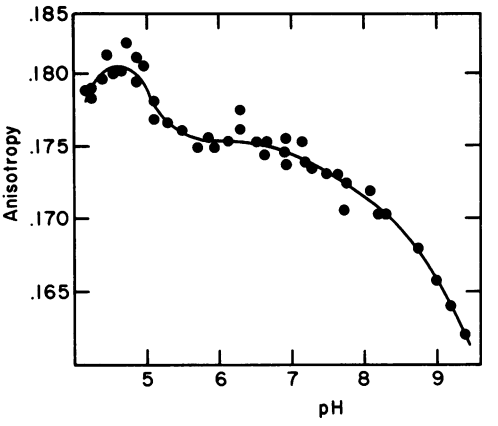

Figure 4. Effect of pH on core particle tyrosine fluorescence at 1 mM monovalent cation. Core particles were diluted to a concentration of 1 μM (A_{260} = 2) and an NaCl concentration of 0.8 mM. The core particle stock solution had been dialyzed to low salt as described (8). The monovalent cation concentration contributed by the core particles has been estimated to be 0.2 mM for A_{260} = 2; thus the total monovalent cation (Na^+) was near 1 mM. The pH, which was initially 7, was adjusted to 9.4 with 1 mM NaOH. Lower pHs were then obtained by additions of 1 mM HCl. A titration was also done starting at pH 5.5 and going to higher values (data not shown); the small differences in the curves started at 5.5 and 9.4 were not significant. For this experiment it was necessary to maintain an atmosphere of N_2 over the solution to avoid pH drifts resulting from absorption of CO_2. The solution before addition of core particles was degassed under vacuum in order to minimize dissolved CO_2.

Considering that electrostatic repulsion within the core particle is the most likely driving force for the low-salt transition,[8,11] one can predict that a loosening of the core particle structure would result in a shift of the low-salt transition to higher ionic strength (i.e., it would require less electrostatic repulsion). This is exactly what occurred, suggesting a causal relationship between the pH transition and the observed effect of pH on the low-salt transition.[8]

In an effort to test this suggestion the experiment shown in Figure 4 was done. The monovalent cation concentration was held at about 1 mM while the pH was varied. This cation concentration was chosen because the effect of pH on the core particle tyrosine anisotropy would be near maximal. Changes in $\underline{r}$ will then give an indication of how much the transition shifts with pH. Instead of showing a relationship between the pH transition and the effects of pH on the low-salt transition, the results in Figure 4 suggest a titration of groups with pK_a's on the order of 9 or 10. Since there are many groups within the particle which would begin to titrate in

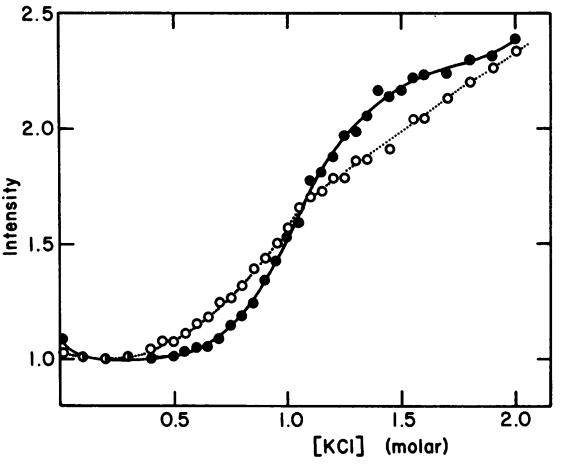

Figure 5. Effect of low and high pH on salt dissociation of core particles. The pH was controlled by 0.01 M sodium cacodylate, pH 6.0 (filled circles) or 0.01 M Tris/HCl, pH 8.2 (open circles). Samples for each KCl concentration and pH were made up by diluting a core particle stock solution (A_{260} = 45) into an appropriate salt-buffer solution. The final core particle concentration was 1 μM (A_{260} = 2). Measurement of tyrosine fluorescence anisotropy was done 30 minutes after the sample was prepared.

this pH range (lysine, tyrosine), it appears that the pH induced shift of the low-salt transition results from an increase in overall charge at higher pH. The accompanying increase in electrostatic repulsion would be expected to cause a shift of the low-salt transition to higher ionic strength.

