Unfolded Structure and Reactivity of Nucleosome Core DNA–Histone H2A,H2B Complexes in Solution As Studied by Synchrotron Radiation X-ray Scattering†

Montserrat Samsó and Joan-Ramon Daban*

Departament de Bioquímica i Biologia Molecular, Facultat de Ciències, Universitat Autònoma de Barcelona, 08193-Bellaterra (Barcelona), Spain

Received July 13, 1992; Revised Manuscript Received February 11, 1993

ABSTRACT: It has been previously found using different physicochemical techniques [Aragay, A., Diaz, P., & Daban, J.-R. (1988) *J. Mol. Biol. 204*, 141–154] that histones H2A,H2B in the absence of H3,H4 can associate with nucleosome core DNA (146 base pairs). Here we describe a synchrotron X-ray scattering study of core DNA–(H2A,H2B) complexes in solution. Our results obtained using different histone to DNA weight ratios and ionic conditions ranging from very low ionic strength to 0.2 M NaCl show that histones H2A,H2B are unable to fold core DNA. Model calculations indicate that histones H2A,H2B produce very elongated structures even when the reconstituted complexes are prepared at physiological ionic strength. In contrast, our scattering data indicate that the reconstituted complexes prepared at physiological salt concentration either with the four core histones or with histones H3,H4 without H2A,H2B are completely folded particles with a radius of gyration similar to that corresponding to the native nucleosome core (4.2 nm). Furthermore, our results show that the DNA of the extended complexes containing histones H2A, H2B becomes completely folded after the histone pair exchange reaction that occurs spontaneously between preformed DNA–(H2A,H2B) and DNA–(H3,H4) complexes. These observations, together with our previous studies, suggest that the open conformation of DNA–(H2A,H2B) complexes facilitates the involvement of this structure as a transient intermediate in the reaction of nucleosome formation at physiological ionic strength.

Different laboratories have shown that histones H3,H4 in the absence of histones H2A,H2B can organize the typical nucleosome core particle structure (Simon et al., 1978; Read et al., 1985; Hayes et al., 1991; Dong & van Holde, 1991). Furthermore, when in vitro nucleosome assembly is carried out using stepwise salt gradient methods, histones H3,H4 bind first to DNA (from 1.2 to 0.8 M NaCl), followed by histones H2A,H2B (between 0.8 and 0.6 M NaCl) (Wilhelm et al., 1978; Hansen et al., 1991). The essential role of histones H3,H4 in organizing the nucleosome structure has also been suggested from experiments showing that the addition of H2A, H2B to DNA previously complexed with H3,H4 produces normal nucleosome core particles (Ruiz-Carrillo et al., 1979). The ordered histone binding mechanism inferred in these studies could be used by the cell to reassociate the partially dissociated nucleosomes (containing only H3,H4 tetramers) probably produced during chromatin transcription (Jackson, 1990).

The above observations do not imply, however, that histones H2A,H2B in the absence of the other histones are not able to interact with DNA. In fact, it has been found previously using circular dichroism and fluorescence spectroscopy (Oohara & Wada, 1987), and nucleoprotein gel electrophoresis, sedimentation, chemical cross-linking, trypsin and nuclease digestions, and thermal denaturation (Aragay et al., 1988), that histones H2A,H2B in the absence of H3,H4 can form various thermodynamically stable complexes with DNA at low ionic strength and physiological salt concentration. This poses additional problems to the nucleosome self-assembly reaction. Since at physiological ionic strength the octamer is totally dissociated into the H3,H4 tetramer and the H2A, H2B dimer (Eickbush & Moudrianakis, 1978; Ruiz-Carrillo & Jorcano, 1979) even at high histone concentration (Aragay et al., 1991), and since the association of histones with DNA is a very fast process (Daban & Cantor, 1982a; Diaz & Daban, 1986; Pfaffle & Jackson, 1990), it is very likely that during the in vitro assembly both DNA–(H2A,H2B) and DNA–(H3,H4) complexes are rapidly produced. Nevertheless, the rapid histone exchange reactions demonstrated using fluorescence (Daban & Cantor, 1982b) and electrophoretic (Aragay et al., 1988) methods allow the spontaneous formation of core particles from mixtures of DNA–(H2A,H2B) and DNA–(H3,H4) complexes. A mechanism of nucleosome assembly involving an initial random association of separate H2A,H2B dimers and H3,H4 tetramers with different DNA molecules (or different regions of the same DNA molecule) may participate in the formation of new nucleosomes during chromatin replication, and in the reassembly of the completely dissociated nucleosomes observed in actively transcribed genes (Karpov et al., 1984) and in transcription experiments using SP6 RNA polymerase (Lorch et al., 1988).

