linear kinetics. Both studies show that the *Rp* isomer is by far the preferred substrate for the snake venom exonuclease. It cannot be excluded that the small activity observed with the *Sp* isomer is due to a contamination by the *Rp* isomer. The fact that the *Sp* isomer is such a poor substrate is not due to a lack of binding. Both isomers are competitive inhibitors for the hydrolysis of thymidine 5'-*O*-*p*-nitrophenyl phosphate and have similar K_i values. Analogous results were found for the hydrolysis of the diastereomers of adenosine 5'-*O*-(1-thiotriphosphate) by snake venom phosphodiesterase (Burgers & Eckstein, 1978b). The formation of an abortive complex of the *Sp* diastereomer with the enzyme suggests that the functional group or groups at the active site involved in the catalysis of hydrolysis cannot function when one particular oxygen is replaced by sulfur. It is conceivable that protonation of a phosphate oxygen which might be necessary for hydrolysis becomes impossible or difficult when this oxygen is replaced by sulfur in the *Sp* diastereomer of the phosphorothioate.

Our data on the hydrolysis of Up(S)A by snake venom phosphodiesterase establish the stereoselectivity of this enzyme. To arrive at the stereochemistry of enzymatic hydrolysis, experiments with $H_2{}^{18}O$ will have to be performed. For such studies these compounds will also be suitable.

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Role of Histone H1 in the Conformation of Oligonucleosomes as a Function of Ionic Strength†

Wolf H. Strätling

ABSTRACT: The role of histone H1 with respect to the conformation of oligonucleosomes and the dependence on ionic strength was investigated by studying two functions: (1) compaction of single oligonucleosomal chains; (2) packaging of several nucleosomal chains into composite particles. The first function was examined by sedimenting homogeneous mono- to hexanucleosomes in isokinetic sucrose gradients containing various NaCl concentrations. Homogeneous oligonucleosomes were prepared from mildly digested rat liver nuclei using a NaCl concentration of 170 mM being present during nuclear lysis and sedimentation in isokinetic sucrose gradients. The high resolution obtained at this salt concentration results from the dissociation of composite particles into single nucleosomal chains. The second function was studied by analyzing the salt-dependent chain length heterogeneity of trimers and tetramers obtained by centrifugation in isokinetic sucrose gradients. The results show that, depending on the NaCl concentration, oligonucleosomes can exist in four discrete conformations. First, at very low ionic strength, oligonucleosomal chains exhibit an extended conformation and packaging of different chains into composite particles is negligible. Second, at about 5–20 mM NaCl, oligonucleosomes undergo a cooperative transition to a compact conformation. Above the transition, H1 mediates packaging of different nucleosomal chains into composite particles. Third, at 150 mM NaCl, single oligonucleosomes occur in a conformation slightly more compact than at 50 mM NaCl. At this salt concentration, however, packaging of several nucleosomal chains into composite particles is not observed. Fourth, above 200 mM NaCl, oligonucleosomes exhibit an extended conformation.

Nucleosomes are now recognized as the basic structural entities of chromatin. They are built by four of the five major histone classes forming a histone core around which the DNA helix is wrapped. Histone H1 appears not to be a structural component of the nucleosomal protein core, but to be located in the internucleosomal space (Varshavsky et al., 1976; Noll & Kornberg, 1977). Furthermore, histone H1 seems to specify the length of the internucleosomal DNA segment (Morris, 1976; Lipps & Morris, 1977; Spadafora & Geraci, 1975; Spadafora et al., 1976). In a number of tissues the nucleosomal repeat length was related to the size and the basicity of histone H1 (and histone H5). Studies on the structure of H1 under physiological salt conditions have revealed that a central region of H1 assumes a globular structure while the ends form unstructured tails (Hartman et al., 1977). These

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properties support the notion that H1 plays a cross-linking role in chromatin.

