Volume 8 Number 21 1980 Nucleic Acids Research

The roles of H1, the histone core and DNA length in the unfolding of nucleosomes at low ionic strength

John B.E.Burch and Harold G.Martinson

Division of Biochemistry, Department of Chemistry, and the Molecular Biology Institute, University of California, Los Angeles, CA 90024, USA

Received 4 August 1980

ABSTRACT

Calf thymus nucleosomes exhibit two different and independent hydrodynamic responses to diminishing salt concentration. One change is gradual over the range 40 to 0.2 mM Na^+ and is accompanied by decreases in contact-site cross-linking efficiency. The other change is abrupt, being centered between 1 and 2 mM Na^+. We find only one abrupt change in sedimentation rate for particles ranging in DNA content from 144 to 230 base pairs. This response to decreasing ionic strength is similar for particles of both 169 and 230 base pairs. Core particles (144 base pairs) exhibit a somewhat diminished response. The abrupt change is blocked by formaldehyde or dimethylsuberimidate cross-linking. The blockage by dimethylsuberimidate demonstrates that the abrupt conformational change requires the participation of the core histones. H1 completely blocks the abrupt but not the gradual conformational change. Thus H1 uncouples the different responses to low ionic strength and exerts an important constraint on the conformational states available to the nucleosome core.

INTRODUCTION

Much progress has been made recently in understanding the basic structure of chromatin[1]. Chromatin is comprised of repeating subunits termed nucleosomes which are organized along the DNA with a periodicity of about 200 base pairs. Nucleosomes can be prepared in monodisperse form by excision from high molecular weight chromatin using micrococcal nuclease which preferentially attacks the DNA between nucleosomes. The average amount of DNA per nucleosome in the nucleus is somewhat variable, each tissue and organism having a characteristic average. However, under appropriate conditions of digestion by micrococcal nuclease a well-defined elementary particle is obtained which contains 168 base pairs of DNA[2] wrapped probably in two turns[1,3,4] around the eight "core" histones (2 each of H2A, H2B, H3, H4). Further digestion yields the familiar and more stable 146 base pair subnucleosomal entity known as the core particle[1].

Chromatin also contains a fifth histone--H1, bound at a peripheral location on the nucleosome. H1 is not present on 146 base pair core particles

but the 168 base pair particles can be prepared without the loss of this histone[3]. Such particles, termed "chromatosomes," contain one molecule of H1 at the periphery, and this H1 apparently contacts both the DNA and the core histones[5]. Simpson[3] has proposed that the chromatosome is the minimal complete subunit of chromatin.

The mechanism by which nucleosomes accommodate the metabolic functions of their DNA remains an intriguing problem. However, it is now apparent that conformational flexibility is an important, physiologically relevant design feature of chromatin subunits[6-10]. Therefore, we have been engaged in characterizing the conformational states which chromatin subunit particles may assume in response to various types of stress *in vitro*[11,12]. In a previous paper we reported that specific histone-histone contacts are disrupted when nuclei are stressed by subjecting them to conditions of very low ionic strengths[11]. In addition, we and others have observed that the sedimentation coefficient of isolated nucleosomes decreases over a similar range of salt concentration[11,13,14]. However, there has been uncertainty concerning whether the observed decrease in sedimentation coefficient reflects a discrete conformational change which occurs in one step[11,15,16], or in two steps[13], or whether it even reflects a conformational change at all[17]. It has been suggested that the length of DNA associated with the particle may determine the number of steps[14,18].

In this report we show that as the ionic strength is lowered, calf thymus nucleosomes experience a substantial decrease in sedimentation rate. This decrease is largely gradual but at about 3 mM Na^+ a sharper decrease commences which is clearly attributable to an abrupt conformational change involving the histone core. We show that this abrupt change occurs in a single step centered between 1 and 2 mM Na^+ under our conditions regardless of the length of the DNA associated with the particle. We also have investigated the effect of the non-core histone, H1, on the ability of nucleosomes to undergo this discrete conformational change. We find that, as measured by sedimentation, H1 completely blocks the abrupt unfolding of nucleosomes which occurs near 2 mM Na^+.

MATERIALS AND METHODS

Isolation of Nuclei. Calf thymus nuclei were prepared in phosphate-buffered saline-EDTA, pH 7.5, as previously described[11]. Two 40 g portions of frozen calf thymus were homogenized separately in 600 ml of 24 mM EDTA, 20 mM sodium phosphate, 45 mM NaCl, pH 7.5, containing phenylmethylsulfonyl

fluoride (6.0 ml of a 0.1 M solution in dimethylsulfoxide) and octanol (1.0 ml) as protease and antifoaming agents, respectively. The homogenates were filtered, centrifuged, combined, washed once by homogenization in 400 ml of the same buffer containing 1 mM phenylmethylsulfonyl fluoride and centrifuged. All steps were carried out at 4°C.

Preparation of Chromatosomes. Chromatosomes were prepared following the protocol of Simpson[3]. The nuclear pellet was resuspended in approximately 200 ml of 0.25 M sucrose, 10 mM Tris, 1 mM $CaCl_2$, pH 8.0, containing phenylmethylsulfonyl fluoride (2.0 ml of a 0.1 M solution in dimethylsulfoxide), homogenized and centrifuged. After a second wash in the same volume of this buffer, the nuclei were resuspended in 0.25 M sucrose, 10 mM Tris, 1 mM $CaCl_2$, pH 8.0, with a Dounce homogenizer to give an A_{260} of approximately 100.

