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IONIC STRENGTH AND TEMPERATURE INDUCED CONFORMATIONAL CHANGES IN MONONUCLEOSOMES AND OLIGONUCLEOSOMES

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Chromatin is a nucleohistone complex which exhibits a repeat unit structure as inferred from nuclease digestion studies. The repeat unit, or nucleosome, is defined as ~200 base pairs of DNA wrapped about the surface of an octameric histone complex (two copies each of the histones H2A, H2B, H3, and H4). We report in this communication preliminary studies on the conformation of chromatin mononucleosomes and oligonucleosomes as a function of temperature and ionic strength. The methods used were conductivity, fluorescence of bound proflavine, and quasielastic light scattering.

Chicken erythrocyte chromatin was digested at 37°C with micrococcal nuclease as described by Rill et al. (1978). The sample was placed on an A5m column (200–400 mesh) and the fractions designated by tube number (200 drops/tube).

The monomer pool from the A5m column was rechromatographed on a second column (A0.5m,100–200 mesh) and the tube with maximum absorption at 260 nm was designated as sample D. The tubes on either side were designated D–1 and D+1. Polyacrylamide gel electrophoresis indicated that no proteolysis of the histones occurred and that the distribution of DNA lengths of the monomer was biomodal (144–153,162–181 base pairs). Some dimer contamination was observed in sample D-1 but not in sample D+1. The conductivity increment (C'-C)/C for mononucleosomes in 1 mM cacodylate was determined for selected fractions at several temperatures. (cf. Fig. 1*A* caption for definitions of terms). A decrease in (C'-C)/C with temperature from 5° to 35°C suggests the mononucleosomes absorb ions from solution, which is then followed by an apparent release of ions at higher temperatures. These data suggest at least one conformational change occurs at 35°C. Proflavine was used as a probe to study the thermally induced conformational change of mononucleosomes in 1 mM cacodylate and reported as the function (D/P)(F'-F)/F. (cf. Fig. 1*B* caption for definitions of terms). These data suggest conformational changes may occur at 30°, 35°, 37°, and 42°C.

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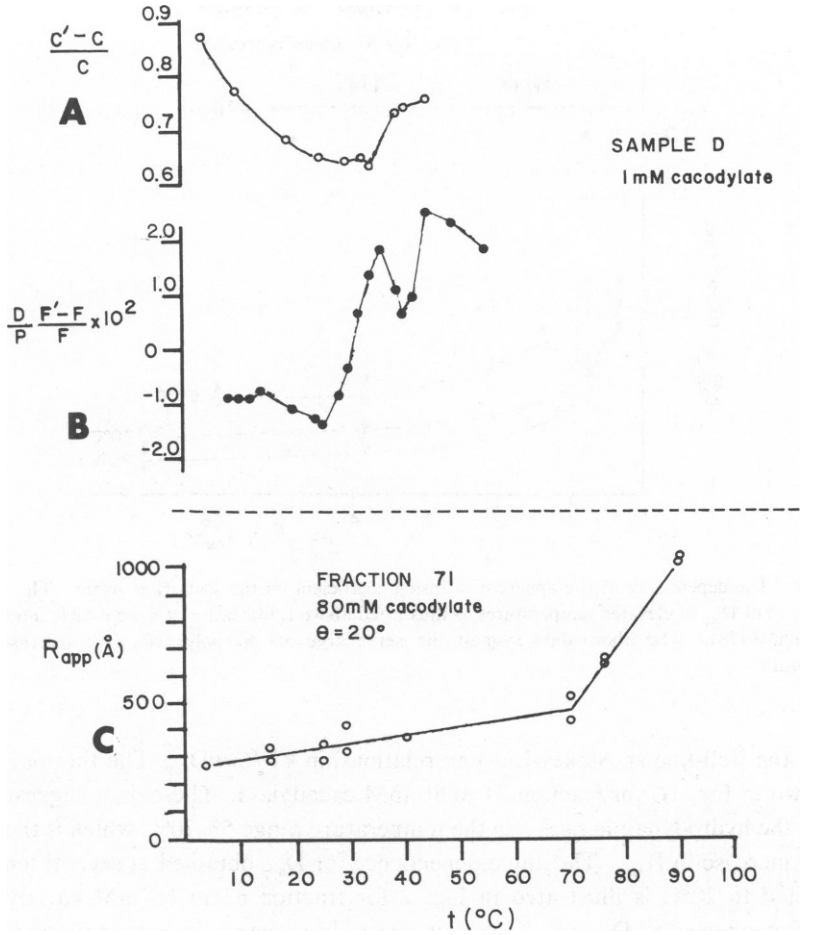