In this work, we have used synchrotron radiation X-ray scattering techniques to study the structural properties of DNA–(H2A,H2B) complexes in solution. These techniques have been used previously to study the superstructure of chromatin (Bordas et al., 1986; Koch et al., 1989; Fujiwara et al., 1989) and the structural changes of nucleosome cores in solutions of low ionic strength (Hirai et al., 1988). The high flux of X-rays from the storage ring has allowed us to obtain scattering patterns using relatively low concentrations and short irradiation times. This has made possible the analysis of many DNA–(H2A,H2B) samples with different histone to

† This work was supported in part by a DGICYT grant (PB86-0029). Synchrotron radiation experiments performed at SERC Daresbury Laboratory (Warrington, U.K.) were supported by the European Community Large Scale Facilities Programme. M.S. has received support from a predoctoral fellowship from the Plan de Formación de Personal Investigador (Ministerio de Educación y Ciencia, Spain) and a CIRIT fellowship (Generalitat de Catalunya).

*Author to whom correspondence should be addressed.

0006-2960/93/0432-4609$04.00/0

DNA weight ratios and under different conditions. Furthermore, we have compared the scattering data of DNA–(H2A, H2B) complexes with the data obtained for core particles and DNA–(H3,H4) complexes under identical conditions. The results of this study have led us to a structural interpretation of the reactivity and dynamic properties of DNA–(H2A, H2B) complexes.

MATERIALS AND METHODS

Preparation of Histones, Nucleosome Cores, and DNA. Core histones, nucleosome core particles, and core DNA [146 base pairs (bp)][1] were prepared from chicken erythrocyte nuclei as described (Diaz & Daban, 1986; Aragay et al., 1988). The histone pairs H2A,H2B and H3,H4 were prepared from core histones by chromatography on a Sephadex G-100 (superfine) column, eluted with 0.2 M NaCl, 0.4 mM phenylmethanesulfonyl fluoride, 0.03% 2-mercaptoethanol, and 10 mM Tris-HCl, pH 7.4 (Aragay et al., 1991). The fractions of the H2A,H2B and H3,H4 peaks were dialyzed against 20 mM HCl, precipitated with acetone, and dried under vacuum. The resulting histones were analyzed on SDS–polyacrylamide gels stained with the fluorescent dye Nile red (Daban et al., 1991b). The concentration of histone solutions was determined spectrophotometrically, using $A_{230nm} = 4.2$ for 1 mg of histone/mL (Thomas & Oudet, 1979). The concentration of DNA (free or complexed with histones) was calculated by using $A_{260nm} = 20.0$ for 1 mg of DNA/mL, and by assuming that the absorbance of histones at 260 nm is negligible.

Preparation of Samples. Nucleosome core particles and core DNA were dialyzed extensively against the different buffers indicated below. To prepare DNA–histone complexes, histones were dissolved in water before the addition of the required buffer solution. In general, a fresh solution of H2A, H2B, H3,H4, or the four core histones in a given buffer (see Results) was added to a core DNA solution in the same buffer. The histone to DNA weight ratios in these samples were 0.6, 0.8, or 1.1, and the final DNA concentration was 1 mg/mL. In some experiments, the DNA–histone solutions were prepared in TEA buffer containing 2.0 M NaCl, and the reassociation was carried out by stepwise dialysis against solutions of decreasing salt concentration (Aragay et al., 1988) to a final NaCl concentration of 0.2 M. In the transfer reaction experiments, a solution of DNA–(H2A,H2B) in TEA buffer containing 0.2 M NaCl was mixed with a solution of DNA–(H3,H4) in the same buffer. The different DNA–histone samples were centrifuged for 2 min at 10000*g*, and the resulting supernatant was used for the X-ray scattering measurements. After irradiation, samples were analyzed on nondenaturing 6% polyacrylamide gels containing 0.1×TBE as described previously (Aragay et al., 1988).

X-ray Scattering. Small-angle X-ray scattering experiments were carried out on stations 2.1 (Towns-Andrews et al., 1989) and 8.2 (Gerritsen & Robertus, 1990) of the Daresbury Laboratory Synchrotron Radiation Source (Warrington, United Kingdom) using a quadrant detector system (Lewis et al., 1988). Scattering measurements were carried out essentially following a previously described procedure for samples in solution (Daban et al., 1991a). All samples and buffer solutions were degassed for 15 min. Exposure time was 5 min in small cells (80 μL) with thin mica windows. Under these conditions, a constant intensity was observed for the low-angle channels in time-frame control experiments, indicating that radiation damage was negligible. Data were analyzed using the Otoko Program (Synchrotron Radiation Source Program Library, Daresbury Laboratory). The results are plotted as $\log[I(S)]$ versus S, where $I(S)$ is the scattering intensity at $S = 2 \sin \theta/\lambda$, 2θ is the scattering angle, and λ is the wavelength (1.5 Å), and as $\log[I(S)]$ versus S^2 [Guinier plots (Porod, 1982)]. The results obtained were reproducible when equivalent DNA–histone complexes were analyzed in stations 2.1 and 8.2 using camera lengths ranging from 6 to 3.5 m. The radii of gyration were determined from the slope of Guinier plots, and the radii of gyration of the cross section were obtained from plots of $\log[SI(S)]$ versus S^2 (Porod, 1982). In general, these structural parameters were calculated from the scattering intensities corresponding to the S values ranging from 0.005 to 0.01 Å^{-1}. Calculated scattering patterns for models of unfolded DNA–histone complexes were obtained using an X-ray solution scattering simulation program developed by Pantos et al. (E. Pantos, D. Holden, J. West, and J. Bordas, unpublished results).