A 40 ml aliquot of the nuclear suspension was warmed to 37°, the pH was readjusted to 8.0, if necessary, by addition of 1 M Tris, then 100 units of micrococcal nuclease (Sigma) were added. After 30-35% of the DNA was rendered acid soluble (as determined in 1 M $HClO_4$, 1 M NaCl), the digestion was terminated by cooling on ice and adding 1/5 volume 100 mM EDTA, pH 7.0. The nuclei were centrifuged at 16,000 g for 15 min, resuspended in approximately 1/10 volume 0.25 mM EDTA, pH 7.0, and dounced to homogeneity. The solution was dialyzed against the same buffer overnight, dounced and clarified by centrifuging for 30 min at 16,000 g. Phenylmethylsulfonyl fluoride was added to a final concentration of 1 mM (1/100 vol of a 0.1 M solution in dimethylsulfoxide) and the sample was fractionated at 4°C using a Sepharose 4B column (2.3 cm x 95 cm) equilibrated with 25 mM Tris, 1 mM EDTA, 0.02% NaN_3, pH 8.0. Fractions (5 ml) were collected and assayed for DNA size and histone content by gel electrophoresis[19]. The monomer fractions were pooled, concentrated using a Millipore immersible separator and dialyzed overnight against 1 mM EDTA, pH 7.6. The A_{260} was adjusted to 40-60 and the particles were fractionated by slowly adding 1/9 volume of 1 M KCl with continuous vortexing. The material insoluble in 0.1 M KCl is predominantly chromatosomes with some oligosome contamination which was removed by sedimenting into a 5-20% sucrose gradient containing 1 mM Tris, 1 mM EDTA, pH 7.5 (SW41; 5°C; 16 hr; 35 K). Fractions (0.4 ml) were collected and monitored for absorbance at 260 nm. Monomer peak fractions were pooled, dialyzed exhaustively against 0.1 mM sodium phosphate, 0.01 mM EDTA, pH 7.2, and stored frozen at -25°C.

Preparation of Chromatosomes Lacking H1. H1 was removed from chromatosomes using 0.45 M salt as described by Simpson[3]. Chromatosomes at an A_{260} of 40-60 in 1 mM EDTA, pH 7.6, were precipitated with KCl as above and resuspended

in 0.45 M NaCl, 1 mM EDTA, pH 7.5, to yield an A_{260} of approximately 40. The monomer particles were freed of H1 by either Sepharose 4B column chromatography in 0.45 M NaCl, 10 mM EDTA, 1 mM Tris, 0.02% NaN_3, pH 7.5, or by sedimentation in 5-20% sucrose gradients containing 0.4 M NaCl, 25 mM Tris, 1 mM EDTA, pH 7.5.

Particles exposed to 0.4 M NaCl are contaminated with free DNA due to disproportionation. Other products of disproportionation, such as monomer subunit particles containing an extra octamer of histone, are presumably also present[20]. To remove these contaminants, particles which had been exposed at any time to salt concentrations in excess of 0.2 M Na^+ were dialyzed against 24 mM EDTA, 20 mM phosphate, 45 mM NaCl, pH 7.5, in which subunit particles containing extra histone are insoluble[20] and then centrifuged at 10,000 g to remove the precipitates. The particles were then separated from the free DNA on sucrose gradients containing 1 mM Tris, 1 mM EDTA, pH 7.5, dialyzed exhaustively against 0.1 mM sodium phosphate, 0.01 mM EDTA, pH 7.2, and stored frozen at -25°C.

Preparation of Core Particles. Core particles were obtained by redigesting mononucleosomes to 10% acid solubility with micrococcal nuclease as described previously[19]. The core particles generated were purified by centrifugation on a 5-20% sucrose gradient containing 24 mM EDTA, 20 mM phosphate, 45 mM NaCl, pH 7.2 (SW27; 4°C; 24 hr; 27 K). The peak fractions were pooled, dialyzed exhaustively against 0.1 mM phosphate, 0.01 mM EDTA, pH 7.2, and stored frozen at -25°C.

Preparation of Large Nucleosomes Lacking H1. Nuclei were prepared as described for chromatosomes. 25 units of micrococcal nuclease (Sigma) were added to a 50 ml aliquot of nuclei at an A_{260} of 65 and digestion was carried out at 4° (to maximize the yield of nucleosomes containing large DNA[21]) for several hours until approximately 3% of the DNA was rendered acid soluble. The reaction was terminated with EDTA and the nuclei were then lysed by dialysis against 0.45 M NaCl, 10 mM Tris, pH 7.5, followed by douncing. Debris was removed by centrifugation (16,000 g, 15 min), the supernatant was dialyzed against 0.15 M NaCl, 10 mM Tris, pH 7.1, and centrifuged again to remove the precipitated H1-containing material. The supernatant containing mononucleosomes was concentrated to a final volume of 5 ml and fractionated on Sepharose 4B in the 0.45 M NaCl buffer. Fractions containing mononucleosomes with large DNA were pooled and centrifuged in the same buffer on 5-20% sucrose gradients (SW41; 37 K; 16.5 hr; 4°C) to remove contaminating dinucleosomes. The mononucleosome fractions were pooled, dialyzed against 24 mM EDTA, 20 mM phosphate, 45 mM NaCl, pH 7.5, clarified by centrifugation for 10 min at 10,000 g

to remove particles containing extra histone (see above), concentrated to 2 ml and dialyzed into 1 mM Tris, 1 mM EDTA, pH 7.2. Phenylmethylsulfonyl fluoride was then added and the sample was centrifuged on 5-20% sucrose gradients (SW41; 37 K; 18 hr; 4°C) at low ionic strength (1 mM Tris, 1 mM EDTA, pH 7.2) to remove free DNA. The peak fractions were pooled, dialyzed exhaustively against 0.1 mM sodium phosphate, 0.01 mM EDTA, pH 7.2, and stored on ice.

Cross-Linking Procedures. Particles prepared and stored as described above were used. Formaldehyde fixation was carried out using a slight variation of the method of Simon et al.[22]. Subunit particles at an A_{260} of 1.0-1.5 were dialyzed at 4°C for 3 hr against 2.5 mM phosphate, 0.1 mM EDTA, 20 mM NaCl, pH 6.7, followed by 16 hr against 1% formaldehyde in the same buffer. The cross-linked particles were then dialyzed exhaustively against 0.1 mM sodium phosphate, 0.01 mM EDTA, pH 7.2, and stored on ice. Sedimentation studies were performed within 48 hr after cross-linking. Initially we attempted to carry out the formaldehyde cross-linking following the protocol described by Gordon et al.[13] in which cross-linking is done at an A_{260} of 20. However, analysis of the cross-linked material by gel electrophoresis or sedimentation indicated that much of the material was cross-linked into higher order aggregates. It was to minimize such interparticle cross-linking that we carried out the procedure at the greatly reduced particle concentrations described above. Figure 3 shows that very little interparticle cross-linking occurs under our conditions.