Figure 1 Thermal profiles of: (*A*) Conductivity increment for mononucleosomes in 1 mM cacodylate. The conductivity of sample (C') and solvent (C) was determined with a Barnstead conductivity bridge. The conductivity increment (C'-C)/C illustrated above suggest a transition in the mononucleosome occurs at ~35°C. (A_{260} 0.2). (*B*) Reduced fluorescence of mononucleosome—proflavine complex. The reduced fluorescence, D(F'-F)/PF, where D is the proflavine concentration, P is the phosphate concentration, and F'(F) is the fluorescence at 505 nm of proflavine in the presence (absence) of mononucleosomes (D/P) = 1/24). These data suggest the mononucleosome undergoes at least four conformational changes. (*C*) The apparent Stokes radius. The apparent Stokes radius was calculated from the diffusion coefficient at a scattering angle of 20°. The gradual expansion followed by a rapid change in radius at 70°C suggests the polynucleosome conformation is flexible.

We could not detect similar changes in the conductivity or fluorescence for mononucleosomes in buffers above 10 mM ionic strength. This apparent null result may be due either to the absence of such changes or to the insensitivity of the measurement to changes in the higher ionic strength buffer. Studies on the conformation of oligonucleosomes (fractions 67 and 71) were performed, therefore, in ionic strengths of 65 and 80 mM cacodylate in an attempt to avoid complications that might arise from changes in the conformation of the repeat unit.

Quasielastic light scattering studies were carried out on oligonucleosomes as previously described (Shaw and Schmitz, 1979). The apparent diffusion coefficient D_{app} is defined as $1/(2\tau K^2)$, where τ is the relaxation time for the autocorrelation function and K is the scattering vector, which is defined as $(4\pi n/\lambda)\sin(\theta/2)$. The apparent Stokes radius R_{app} is

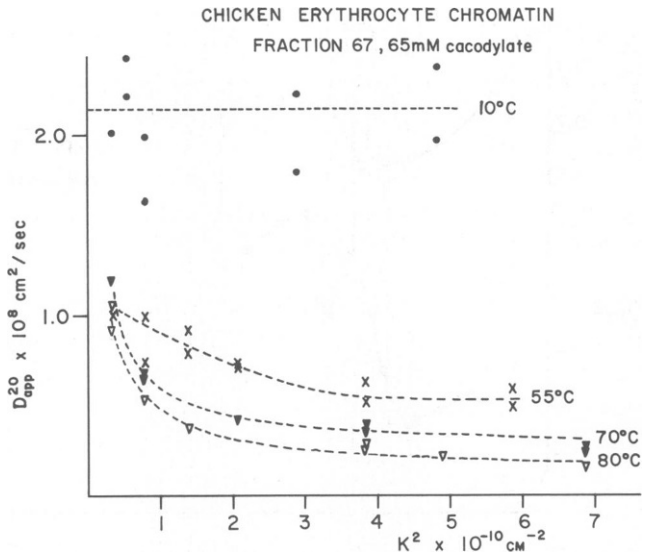

Figure 2 The dependence of the apparent diffusion coefficient on the scattering vector. The angular dependence of D_{app} at elevated temperatures as illustrated above is indicative of interparticle interactions (Ackerson, 1978). The above data suggest the net charge on the polynucleosome increases with temperature.

defined by the well-known Stokes-Einstein relationship $kT/6\pi nD_{app}$. The thermal profile for R_{app} is shown in Fig. 1*C* for fraction 71 in 80 mM cacodylate. These data suggest a gradual increase in the hydrodynamic radius in the temperature range 5°–70°C which is then followed by a sharp increase in R_{app}. The angle dependence for D_{app} obtained at several temperatures and corrected to 20°C is illustrated in Fig. 2 for fraction 67 in 65 mM cacodylate. The apparent dependence of D_{app} on K for low scattering vectors is indicative of interactions between particles (Ackerson, 1978).

Our data suggest the following model: (*a*) Depending on the temperature range, the mononucleosome can either absorb or release counterions as inferred from the 1 mM cacodylate data; (*b*) The conformation of the oligonucleosome appears to be stabilized by two forces, an attractive force between nucleosome units which tends to make the structure more compact and opposing Brownian (thermal) forces; (*c*) The attractive forces between nucleosome units of an oligonucleosome are short-ranged and eventually are overcome by Brownian forces at a temperature that depends on the ionic strength and molecular weight (data not shown); and, (*d*) The apparent charge on the oligonucleosome increases with increasing temperature.

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