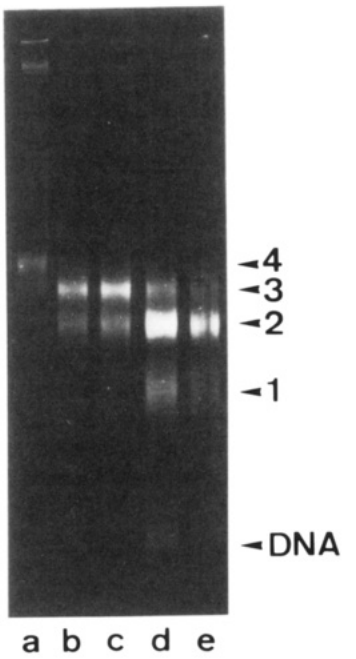

FIGURE 1: Stability of DNA–histone complexes after irradiation with the Synchrotron X-ray beam. Different irradiated (c, e) and unirradiated (a, b, d) DNA–(H2A,H2B) samples in 1×TBE were analyzed on nondenaturing polyacrylamide gels containing 0.1×TBE. The H2A,H2B to core DNA weight ratio was 1.1 (a), 0.8 (b, c), and 0.6 (d, e). The positions of free core DNA and the four bands (Aragay et al., 1988) corresponding to the different DNA–(H2A,H2B) complexes are indicated.

RESULTS

Synchrotron X-ray Solution Scattering Measurements. Low particle concentrations (0.5 mg of DNA/mL) are enough to obtain small-angle scattering curves using short irradiation time (5 min). These conditions do not produce radiation damage. As can be seen in Figure 1, the irradiated DNA–(H2A,H2B) complexes show the same electrophoretic behavior as control unirradiated samples. The main bands produced by the successive binding of H2A,H2B dimers to core DNA [bands 1–4 in Figure 1; see Aragay et al. (1988)] are not altered by synchrotron X-rays. Similarly, the electrophoretic

[1] Abbreviations: bp, base pair(s); EDTA, sodium ethylenediaminetetraacetate; SDS, sodium dodecyl sulfate; 1×TBE, 90 mM Tris–borate and 2.5 mM EDTA, pH 8.3; 0.1×TBE, 9 mM Tris–borate and 0.25 mM EDTA, pH 8.3; TE, 10 mM Tris-HCl and 0.2 mM EDTA, pH 7.4; TEA, 10 mM triethanolamine hydrochloride and 0.2 mM EDTA, pH 7.4.

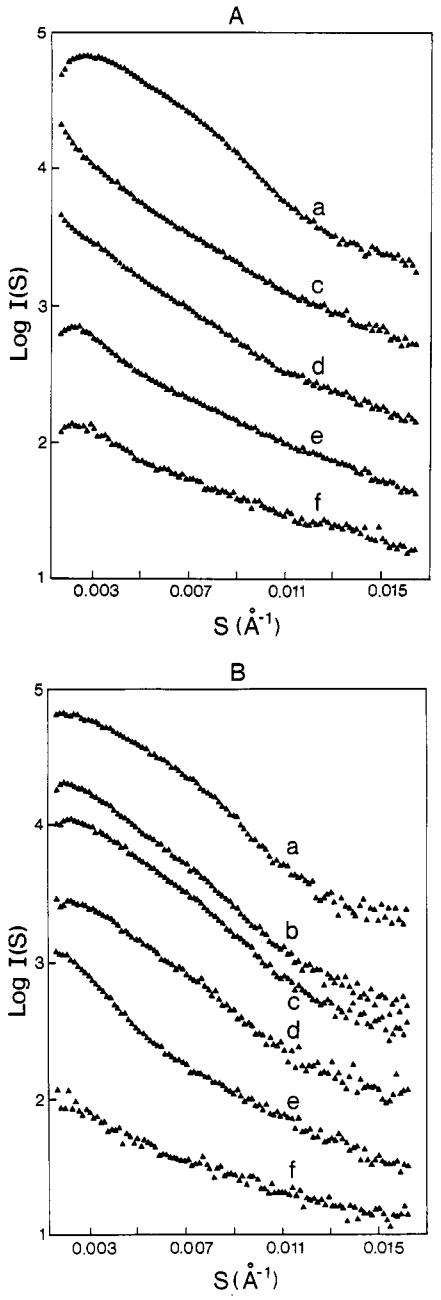

FIGURE 2: Small-angle X-ray scattering curves of reconstituted core DNA–(H2A,H2B,H3,H4) (b, c), DNA–(H3,H4) (d), and DNA–(H2A,H2B) (e) complexes. The curves corresponding to nucleosome core particles (a) and free core DNA (f) are shown as reference. In (A), the complexes were prepared by direct mixing of histone oligomers with core DNA in 0.1×TBE buffer. In (B), the buffer was TEA containing 0.2 M NaCl, and the complexes were prepared by stepwise dialysis, except (b) which was prepared by direct mixing of histones and core DNA. In all complexes, the input histone to DNA weight ratio was 0.8. The curves are displaced vertically by an arbitrary amount for clarity.