Dimethylsuberimidate cross-linking was carried out at room temperature using a variation of the procedure of Stein et al.[23]. Particles at an A_{260} of 20-40 in 0.1 mM phosphate, 0.01 mM EDTA, pH 7.2, were added very slowly with vortexing to 4.0 ml of 0.1 M sodium borate, pH 10.0, containing freshly added dimethylsuberimidate at 5 mg/ml to give a final A_{260} of approximately 2.0. The reaction was quenched after 40 min by adding 4.0 ml of 0.8 M sodium acetate, pH 5.0, and transferring to ice. The solution was dialyzed overnight against 1 mM phosphate, 1 mM EDTA, pH 7.2, concentrated to 0.5 ml, clarified by centrifugation and run into 5-20% sucrose gradients containing 1 mM phosphate, 1 mM EDTA, pH 7.2 (SW41; 5°C; 16 hr; 35 K) to separate the cross-linked monomer from higher order aggregates which are also produced. The monomer peak was pooled, dialyzed exhaustively against 0.1 mM sodium phosphate, 0.01 mM EDTA, pH 7.2, and stored on ice.

Analytical Ultracentrifugation. Sedimentation coefficients for the monomer subunit particles were obtained using a slight variation of the procedure we described earlier[11]. Subunit particles previously dialyzed exhaus-

tively against 0.1 mM sodium phosphate, 0.01 mM EDTA, pH 7.2, were diluted to an A_{260} of 0.55-0.60 with appropriate mixtures of 0.1 mM sodium phosphate, 0.01 mM EDTA, pH 7.2, and 0.1 mM sodium phosphate, 0.01 mM EDTA, 50 mM NaCl, pH 7.2, to yield the desired $[Na^+]$. For each sample, a 0.152 ml aliquot was diluted with 8 µl 0.1 mM phosphate, 0.01 mM EDTA, pH 7.2, to a final volume of 0.160 ml, transferred to a double sector cell and underlaid with a 0.220 ml aliquot which contained 11 µl of a 16% sucrose solution in 0.1 mM phosphate, 0.01 mM EDTA, pH 7.2. The cells were spun at 40,000 rpm in a Beckman Model E analytical ultracentrifuge at room temperature and the boundaries were monitored at 265 nm using a photoelectric scanner. The sedimentation coefficients obtained from a plot of ln r vs. time were corrected for viscosity and temperature to water at 20°C.

RESULTS

Characterization of the Chromatin Subunit Particles. The subunit particles which we have used in this study are listed in Table I. Their characterization is presented in Figure 1. They fall into three size classes with respect to DNA length--144 ± 6, 169 ± 10, and 230 ± 45 base pairs. Lanes 1, 2a and 2b of Figure 1A are overloaded with DNA to show that the most similar size classes are essentially non-overlapping. For studies on the effect of DNA length, particles of classes 2 and 3 were depleted of H1 so that the only variable among classes 1, 2a and 3 was the length of the DNA. For studies on the role of H1, class 2 particles possessing or lacking H1 were compared. Class 2b particles, from which H1 has not been removed (chromatosomes), are the minimal defined subunits of chromatin which contain all five histones[3].

TABLE I

DNA and Histone Content of the Chromatin Subunit Particles

Class #	Class name	DNA length	Histone Content
1	Core particle	144 ± 6[a]	core histones only
2a	H1-depleted chromatosome	169 ± 10[a]	core histones only
2b	Chromatosome	169 ± 10[a]	core histones + H1
3	Large nucleosome	∿ 230 ± 45[b]	core histones only

[a] Width of overloaded main band in lanes 1 & 2 of Figure 1A.

[b] As determined densitometrically, 80% of the DNA is 185-285 base pairs. Only 5% is distributed (broadly) at < 170 base pairs.

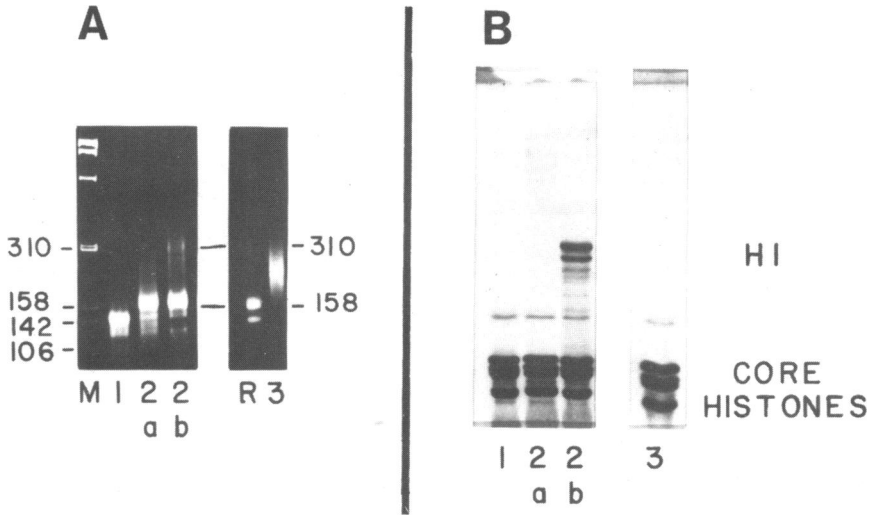

Figure 1. Characterization of chromatin monomer subunit particles. The DNA and protein components of core particles (lanes 1), H1-depleted chromatosomes (lanes 2a), chromatosomes (lanes 2b) and large nucleosomes (lanes 3) are displayed in A and B using the gel methods described previously[19]. Lane M is a *Hae* III restriction digest of phage M13 replicative form DNA. The restriction fragment sizes indicated (in base pairs) are derived from the known sequence of phage M13 DNA[28]. Lane R is DNA obtained from an unfractionated core particle-chromatosome mixture. The two panels in part A were different gels which were calibrated separately. Lanes 1 and 2 were heavily overloaded to reveal minor components. Lane R was not overloaded. The dark band below the position of chromatosome DNA in lane 2b reflects the presence of H1 in that sample[29]. In part B the minor band migrating above the four core histones in each lane is the core semi-histone, A24[19]. The faintly stained material below the core histones is the monomer DNA which migrates more rapidly than histones on our 18% gels.

We have analyzed the DNase I cutting patterns of the core particles and H1-depleted chromatosomes after 5' end-labeling (data not shown) using the method of Simpson and Whitlock[24]. The H1-depleted chromatosomes gave a pattern in agreement with Simpson[3] and Lilley et al.[25] for this species. The core particles similarly yielded a pattern in agreement with that obtained in most other laboratories[21,24,26,27].