Investigation of the physical properties of chromatin by sedimentation studies is heavily impaired by the fact that its solubility is extremely sensitive to changes in ionic strength. At physiological salt concentrations (about 0.15 M NaCl), chromatin aggregates and precipitates. Either lower or higher ionic strengths cause an expansion of chromatin and lead to an increase in solubility. A number of authors have described that oligonucleosomes, however, generated by limited nuclease digestion of nuclei are—at least in part—soluble at physiological salt concentrations (Lewis et al., 1976; Wittig & Wittig, 1977). Therefore, oligonucleosomes lend themselves particularly well to physical studies on the conformation of chromatin. Several authors have already attempted to investigate the conformation of nucleosomal chains as a function of ionic strength by hydrodynamic measurements (Lewis et al., 1976; Li et al., 1977; Renz et al., 1977). But in these instances the preparations used were heterogeneous in molecular weight. I have eliminated problems evoked by heterogeneity by using oligonucleosomes homogeneous with respect to chain length. They were prepared from nuclease digests by using a NaCl concentration of 170 mM during nuclear lysis and the subsequent sedimentation of the "soluble" chromatin fraction in isokinetic sucrose gradients. Secondly, the soluble oligonucleosomal preparations obtained by other investigators were often depleted in histone H1 (Wittig & Wittig, 1977). The present paper describes experimental conditions for the preparation of mono- and oligonucleosomes with a full complement of H1. This provides an experimental basis to test the hypothesis that H1 plays a role in cross-linking neighboring nucleosomes. By comparing the conformation of H1-containing oligonucleosomes with that of oligonucleosomes stripped of H1 the function of H1 in packaging nucleosomes on single chains was investigated at various salt concentrations. Purifying oligonucleosomes in the presence of 50 mM NaCl instead of 170 mM generated particles which contained more than one nucleosomal chain. These particles, called composite or pseudo-particles, may have arisen by chain scissions within supranucleosomal particles (Strätling et al., 1978a,b). The structural requirements of composite particles and the effect of ionic strength on their conformation are therefore of considerable importance for the understanding of the higher order structure of chromatin.

Experimental Procedures

Preparation and Isolation of Oligonucleosomes. All steps were performed at 4 °C unless otherwise stated. Nuclei were prepared from rat liver by the method of Strätling et al. (1976) using buffer "O" (25 mM KCl, 0.9 mM $MgCl_2$, 0.9 mM $CaCl_2$, 0.14 mM spermidine, 5 mM Tris-HCl, pH 7.5) of Chevaillier & Philippe (1973). Nuclei were suspended in this buffer giving an OD_{260nm} of 50. After addition of 30 units of micrococcal nuclease (P-L Biochemicals) per mL of the nuclear suspension, the reaction vial was placed for 1 min in a 37 °C water bath and then for 60 min at 0 °C. The reaction was stopped by the addition of 0.2 M EDTA up to a final concentration of 3.6 mM. After further addition of 5 M NaCl to a final concentration of 145 mM, the suspension was dialyzed overnight against 2 L of TE buffer (5 mM Tris-HCl, pH 7.5, 1 mM EDTA) containing 170 mM NaCl. The precipitated material was removed by centrifugation for 20 min at 23000*g*. The solubilized chromatin in the supernatant fraction was then centrifuged in isokinetic sucrose gradients containing TE buffer and 170 mM NaCl for 9 h at 40000 rpm and 4 °C in a Beckman SW 40 rotor, with c_t = 5%, c_r = 26.9%, V_m = 9.4 mL, and particle density = 1.51 (McCarty et al., 1974). Gradients were monitored for absorbancy at 260 nm by use of a turbulence-free flow-through cell (ISCO).

Removal of Histone H1. To remove H1 from the solubilized chromatin fraction the limited nuclease digest was first dialyzed against TE buffer containing 0.5 M NaCl. The dialysate was cleared by centrifugation at 23000*g* for 20 min and loaded onto a linear 5–20% sucrose gradient which was placed on top of a 3-mL 60% sucrose cushion (in TE buffer containing 0.5 M NaCl). For removal of H1 from isolated oligonucleosomes, the low-speed centrifugation of the dialysate was omitted. After centrifugation for 18 h at 40000 rpm in an SW40 rotor, H1-stripped chromatin and oligonucleosomes, respectively, were collected from the upper part of the 60% sucrose cushion.