mobility of core particles and DNA–(H3,H4) complexes on nondenaturing gels (Aragay et al., 1988, 1991) does not change after X-ray irradiation (not shown).

Nucleosome core particles in the different buffers used in the study of the DNA–histone complexes considered below show a radius of gyration of 4.2 nm (Table I). As expected, this value approaches the radius of gyration of the DNA component (4.8 nm) and is higher than the radius of gyration of the protein component (3.3 nm) determined for nucleosome core particles using neutron scattering techniques (Suau et al., 1977; Imai et al., 1986). Salt concentrations higher than

Table I: Radii of Gyration of Nucleosome Core Particles in Different Solvent Conditions

buffer	[NaCl] (M)	radius of gyration (Å)[a]	buffer	[NaCl] (M)	radius of gyration (Å)[a]
0.1×TBE		42.1	TE	0.2	41.9
1×TBE		42.5 ± 0.5[b]		0.6	44.2
TEA	0.2	42.2		0.8	47.6

[a] Calculated from Guinier plots (see Materials and Methods) obtained with nucleosome core particle solutions at concentrations ranging from 0.5 to 1.75 mg of DNA/mL. [b] Mean ± 1 standard deviation for 6 determinations.

Table II: Radii of Gyration of Various DNA–Histone Complexes under Different Conditions

reconstituted complex	histone to DNA weight ratio	radius of gyration (Å)[a] 1×TBE	radius of gyration (Å)[a] TEA–0.2 M NaCl
DNA–(H3,H4)	0.6	40.1	40.4 (39.2)
	0.8	43.1	38.6 (40.3)
DNA–(H2A,H2B,H3,H4)	0.6	40.2	39.9 (40.1)
	0.8	41.7	41.2 (40.7)

[a] Calculated from Guinier plots corresponding to complexes prepared by direct mixing of histones and core DNA (values without parentheses) or by stepwise dialysis (values in parentheses).

0.2 M NaCl produce significant changes in the general shape of the scattering curves. The increase of the apparent radius of gyration of nucleosome cores found at 0.6 M NaCl (see Table I) is probably related to the conformational change detected in this ionic strength region using different physicochemical techniques (Daban & Cantor, 1982a; Ausio et al., 1989).

Studies at Low Ionic Strength. Nucleosome cores in 0.1×TBE buffer produce the typical scattering pattern of folded particles presented in Figure 2A (curve a). This pattern is completely different from that found for core DNA in the absence of histones (curve f). In contrast, the scattering patterns obtained with reconstituted complexes containing core DNA and the four core histones (curve c), or the pairs H3,H4 (curve d) or H2A,H2B (curve e), are not so markedly different from that observed for free core DNA, indicating that in 0.1×TBE these histone oligomers are unable to fold the DNA. Assuming an elongated structure for these complexes, the apparent radii of gyration of the cross section can be estimated from the scattering data (Table III). Model calculations with prolate ellipsoids of revolution indicate that the axial ratio of these unfolded DNA–histone complexes is higher than 5. The best fits for complexes containing core histones, H3,H4, and H2A,H2B, in 0.1×TBE at a histone to DNA weight ratio of 0.8, are obtained using ellipsoids of revolution with axis of 35:4.5, 25:4.7, and 33:3.4 nm, respectively.

Studies at Intermediate Ionic Strength. The unfolded structure produced by core histones in 0.1×TBE (see the preceding section) is probably due to the fact that these complexes have been prepared by direct mixing of histones and core DNA at low ionic strength. Presumably, the strong electrostatic interactions existing in these conditions preclude the dynamic rearrangement of histones required for the correct placement of histones on the core DNA. In keeping with this interpretation, we have found that when DNA–core histone complexes are prepared by direct mixing of histones and core DNA in the presence of a buffer of higher ionic strength (1×TBE), the resulting scattering patterns correspond to folded structures having a radius of gyration similar to that

Table III: Apparent Radii of Gyration of the Cross Section of Various Unfolded DNA–Histone Complexes and Core DNA under Different Conditions

sample	histone to DNA weight ratio	radius of gyration of cross section (Å)[a] 0.1×TBE	1×TBE	TEA–0.2 M NaCl
DNA		10.7	9.2	7.7
DNA–(H2A,H2B)	0.6	13.8	18.6	18.6
	0.8	15.0	20.4	17.8
DNA–(H3,H4)[b]	0.6	20.0		
	0.8	21.4		
DNA–(H2A,H2B,H3,H4)[b]	0.6	18.8		
	0.8	19.9		

[a] Values calculated from plots of $\log[SI(S)]$ versus S^2 (see Materials and Methods). [b] The radii of gyration of the cross section corresponding to these complexes in 1×TBE and TEA–0.2 M NaCl were not determined because they produce folded structures under these conditions (see Table II).

found for typical nucleosome core particles (see Table II). Even histones H3,H4 alone bound to DNA in 1×TBE buffer produce folded structures (Table II).