H1-Depleted Chromatosomes Undergo a Single Discrete Sedimentation Transision Which Involves the Histone Core. The dependence of $S_{20,w}$ on $[Na^+]$ for 169 base pair H1-depleted chromatosomes is shown in Figure 2 (solid circles). It can be seen that there is a major decrease in $S_{20,w}$ between 3 mM and 1 mM Na^+. The decrease in $S_{20,w}$ in this range reflects a conformational change in

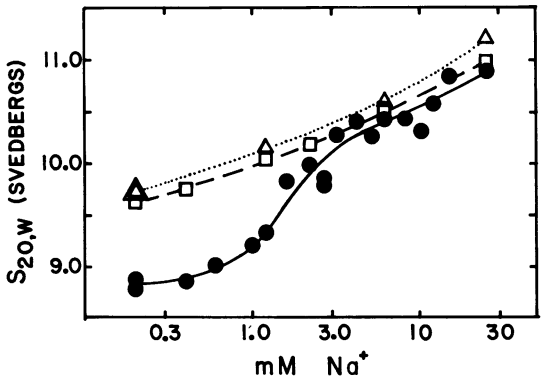

Figure 2. Sedimentation coefficients for H1-depleted chromatosomes as a function of salt concentration: non-cross-linked (●); formaldehyde-cross-linked (□); dimethylsuberimidate-cross-linked (△). The lines shown were fitted by eye to each set of points. The transition is centered at 1.5 mM Na^+.

the particles as demonstrated by the fact that it is blocked by formaldehyde cross-linking (Figure 2, open squares). Both non-cross-linked and cross-linked H1-depleted chromatosomes show a similar gradual decrease in $S_{20,w}$ as the [Na^+] is decreased from 25 mM to 3 mM. However, non-cross-linked H1-depleted chromatosomes exhibit a markedly accentuated decrease in $S_{20,w}$ between 3 mM and 1 mM whereas the decrease for the cross-linked particles remains gradual over the entire range. Below 1 mM the curves, though now displaced, are again parallel.

The decrease in $S_{20,w}$ for the cross-linked particles, though gradual, is nevertheless substantial over the range of ionic strengths examined. We have made no attempt to account for this decrease in quantitative terms. It may be due to an increasing charge effect on $S_{20,w}$, a decreasing tendency of particles to associate or a gradual slight swelling of the cross-linked particles. Regardless of the exact explanation, however, we have verified that the formaldehyde-treated particles were indeed cross-linked by recovering them from the analytical cell following centrifugation and examining them by gel electrophoresis in the presence of sodium dodecyl sulfate. Figure 3 shows that the DNA of these particles has considerably reduced electrophoretic mobility reflecting the presence of covalently bound histones. The reduced mobility of the DNA on the gel does not reflect particle aggregation since $S_{20,w}$ values characteristic of monomer particles are obtained (Figure 2).

We wished to determine whether the abrupt conformational change between 3 mM and 1 mM Na^+ involves simply release of DNA segments from the histone core or whether mobility about histone-histone contacts is required. To address this, we repeated the cross-linking controls described above except that dimethylsuberimidate instead of formaldehyde was used. Unlike formaldehyde,

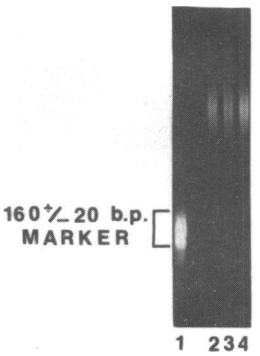

Figure 3. Analysis of formaldehyde-cross-linked H1-depleted chromatosomes. The samples which gave the 1.2 mM and 0.2 mM data points of Figure 2 were recovered from the analytical ultracentrifuge cells after vigorous shaking, electrophoresed in the presence of 0.1% sodium dodecyl sulfate (which removes noncovalently-bound protein from the DNA) and stained for DNA with ethidium bromide (lanes 2 and 3). Lane 4 is a heavier load of similar material prior to sedimentation. When gels such as this one are stained for protein, lanes loaded with formaldehyde-cross-linked particles reveal comigration of the histone and DNA (not shown). DNA from non-cross-linked chromatosomes would migrate alongside the upper portion of the heterogeneous 140-180 base pair marker DNA shown in lane 1.

which induces both histone-histone as well as histone-DNA cross-links[30], dimethylsuberimidate induces only histone-histone cross-links without covalently attaching histone to DNA[23]. Figure 2 shows that the dimethylsuberimidate (open triangles) and the formaldehyde (open squares) cross-linked particles are essentially identical in their response to decreasing ionic strength. In particular, dimethylsuberimidate is as effective as formaldehyde in blocking the discrete transition in $S_{20,w}$ which occurs between 3 mM and 1 mM Na^+ for non-cross-linked particles. Therefore, the low ionic strength-induced conformational change involves and requires participation of the histone core.

Figure 4 verifies that the dimethylsuberimidate-cross-linked particles contained cross-linked histones but no histone-DNA cross-links. Gel A shows that dimethylsuberimidate cross-linked particles, repurified as described in Materials and Methods, contain histones which are present almost exclusively as cross-linked octamers. Figure 4, Gel B shows that dimethylsuberimidate cross-linking, in contrast to formaldehyde cross-linking (Figure 3), has no detectable effect on the electrophoretic mobility of the DNA of the cross-linked particles. Thus the ability of dimethylsuberimidate to block the low ionic strength-induced conformational change of H1-depleted chromatosomes is attributable to the immobilization of the histone core of the particles and is not the result of any cross-linking between histones and DNA.