In Figure 4 a small change in r is observed centered near pH 5. This result corresponds to a change, also centered at pH 5, which was observed at very low salt (the lowest obtainable, ca. 0.1 mM; Figure 4B in reference 8). This was attributed to an ability of hydrogen ions to substitute for metal cations in effecting the transition of the particle to the high salt form.

The effect of pH on high salt dissociation of core particles is illustrated in Figure 5. Both r and I show large changes within the range of salt concentration over which the core histones are known to dissociate from the DNA (0.5 to 2 M). In this case I is the more informative parameter since a 2.6-fold increase occurs between 0.1 and 2.5 molar KCl. Figure 5 shows that core particles at pH 8 are distinctly more sensitive to KCl concentration between 0.3 and 1.0 M than particles at pH 6. At the higher pH an increase in fluorescence intensity becomes noticeable just

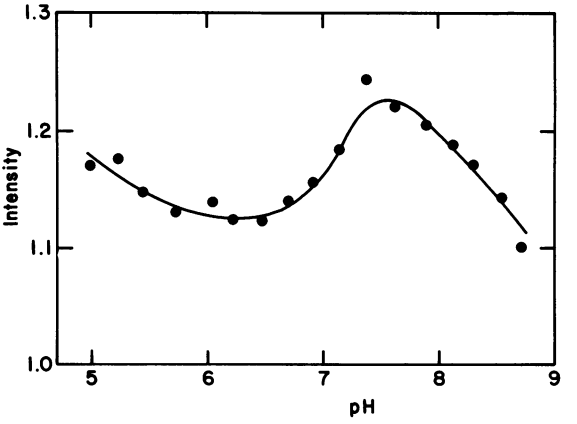

Figure 6. Effect of pH on core particle tyrosine fluorescence intensity at 0.7 M ionic strength. Core particles were diluted to 1 μM (A_{260} = 2) and 0.7 M NaCl. The pH was adjusted to 5, then titrated using 0.7 M NaOH. Measurements of the tyrosine fluorescence on samples removed during the titrations was done 1 to 3 hours later. The pH was then determined directly on the individual samples. Exposure to pH 5 before the titration does not appear to affect the shape of the curve since comparable results are obtained when a core particle stock at pH 7 is diluted directly to 0.7 M salt and various pHs.

above 0.3 M salt while, at pH 6, 0.5 M KCl is required. The much steeper rise in I at pH 6 further suggests that the steps involved in the dissociation process at pH 6 are more cooperative than at pH 8.

Figure 6 shows the effect of pH on tyrosine fluorescence intensity for a constant salt concentration of 0.7 M. As expected, raising the pH from 6 to 8 results in an intensity increase which reflects a decreased stability of the core particles at the higher pH (Figure 5). Near pH 6 the curve clearly plateaus as expected for a titration centered near pH 7. Below 6 an effect of unknown origin causes the curve to turn upward. Above 7.5 the shape of the titration is distorted by partial ionization of tyrosine residues to the much less fluorescence tyrosinate ion, an effect which was also noted at 0.1 M salt (Figure 1B). In spite of the distortion these results still suggest a relationship between the pH transition noted at near physiological ionic strength (Figure 1A) and the decreased stability of core particles to salt dissociation at higher pH (Figure 5).

CONCLUSION

Our results show that core particles undergo a reversible transition with increasing pH, centered at pH 7 and occurring at near physiological

ionic strengths. Changes in the protein core are suggested by dimethylsuberimidate cross-linking which blocks the transition. Sedimentation results indicate that no gross changes in size or shape are involved. Changes in tyrosine fluorescence anisotropy and in the CD spectrum both indicate a somewhat looser structure at high pH. A looser structure is further indicated by the observation that core particles begin to dissociate at considerably lower salt concentration at pH 8 as compared to pH 6.

A small increase in intracellular pH (about 0.3 units) is correlated with the DNA synthesis which occurs during the S phase of the cell cycle.[1-4] Such a pH increase, by causing a destabilization of the core protein structure, might help to facilitate replication either directly or by effects on the higher order structure of chromatin. Although the intracellular pH changes are small, they could have large effects on chromatin structure if a high degree of cooperativity existed.

ACKNOWLEDGMENT

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