On the other hand, there is a marked contrast between DNA–(H2A,H2B) complexes and DNA–(H3,H4) or DNA–core histone complexes in the presence of 1×TBE. Although the shape of the scattering curve of DNA–(H2A,H2B) complexes in 1×TBE is different from that corresponding to free DNA, the observed scattering indicates that under these conditions the complexes containing H2A,H2B are unfolded (Table III). These results are obtained with complexes prepared using histone to DNA weight ratios of 0.6 and 0.8. Higher values of this ratio produce a fraction of aggregated material (see Figure 1, lane a) that complicates the analysis of the scattering results. However, the remarkable differences between DNA–(H2A,H2B) complexes and the complexes containing H3,H4 or the four core histones are also clearly detected in samples with a histone to DNA weight ratio of 1.1.

DNA–(H2A,H2B) Complexes Are Unfolded Even at Physiological Ionic Strength. As shown in Figure 2B, DNA–core histone complexes prepared either by direct mixing of histones and core DNA in 0.2 M NaCl (curve b) or by stepwise dialysis from 2 to 0.2 M NaCl (curve c) produce scattering patterns similar to that observed for native core particles. Equivalent results were obtained with DNA–(H3,H4) complexes in 0.2 M NaCl prepared by stepwise dialysis (curve d) or by direct mixing (not shown). The Guinier plots corresponding to DNA–(H3,H4) complexes (Figure 3, curve b) show a small increase of slope in the low-angle region. The values of this region have not been taken into account in the calculation of the radius of gyration because they are probably modified by the presence of a small amount of aggregated material. The calculated radii of gyration of all these complexes (Table II) are similar to that of native nucleosomes under the same conditions.

In contrast, independent of the preparation procedure, DNA–(H2A,H2B) complexes in 0.2 M NaCl give rise to scattering patterns (Figure 2B, curve e) very different from those corresponding to the folded core particle and DNA–(H3,H4) complexes considered above. These results indicate that histones H2A,H2B in 0.2 M NaCl are unable to produce the folding of DNA found in the nucleosome. The scattering curve obtained with DNA–(H2A,H2B) complexes under these conditions is different from that found for these complexes at very low ionic strength (see Figure 2A, curve e). Comparison of the values of the apparent radius of gyration of the cross

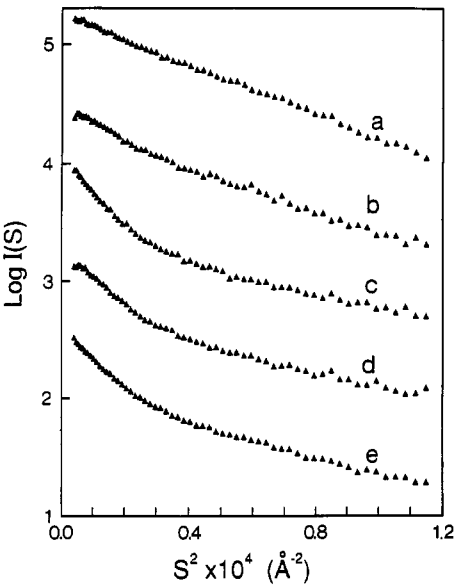

FIGURE 3: Guinier plots of DNA–(H2A,H2B) (c–e) and DNA–(H3,H4) (b) complexes under different conditions. The complexes were prepared by stepwise dialysis from 2 to 0.2 M NaCl in the presence of TEA buffer (b, c) or by direct mixing in TEA–0.2 M NaCl (d) or 1×TBE (e). In all complexes, the input histone to DNA weight ratio was 0.8. The plot corresponding to native core particles in 0.2 M NaCl is shown as a reference (a). The plots are displaced vertically for clarity. The values of the radii of gyration calculated from the slope of the low-angle region of plots c, d, and e are 7.0, 6.2, and 6.3 nm, respectively.

section and model calculations indicates that DNA–(H2A, H2B) complexes are less extended at physiological salt concentration (or at intermediate ionic strength) than at low ionic strength.