Wasylyk and Chambon[31] have suggested that histone-DNA interactions may be significantly strengthened as a result of the reaction of dimethylsuberimidate with histones because the pK of the derivatized lysines is higher than that of unreacted lysines[32]. It seems questionable to us that at near neutral pH an increase in pK of the lysines could be of significant consequence. Moreover,

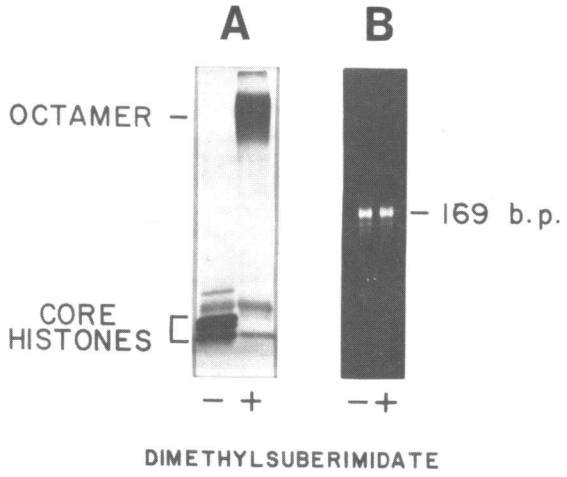

Figure 4. Analysis of dimethylsuberimidate-cross-linked H1-depleted chromatosomes. Following sedimentation at 0.2 mM Na^+ (see Figure 2), the non-cross-linked and dimethylsuberimidate-cross-linked H1-depleted chromatosomes were recovered from the analytical ultracentrifuge cells (after vigorous shaking), dissolved in sodium dodecyl sulfate and then divided in half for electrophoresis. (A) 12% polyacrylamide gel stained for protein with Coomassie blue. The identification of the cross-linked protein species as a histone octamer was deduced from the characteristic dimer, trimer, etc., ladder pattern[23] which was obtained when earlier time points were analyzed on the same gel (data not shown). Note that histones H3 and H2B are not resolved and that DNA stains as a faint band above the core histones on our 12% protein gels. (B) 4% polyacrylamide gel stained for DNA with ethidium bromide. The mobility of the 169 base pair DNA is unchanged by dimethylsuberimidate treatment. Note that the gels for both A and B were run in the presence of 0.1% sodium dodecyl sulfate which dissociates non-covalently-bound protein from DNA.

in one study which directly addresses this point, reaction of chromatin with a monofunctional imidate resulted in weakening, if anything, of the interaction of histone H1 with chromatin[33]. In another study, reaction of core particles with dimethylsuberimidate was found to have no effect on the release of DNA from the histone core under the influence of temperature[34]. All of the results which Wasylyk and Chambon[31] sought to explain based on changes in pK can be readily understood in terms of the immobilization conferred on the histones by dimethylsuberimidate cross-linking.

Core Particles Undergo a Similar Conformational Transition. Core particles (144 ± 6 base pairs DNA) were characterized by sedimentation as a function of $[Na^+]$ as described for the H1-depleted chromatosomes (169 ± 10 base pairs DNA). Figure 5 summarizes the data using symbols comparable to

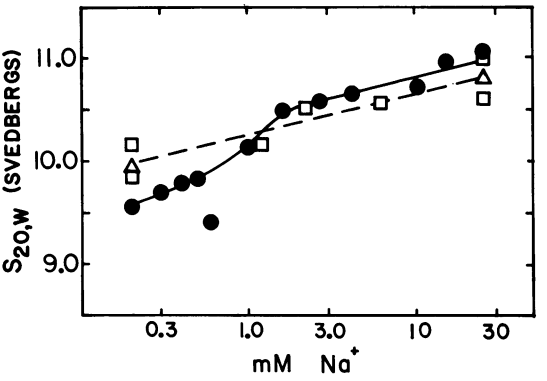

Figure 5. Sedimentation coefficients for core particles as a function of salt concentration: non-cross-linked (●); formaldehyde-cross-linked (□); dimethylsuberimidate-cross-linked (△). Gel analysis of the cross-linked particles gave results equivalent to those of Figures 3 and 4. The lines shown were fitted by eye to the points. The transition is centered at 1.1 mM Na^+.

those of Figure 2. It can be seen that non-cross-linked core particles (solid circles) undergo a discrete transition in $S_{20,w}$ similar to that exhibited by the H1-depleted chromatosomes. However, the transition appears to occur at a slightly lower Na^+ concentration and is somewhat smaller. We will discuss this later. The transition is blocked by either formaldehyde or dimethylsuberimidate cross-linking, demonstrating that it represents, as for the H1-depleted chromatosomes, a conformational change involving the histone core. We note that the $S_{20,w}$ for core particles is higher than for H1-depleted chromatosomes at all ionic strengths tested, a trend which extends also to H1-depleted particles of even longer DNA than chromatosomes (see below).

Large H1-Depleted Nucleosomes Do Not Show a Transition Above 3 mM Na^+. It has been reported that H1-depleted nucleosomes display two transitions in $S_{20,w}$; one at about 1-2 mM as we find and, in addition, another at about 8 mM ionic strength[13,14]. However, the nucleosomal particles of Gordon et al.[13,14] are now known to have been very heterogeneous in DNA length (140-200 base pairs; V. Schumaker, personal communication). We were therefore curious as to whether one of the transitions in $S_{20,w}$ observed by Gordon et al.[13,14] might have been due to a subset of nucleosomes which contained long DNA. Such a subset could account for the 8 mM transition of Gordon et al.[13,14] if large particles either have two transitions or if they have a single transition shifted to a higher concentration of salt.

To determine whether "large nucleosomes" display a transition at 8 mM, we prepared H1-depleted nucleosomes containing 230 ± 45 base pairs of DNA (weight average) and measured the dependence of $S_{20,w}$ on $[Na^+]$ for these particles. The data obtained are shown in Figure 6 as open circles. Also shown in Figure 6 for comparison are the data from Figure 2 for H1-depleted chromatosomes (solid circles). These two sets of data are strikingly similar. Indeed, apparently the only significant difference is the shift to lower $S_{20,w}$ values at all salt concentrations for the particles with longer DNA (a trend already noted for H1-depleted chromatosomes compared to core particles). To illustrate this further, in Figure 6 we have taken the best fit curve for the data from H1-depleted chromatosomes (upper curve) and displaced it to coincide with the data points for the large nucleosomes (lower curve). Clearly, identically shaped curves fit both sets of data comparably well. Thus it is evident that large nucleosomes are qualitatively similar to H1-depleted chromatosomes and also core particles in their response to low ionic strength. In particular, there is no evidence for any discrete transition between 3 and 40 mM Na^+ for the large nucleosomes. The only discrete transition we find for

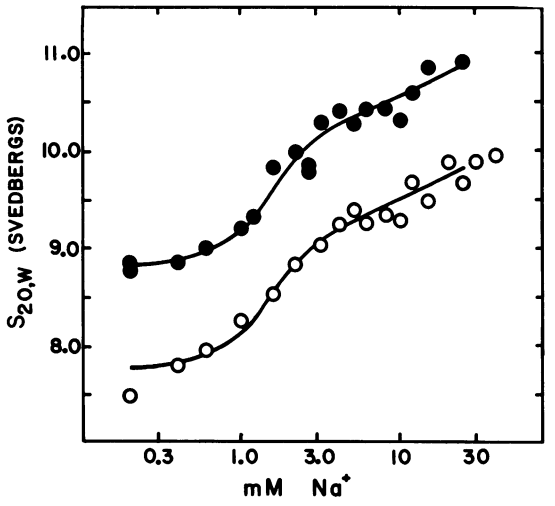

Figure 6. Sedimentation coefficients for H1-depleted large nucleosomes (○) as a function of salt concentration. The chromatosome data of Figure 2 (●) are presented for comparison (see text). Both types of H1-depleted preparation contained about 5% free DNA which, however, did not contribute to the calculated $S_{20,w}$ values. Note that the very similar curves for particles of greatly differing charge show that this parameter contributes negligibly to the dependence of $S_{20,w}$ on salt concentration which we observe.

any particle is that which occurs between 1 and 3 mM Na^+.

The Presence of H1 on Chromatosomes Blocks the Discrete Low Ionic Strength-Induced Conformational Change. We next investigated the effect of H1 on the low ionic strength-induced conformational change by examining the smallest particle possessing all five histones, namely, the chromatosome[3]. The approach was essentially the same as described above for H1-depleted chromatosomes and core particles. The sedimentation data obtained are presented in Figure 7. The dependence of $S_{20,w}$ on $[Na^+]$ is essentially the same for both non-cross-linked (solid circles) and formaldehyde-cross-linked chromatosomes (open squares). Therefore we conclude that the abrupt conformational change induced in H1-depleted particles between 3 mM and 1 mM Na^+ is blocked by the presence of H1.

DISCUSSION

The Low Ionic Strength-Induced Conformational Changes of Calf Thymus Nucleosomes. The above studies demonstrate that native calf thymus chromatin subunit particles containing the eight core histones and monomer length DNA change shape abruptly in a discrete transition between 3 mM and 1 mM Na^+ as the ionic strength is reduced. This conformational transition requires the participation of the histone core and is blocked by H1.

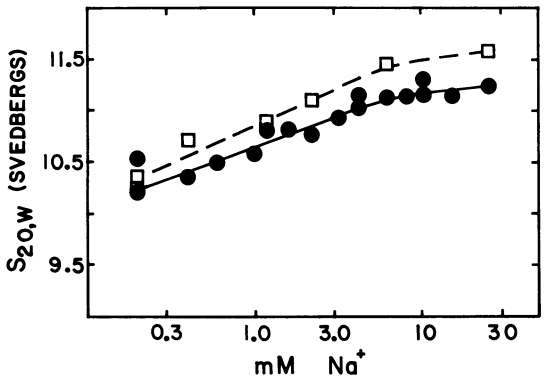

Figure 7. Sedimentation coefficients for (H1-containing) chromatosomes as a function of salt concentration: non-cross-linked (●); formaldehyde-cross-linked (□). Polyacrylamide gel analysis of the formaldehyde cross-linked chromatosomes (data not shown) gave results equivalent to those in Figure 3. The lines shown were fitted by eye to the points. Note that there are two data points (open squares) for cross-linked particles at 0.2 mM Na^+ but the one at about 10.2 S is mostly obscured by the adjacent symbols.

The abrupt transition of H1-depleted subunit particles is superimposed on a gradual decrease in $S_{20,w}$ which is observed over the range 0.2-40 mM Na^+. Unlike the abrupt change, the gradual decrease is not blocked in particles containing H1. Preliminary data obtained using contact-site cross-linking probes (see below) suggest that the gradual decrease may reflect in part a gradual change in conformation which is independent of the abrupt transition.

Effect of DNA Length. The 168 base pair chromatosome is believed to be the true complete fundamental subunit of chromatin[2,3]. Therefore, we will take our 169 base pair H1-depleted particles as the point of departure in discussing DNA length.

Figure 6 shows that increasing the DNA length beyond chromatosome size has no effect on either the magnitude of the transition or the salt concentration at which it occurs. The only effect of the extra DNA is to reduce the sedimentation rates of the particles by essentially the same amount at all the salt concentrations tested. This presumably reflects the added asymmetry at these ionic strengths of particles containing extra DNA not bound by the core. The observation that the DNA in excess of 169 base pairs does not detectably affect the transition is consistent with the notion that this DNA length represents a fundamental feature of nucleosome structure[2].

Particles digested beyond the chromatosome stage (i.e., core particles) exhibit a somewhat diminished transition which is shifted to a slightly lower ionic strength (see Figure 5). This suggests that the terminal segments of chromatosome DNA are involved in driving the abrupt unfolding of nucleosomes at low ionic strength. The amount of terminal DNA responsible must be more than the ∿11 base pairs[2] per end[3] which are removed in generating core particles from chromatosomes because core particles do exhibit a considerable "remnant" of the transition (see Figure 5) despite their trimmed state. Of course, whether the core particle remnant represents the initial or final stage of the complete chromatosome transition, or perhaps even a different transition, cannot be distinguished by our data.

Role of the Histone Core. One function of the histone core of nucleosomes may be to guide the nucleosome through orderly conformational changes[11] as required by various physiological stimuli. The electrostatic stress of low ionic strength may cause nucleosomes to change conformation along pathways similar to those followed in response to stresses encountered *in vivo*[35]. Figures 2 and 5 show that immobilization of the histone core by dimethylsuberimidate cross-linking blocks the abrupt low ionic strength conformational transition of both core particles and H1-depleted chromatosomes. This demon-

strates an intimate and necessary participation of the histones in this conformational change. Our results thus complement those of Wu et al.[16] who made a similar finding based on transient electric dichroism measurements of core particles as a function of both ionic strength and dimethylsuberimidate cross-linking [Gordon et al.[14] also carried out dimethylsuberimidate-cross-linking experiments but their results are questionable (see below)].

The dimethylsuberimidate cross-linking data do not address whether the histones assist in driving the change (e.g., by local repulsions between positively charged amino acid residues) or whether they merely must accommodate a change driven by repulsion between adjacent layers of DNA (to which, of course, the histones are bound). These two possibilities are not mutually exclusive. However, even if the DNA provides the principal driving force for the low ionic strength transition, the histones, which are known to be predisposed to certain pathways of dissociation[35], may govern to a large extent the precise pathway of the conformational change.