The radius of gyration of the whole DNA–(H2A,H2B) structure can be calculated from the small-angle region of the Guinier plots shown in Figure 3 (curves c–e). According to Pilz (1982), the values obtained for the radius of gyration of the whole structure (see legend of Figure 3), together with the radius of gyration of the cross section (Table III), allow us to calculate the length and the axial ratio of elongated ellipsoids. The DNA–(H2A,H2B) complexes prepared at a histone to DNA weight ratio of 0.8 by direct mixing either in 1×TBE or 0.2 M NaCl or by stepwise dialysis from 2 to 0.2 M NaCl show similar axial ratios ranging from 4.2 to 5.0. The experimental X-ray scattering results of these complexes prepared by direct mixing in 0.2 M NaCl can be approximated by the calculated scattering corresponding to a prolate ellipsoid of revolution with axes of 25:5.3 nm.

Reactivity of DNA–(H2A,H2B) Complexes. Previously, we have demonstrated using gel electrophoresis under non-denaturing conditions that there are histone transfer reactions that allow the formation of reconstituted nucleosome core particles from the mixture of DNA–(H2A,H2B) and DNA–(H3,H4) complexes (Aragay et al., 1988). The resulting reconstituted particles show the same electrophoretic mobility (Aragay et al., 1991) and histone stoichiometry (Aragay et al., 1988) as native core particles. Although there is a small increase of the slope of the Guinier plots in the low-angle region (Figure 4), indicating the existence of a small fraction of aggregated material, the scattering properties of the product of the reaction of preformed DNA–(H2A,H2B) with DNA–(H3,H4) complexes in 0.2 M NaCl are essentially equivalent to those corresponding to typical nucleosome core particles. The radii of gyration calculated from the Guinier plots shown in Figure 4 are 4.0 and 4.2 nm for the complexes resulting

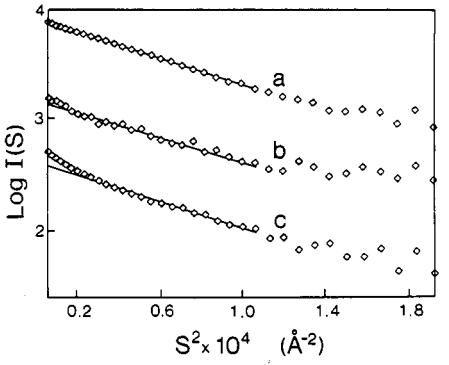

FIGURE 4: Guinier plots of nucleosome core particles produced by the reaction of DNA–(H2A,H2B) with DNA–(H3,H4) complexes. The DNA–(H2A,H2B) and DNA–(H3,H4) complexes used in these experiments were prepared either by direct mixing of histones with core DNA (b) in TEA buffer containing 0.2 M NaCl or by stepwise dialysis (c). The input weight ratio of histones H2A,H2B and H3, H4 to DNA was 0.8. The plot corresponding to native nucleosome cores is shown as a reference (a). The plots are displaced vertically for clarity.

after the mixture of DNA–(H2A,H2B) and DNA–(H3,H4) complexes prepared, respectively, by direct mixing (curve b) or stepwise dialysis (curve c). These results allow us to conclude that the unfolded structure found for DNA–(H2A, H2B) complexes at physiological ionic strength becomes completely folded after the histone pair exchange reaction that takes place between the preformed DNA–(H2A,H2B) and DNA–(H3,H4) complexes.

DISCUSSION

Using the conditions described above, the different DNA–histone samples analyzed in this work were not damaged by the irradiation with the synchrotron X-ray source. This together with the low particle concentration, small volume, and short exposure time required to carry out the scattering measurements, demonstrates that the high flux of X-ray photons from the synchrotron radiation source is very useful to analyze the structural properties of DNA–histone complexes in solution.

Our scattering results show that under all the conditions used in this study histones H2A,H2B are unable to fold core DNA. This unfolded structure is probably responsible for the relatively low electrophoretic mobility and slow sedimentation in sucrose gradients observed for DNA–(H2A,H2B) complexes (Aragay et al., 1988). In contrast, our results indicate that the reconstituted DNA–(H3,H4) complexes prepared using different values of the histone to DNA weight ratio in 0.2 M NaCl or 1×TBE show a folded structure equivalent to that corresponding to native nucleosome core particles under the same conditions. These findings are in keeping with the results obtained using neutron scattering and different physicochemical techniques indicating that depleted nucleosomes containing one H3,H4 tetramer or complexes containing two H3,H4 tetramers are compact particles similar to native nucleosome cores (Read et al., 1985; Read & Crane-Robinson, 1985).