Recently, Chao et al.[12] have shown that the interaction of lac repressor with synthetic lac nucleosomes at physiological ionic strength also requires a conformational change in the histone core. Similarly, Wasylyk and Chambon[31] have presented data based on dimethylsuberimidate cross-linking which suggest that a conformational change in the histone core may be required for efficient utilization of nucleosomal DNA by RNA polymerase. However, Simpson[34] has reported a reversible thermally-induced change in conformation which occurs *despite* dimethylsuberimidate cross-linking. Therefore not all conformational changes require participation of the histone core.

Role of H1. The abrupt low ionic strength transition of nucleosomes is blocked by H1 (Figure 7). Thus H1 acts in part at the level of individual nucleosomes. The effect of H1 is unlikely to be related simply to the decrease in overall net charge conferred on the particle by H1. Chromatosomes, which contain H1, do not undergo the abrupt change whereas core particles, which lack H1, do undergo this transition. However, both particles have about the same overall net charge. Although simple *net* charge considerations cannot account for the effect of H1 on chromatosomes, it is certainly possible that H1 functions by alteration of *local* charge densities on the particle. Additionally, since the interaction of H1 with the histone core is well documented[5,36,37], the stabilizing effect of H1 may reflect a specific direct influence of H1 on the histone core. This is reasonable given the intimate and obligatory role of the histone core in the conformational change.

The model of Thoma et al.[4] readily accounts for the blockage by H1 of

the low ionic strength transition. In their model, H1 simply fastens the ends of chromatosomal DNA along one face of the particle, thus constraining it in the closed conformation. DNA of 168 base pairs is assumed to comprise two full turns. Whatever the exact mechanism by which H1 prevents nucleosome unfolding, the constraints imposed by this histone could be relieved in "active" chromatin either by the modification or the removal of H1.

One or Two Transitions at Low Ionic Strength? Gordon et al.[13,14] were the first to characterize the low ionic strength dependent conformational changes of nucleosomes by hydrodynamic techniques. However, they reported the existence of two conformational transitions--one centered at about 1 to 2 mM and the other at about 7 to 8 mM ionic strength. They have suggested that the 7-8 mM transition is exhibited only by particles containing DNA in excess of core particle length[14]. Using similar techniques, we confirm the transition centered at 1 to 2 mM but have failed in all efforts to find evidence of a second discrete transition at 7 to 8 mM.

Recently Dieterich et al.[18], using different techniques, presented evidence for a conformational transition which occurs at about 7 mM ionic strength for H1-depleted chromatosomes but at lower ionic strength for core particles. Essentially the only similarity between the studies of Dieterich et al.[18] (who monitored the fluorescence of chemically derivatized H3 in reconstituted particles) and Gordon et al.[13,14] (who monitored the hydrodynamic properties of native particles) is that both used chicken erythrocyte material. Since we have used calf thymus chromatin subunit particles, perhaps our failure to find a discrete transition near 7 mM, both in our previous studies[11] and in those reported here, reflects a species specific difference in the response to low ionic strength. This attractive explanation is not without difficulties, however, because Fulmer and Fasman[38] obtained evidence for only the one transition near 3 mM by monitoring the circular dichroism of high molecular weight H1-depleted chicken erythrocyte chromatin up to between 9 and 10 mM ionic strength. Gordon et al.[14] reported that *both* transitions were accompanied by changes in circular dichroism. Using calf thymus H1-depleted chromatosomes, we have confirmed the circular dichroism result of Fulmer and Fasman[39] (not shown). Fulmer and Fasman[38] used a phosphate buffer as have we. The buffers of Gordon et al.[13,14] and Dieterich et al.[18] did not contain phosphate. Therefore, detection of a transition near 7 mM may depend on the nature of the buffer. We emphasize that our data do not bear on whether the transitions exhibited by 144 and 169 base pair particles are physically similar or distinct or whether the "single" transition exhibited by the larger

particles is simple or complex.

A Gradual Conformational Change Not Blocked by H1. Previously we observed, using contact-site cross-linking techniques, an intranucleosomal contact surface between histones H2B and H4 which was gradually disrupted in nuclei as the ionic strength was reduced[11]. To determine the relationship of this gradual conformational change of whole chromatin to the changes in $S_{20,w}$ which occur for monomer particles, we applied the contact-site cross-linking procedures to H1-depleted chromatosomes. Contact-site cross-linking of these particles as a function of salt concentration (Burch and Kunkel, data not shown) gave results similar to those obtained previously with nuclei[11] in that disruption of the individual H2B-H4 contacts is gradual as the salt concentration is reduced. Thus the conformational change detected by cross-linking may be related to the gradual component of the decrease in $S_{20,w}$ between 25 and 0.2 mM Na^+. This suggests that the gradual decrease in $S_{20,w}$ may reflect, in part, a swelling of the particle sufficient to displace contacts over short distances. In addition, we have found that the dependence on ionic strength of the "rupture" of these contact sites is similar in the presence or absence of H1 (Martinson et al.[11], and unpublished results). This suggests, consistent with the sedimentation results, that any gradual swelling which occurs does so independently of the large abrupt transition which occurs between 1 and 3 mM Na^+.

We hasten to add, however, that the gradual change is a complex process[11] and that preliminary data currently available raise the possibility that the gradual decreases in $S_{20,w}$ exhibited by the various particles (e.g., cross-linked core particles vs. chromatosomes, or particles cross-linked using formaldehyde vs. dimethylsuberimidate) may reflect different combinations of underlying molecular events. In particular, we leave open the possibility that even the cross-linked particles may be capable of some swelling, the precise nature of which probably depends on the type of cross-linking employed. It seems unlikely to us, for example, that above 5 mM Na^+ in Figure 2 the striking dependence of $S_{20,w}$ on salt concentration for cross-linked particles is solely the result of charge effects on sedimentation (see also legend to Figure 6). Since neither formaldehyde nor dimethylsuberimidate is capable of immobilizing *all* contacts in the particle, it seems probable that significant particle flexibility remains. Experiments conducted on particles cross-linked by a combination of agents should assist in evaluating this possibility. Also required are determinations of the effect (if any) below an A_{260} of 0.6 of particle concentration on $S_{20,w}$. This would reveal any contribution of

interparticle association to the dependence of $S_{20,w}$ on salt concentration.

In contrast to our results, Gordon et al. have reported that particles cross-linked with formaldehyde[13] or dimethylsuberimidate[14] at 10 mM ionic strength exhibit essentially no dependence of $S_{20,w}$ on ionic strength. Possibly their particles contained more cross-links than ours, thus preventing any swelling from occurring. However, we have some reservations concerning the interpretation of their results since they did not characterize their material following cross-linking. For example, in our hands, their method of formaldehyde cross-linking (particularly the high nucleosome concentration) leads to extensive interparticle aggregation which could complicate the analysis, leading to overestimated $S_{20,w}$ values. Also, for dimethylsuberimidate, we have found it necessary to repurify the mononucleosomes to remove aggregated material following cross-linking. In any event, their additional result that $S_{20,w}$ values are lower for particles cross-linked with dimethylsuberimidate at nominally low ionic strengths[14] appears internally inconsistent when it is realized that this reagent is normally supplied as the dihydrochloride (the free imidate is not water soluble) and thus itself contributes substantially to the ionic strength (> 7 mM at 1 mg/ml). Since the nucleosomes were therefore presumably in all cases in their high ionic strength form during cross-linking, the variability in $S_{20,w}$ values subsequently obtained is difficult to interpret. Owing to these uncertainties, we feel a detailed attempt to reconcile our data on *cross-linked* particles with those of Gordon et al.[13,14] is premature.

ACKNOWLEDGEMENTS

We thank V. N. Schumaker and D. M. Crothers for stimulating and helpful discussions, Dan Ray for the gift of M13 DNA, and Shirley Phillips for preparation of the manuscript. This work was supported by NSF Grant PCM 7910654 and by USPHS Training Grant GM 07185.

REFERENCES

1. McGhee, J. D., and Felsenfeld, G. (1980) Ann. Rev. Biochem. 49, 1115-1156.
2. Weischet, W. O., Allen, J. R., Riedel, G., and Van Holde, K. E. (1979) Nucleic Acids Res. 6, 1843-1862.
3. Simpson, R. T. (1978) Biochemistry 17, 5524-5531.
4. Thoma, F., Koller, T., and Klug, A. (1979) J. Cell Biol. 83, 403-427.
5. Boulikas, T., Wiseman, J. M., and Garrard, W. T. (1980) Proc. Natl. Acad. Sci. 77, 127-131.
6. Felsenfeld, G. (1978) Nature 271, 115-122.

7. Gariglio, P., Llopis, R., Oudet, P., and Chambon, P. (1979) J. Mol. Biol. 131, 75-105.
8. Wasylyk, B., Thevenin, G., Oudet, P., and Chambon, P. (1979) J. Mol. Biol. 128, 411-440.
9. Weisbrod, S., Groudine, M., and Weintraub, H. (1980) Cell 19, 289-301.
10. Giri, C. P. and Gorovsky, M. A. (1980) Nucleic Acids Res. 8, 197-214.
11. Martinson, H. G., True, R. J., and Burch, J. B. E. (1979) Biochemistry 18, 1082-1089.
12. Chao, M. V., Martinson, H. G., and Gralla, J. D. (1980) Biochemistry 19, 3260-3269.
13. Gordon, V. C., Knobler, C. M., Olins, D. E., and Schumaker, V. N. (1978) Proc. Natl. Acad. Sci. 75, 660-663.
14. Gordon, V. C., Schumaker, V. N., Olins, D. E., Knobler, C. M., and Horwitz, J. (1979) Nucleic Acids Res. 6, 3845-3858.
15. Dieterich, A. E., Axel, R., and Cantor, C. R. (1979) J. Mol. Biol. 129, 587-602.
16. Wu, H.-M., Dattagupta, N., Hogan, M., and Crothers, D. M. (1979) Biochemistry 18, 3960-3965.
17. Schmitz, K. S. (1980) Fed. Proc. 39, Abstr. 1471, 1885.
18. Dieterich, A. E., Eshaghpour, H., Crothers, D. M., and Cantor, C. R. (1980) Nucleic Acids Res. 8, 2475-2487.
19. Martinson, H. G., True, R., Burch, J. B. E., and Kunkel, G. (1979) Proc. Natl. Acad. Sci. 76, 1030-1034.
20. Stein, A. (1979) J. Mol. Biol. 130, 103-134.
21. Noll, M. and Kornberg, R. D. (1977) J. Mol. Biol. 109, 393-404.
22. Simon, R. H., Camerini-Otero, R., and Felsenfeld, G. (1978) Nucleic Acids Res. 5, 4805-4818.
23. Stein, A., Bina-Stein, M., and Simpson, R. T. (1977) Proc. Natl. Acad. Sci. 74, 2780-2789.
24. Simpson, R. T. and Whitlock, J. R., Jr. (1976) Cell 9, 347-353.
25. Lilley, D. M. J., Jacobs, M. F., and Houghton, M. (1979) Nucleic Acids Res. 7, 377-399.
26. Whitlock, J. P. and Simpson, R. T. (1977) J. Biol. Chem. 252, 6516-6520.
27. Lutter, L. C. (1978) J. Mol. Biol. 124, 391-420.
28. van Wezenbeek, P., Hulsebos, T., and Schoenmakers, J. G. G. (1980) Gene, in press.
29. Todd, R. D. and Garrard, W. T. (1977) J. Biol. Chem. 252, 4729-4738.
30. Jackson, V. (1978) Cell 15, 945-954.
31. Wasylyk, B. and Chambon, P. (1980) Eur. J. Biochem. 103, 219-226.
32. Peters, K. and Richards, F. M. (1977) Ann. Rev. Biochem. 46, 523-551.
33. Tack, L. O. and Simpson, R. T. (1977) Biochemistry 16, 3746-3753.
34. Simpson, R. T. (1979) J. Biol. Chem. 254, 10123-10127.
35. Martinson, H. G. and True, R. J. (1979) Biochemistry 18, 1089-1094.
36. Bonner, W. M. and Stedman, J. D. (1979) Proc. Natl. Acad. Sci. 76, 2190-2194.
37. Ring, D. and Cole, R. D. (1979) J. Biol. Chem. 254, 11688-11695.
38. Fulmer, A. W. and Fasman, G. D. (1979) Biopolymers 18, 2875-2891.