Our scattering data show that the histone transfer reaction that takes place between DNA–(H2A,H2B) and DNA–(H3, H4) complexes (Daban & Cantor, 1982b; Aragay et al., 1988) produces completely folded particles. These results suggest that although both DNA–(H2A,H2B) and DNA–(H3,H4) complexes are thermodynamically stable, these complexes are less stable than the normal particle containing an octamer of the four core histones. On the other hand, from a dynamic point of view, the unfolded structure of DNA–(H2A,H2B) complexes may facilitate the histone transfer reaction. If as suggested from kinetic studies (Aragay et al., 1988) the transfer reaction involves direct contact between the DNA–(H2A, H2B) and DNA–(H3,H4) complexes before dissociation of the histone pairs from DNA, the open structure of the complex containing histones H2A,H2B could facilitate the transfer of these histones to the folded DNA fragment initially associated with H3,H4. The fact that histones H2A,H2B show a higher tendency than histones H3,H4 to migrate from one incomplete core particle to another (Aragay et al., 1988) is also probably related to the structural properties of DNA–(H2A,H2B) complexes. Presumably, the correct association of the transferred H2A,H2B dimers with the core DNA tails (Shick et al., 1980; Richmond et al., 1984; Bentley et al., 1984; Uberbacher & Bunick, 1989; Arents et al., 1991) can take place even when the acceptor core DNA is initially folded due to the presence of histones H3,H4.

Furthermore, taking into account that it has been observed that histones H3,H4 can also be transferred from DNA–(H3,H4) to DNA–(H2A,H2B) complexes (Aragay et al., 1988), it is also reasonable to speculate that the unfolded structure of DNA–(H2A,H2B) complexes may facilitate the correct placement of the transferred H3,H4 tetramer in the central region of the core DNA, as expected for typical nucleosomes (Shick et al., 1980; Richmond et al., 1984; Bentley et al., 1984; Uberbacher & Bunick, 1989; Arents et al., 1991). The observation that H3,H4 tetramers alone can recognize nucleosome-positioning sequences (Hayes et al., 1991; Dong & van Holde, 1991) suggests that these histones may have the capability to locate the central region of core DNA even when the H2A,H2B dimers are previously bound to the same DNA molecule. Finally, from our results showing that the complexes prepared by direct mixing of core histones and core DNA at low ionic strength remain unfolded, it can be concluded that the rearrangement of histones required in the nucleosome formation reactions can only occur under ionic strength conditions that favor a dynamic interaction of histones with DNA.

The structural and dynamic properties of the DNA–histone complexes considered above provide a mechanism for nucleosome formation by spontaneous exchange of histones between the incomplete nucleosome cores produced after the random association of H2A,H2B dimers and H3,H4 tetramers with different DNA molecules or with different regions of the same DNA molecule. Nevertheless, due to the fact that the binding of histones to DNA (Daban & Cantor, 1982a; Diaz & Daban, 1986; Pfaffle & Jackson, 1990) and the reactions of histone transfer are fast (Daban & Cantor, 1982b; Aragay et al., 1988), the main intermediates of this mechanism [i.e., DNA–(H2A,H2B) and DNA–(H3,H4) complexes] cannot be detected using conventional procedures. This explains why in the in vitro assembly experiments performed by direct mixing of core histones and DNA at physiological ionic strength or by salt-jump of nucleosome cores from 2 to 0.2 M NaCl the only complex detected is the rapidly formed final core particle (Diaz & Daban, 1986). On the other hand, this may also occur in assembly systems containing nucleoplasmin and proteins N1/N2 (Kleinschmidt et al., 1985; Dilworth et al., 1987). In these systems, the transfer of histones from nucleoplasmin–(H2A,H2B) and N1/N2–(H3,H4) complexes to DNA could produce intermediate incomplete nucleosomes which are not detected because presumably they undergo a spontaneous rapid exchange of histone pairs that rapidly produces complete nucleosome cores. In vivo, the unfolded

structure of DNA–(H2A,H2B) complexes may facilitate nucleosome formation and other nuclear functions involving a transient association of histones H2A,H2B with DNA.

ACKNOWLEDGMENT

We thank the staff of the Daresbury Laboratory for their assistance. We are especially grateful to Drs. J. Bordas, E. Towns-Andrews, G. Jones, and E. Pantos for advice and helpful discussions. We thank Dr. J. A. Subirana for a helpful suggestion concerning the data analysis of the unfolded structures.

REFERENCES

Aragay, A. M., Diaz, P., & Daban, J.-R. (1988) *J. Mol. Biol. 204*, 141–154.

Aragay, A. M., Fernandez-Busquets, X., & Daban, J.-R. (1991) *Biochemistry 30*, 5022–5032.

Arents, G., Burlingame, R. W., Wang, B.-C., Love, W. E., & Moudrianakis, E. N. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 10148–10152.

Ausio, J., Dong, F., & van Holde, K. E. (1989) *J. Mol. Biol. 206*, 451–463.

Bentley, G. A., Lewit-Bentley, A., Finch, J. T., Podjarny, A. D., & Roth, M. (1984) *J. Mol. Biol. 176*, 55–75.

Bordas, J., Perez-Grau, L., Koch, M. H. J., Vega, M. C., & Nave, C. (1986) *Eur. Biophys. J. 13*, 157–173.

Daban, J.-R., & Cantor, C. R. (1982a) *J. Mol. Biol. 156*, 749–769.

Daban, J.-R., & Cantor, C. R. (1982b) *J. Mol. Biol. 156*, 771–789.

Daban, J.-R., Samsó, M., & Bartolomé, S. (1991a) *Anal. Biochem. 199*, 162–168.

Daban, J.-R., Bartolomé, S., & Samsó, M. (1991b) *Anal. Biochem. 199*, 169–174.

Diaz, P., & Daban, J.-R. (1986) *Biochemistry 25*, 7736–7744.

Dilworth, S. M., Black, S. J., & Laskey, R. A. (1987) *Cell 51*, 1009–1018.

Dong, F., & van Holde, K. E. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 10596–10600.

Eickbush, T. H., & Moudrianakis, E. N. (1978) *Biochemistry 17*, 4955–4964.

Fujiwara, S., Inoko, Y., & Ueki, T. (1989) *J. Biochem. 106*, 119–125.

Gerritsen, H. C., & Robertus, C. (1990) in *Applications of Synchrotron Radiation* (Catlow, C. R. A., & Greaves, G. N., Eds.) pp 100–120, Blackie, London, U.K.

Hansen, J. C., van Holde, K. E., & Lohr, D. (1991) *J. Biol. Chem. 266*, 4276–4282.

Hayes, J. J., Clark, D. J., & Wolffe, A. P. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 6829–6833.

Hirai, M., Niimura, N., Zama, M., Mita, K., Ichimura, S., Tokunaga, F., & Ishikawa, Y. (1988) *Biochemistry 27*, 7924–7931.

Imai, B. S., Yau, P., Baldwin, J. P., Ibel, K., May, R. P., & Bradbury, E. M. (1986) *J. Biol. Chem. 261*, 8784–8792.

Jackson, V. (1990) *Biochemistry 29*, 719–731.

Karpov, V. L., Preobrazhenskaya, O. V., & Mirzabekov, A. D. (1984) *Cell 36*, 423–431.

Kleinschmidt, J. A., Fortkamp, E., Krohne, G., Zentgraf, H., Franke, W. W. (1985) *J. Biol. Chem. 260*, 1166–1176.

Koch, M. H. J., Sayers, Z., Michon, A. M., Sicre, P., Marquet, R., & Houssier, C. (1989) *Eur. Biophys. J. 17*, 245–255.

Lewis, R., Summer, I., Berry, A., Bordas, J., Gabriel, A., Mant, G., Parker, B., Roberts, K., & Worgan, J. (1988) *Nucl. Instrum. Methods Phys. Res., Sect. A A273*, 773–777.

Lorch, Y., LaPointe, J. W., & Kornberg, R. D. (1988) *Cell 55*, 743–744.

Oohara, I., & Wada, A. (1987) *J. Mol. Biol. 196*, 389–397.

Pfaffle, P., & Jackson, V. (1990) *J. Biol. Chem. 265*, 16821–16829.

Pilz, I. (1982) in *Small Angle X-ray Scattering* (Glatter, O., & Kratky, O., Eds.) pp 239–293, Academic Press, New York.

Porod, G. (1982) in *Small Angle X-ray Scattering* (Glatter, O., & Kratky, O., Eds.) pp 17–51, Academic Press, New York.

Read, C. M., & Crane-Robinson, C. (1985) *Eur. J. Biochem. 152*, 143–150.

Read, C. M., Baldwin, J. P., & Crane-Robinson, C. (1985) *Biochemistry 24*, 4435–4450.

Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D., & Klug, A. (1984) *Nature 311*, 532–537.

Ruiz-Carrillo, A., & Jorcano, J. L. (1979) *Biochemistry 18*, 760–768.

Ruiz-Carrillo, A., Jorcano, J. L., Eder, G., & Lurz, R. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3284–3288.

Shick, V. V., Belyavsky, A. V., Bavykin, S. G., & Mirzavekov, A. D. (1980) *J. Mol. Biol. 139*, 491–517.

Simon, R. H., Camerini-Otero, R. D., & Felsenfeld, G. (1978) *Nucleic Acids Res. 5*, 4805–4818.

Suau, P., Kneale, G. G., Braddock, G. W., Baldwin, J. P., & Bradbury, E. M. (1977) *Nucleic Acids Res. 4*, 3769–3786.

Thomas, J. O., & Oudet, P. (1979) *Nucleic Acids Res. 7*, 611–623.

Towns-Andrews, E., Berry, A., Bordas, J., Mant, G. R., Murray, P. K., Roberts, K., Summer, I., Worgan, J. S., & Lewis, R. (1989) *Rev. Sci. Instrum. 60*, 2346–2349.

Uberbacher, E. C., & Bunick, G. J. (1989) *J. Biomol. Struct. Dyn. 7*, 1–18.

Wilhelm, F. X., Wilhelm, M. L., & Daune, M. P. (1978) *Nucleic Acids Res. 5*, 505–521.