Various Physical and Chemical Methods. Apparent sedimentation coefficients (S_{app}) were determined by centrifugation in isokinetic sucrose gradients containing TE buffer and the indicated NaCl concentrations at 40000 rpm and 4 °C in a Beckman SW40 rotor. Calibration was performed by centrifugation of 30S and 50S ribosomal subunits of *Escherichia coli* in parallel isokinetic sucrose gradients containing 5 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, and the indicated NaCl concentrations.

Chromatin concentration was expressed as DNA concentration and was determined spectrophotometrically at 260 nm as described by Strätling et al. (1976). Acid solubility was determined by addition of an equal volume of 10% Cl_3CCOOH to an aliquot of the digested nuclear suspension on ice and centrifugation at 23000*g* for 20 min. The optical density at 260 nm in the supernatant fraction was corrected for the optical density measured in the supernatant fraction from a nondigested nuclear preparation. DNA was extracted from isolated oligonucleosomes with chloroform/isoamyl alcohol as described by Strätling et al. (1976). Histone H1 was prepared from isolated rat liver nuclei by the trichloroacetic acid extraction procedure of De Nooij & Westenbrink (1962). Histones were extracted with 0.4 N H_2SO_4 and analyzed by polyacrylamide gel electrophoresis in the presence of 2.5 M urea at pH 3.2 (Panyim & Chalkley, 1969). Quantitation of each histone fraction was performed by scanning the gels at two selected wavelengths using isolated histones as standards in order to correct for the differences in the absorption spectra of the histone–dye complexes (McMaster-Kaye & Kaye, 1974; Strätling & Seidel, 1976). Total proteins in oligonucleosomes were resolved in 8.75% cylindrical polyacrylamide gels in the presence of 1% sodium dodecyl sulfate as described by Laemmli (1970). DNA fragments were resolved by electrophoresis in 1.1% (w/v) agarose or 3.5% polyacrylamide slab gels in 36 mM Tris, 30 mM NaH_2PO_4, 1 mM EDTA, pH 8.0 at 20 mA (Loening, 1969). Gels were stained with 1 μg/mL of ethidium bromide in 1 mM EDTA, pH 7.5, for 1 h, destained in 1 mM EDTA, and photographed under UV light using a red filter with Kodak Tri X Pan film.

Results

Preparation of Homogeneous Oligonucleosomal Fractions. As it is the main aim of the present paper to obtain mono- and oligonucleosomes (and to study their conformation) in their native organization, extreme care was taken to choose mild and physiological conditions for their isolation. Rat liver nuclei in a physiologically ionic milieu (Chevaillier & Philippe, 1973) were subjected to mild nuclease digestion with the endogeneous Ca/Mg-dependent nuclease and low concentrations of added micrococcal nuclease until the acid solubility reached 0.7%. The digested nuclei were lysed by dialysis against Tris–EDTA

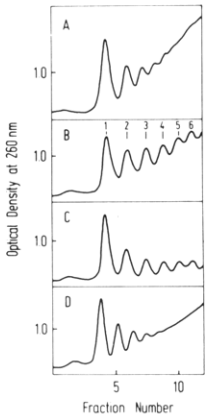

FIGURE 1: Sedimentation profiles of a limited nuclease digest on sucrose gradients containing various NaCl concentrations. Aliquots of a limited nuclease digest of rat liver nuclei (30 units/mL, 1 min at 37 °C and 60 min at 0 °C) were dialyzed against TE buffer containing no salt (A), 50 mM NaCl (B), 150 mM NaCl (C), and 350 mM NaCl (D). The solubilized chromatin was centrifuged for 9 h in isokinetic sucrose gradients containing the specified NaCl concentrations. The numbers in B refer to the position of mono- to hexanucleosomes.

buffer containing NaCl at various concentrations from 0 to 350 mM. Lysis by dialysis instead of resuspension of the sedimented nuclei in dilute solutions of EDTA avoided that oligonucleosomes leaking the nuclei after digestion were discarded (Wittig & Wittig, 1977). The lysed nuclear preparations were clarified by centrifugation at 23000*g* for 20 min. The chromatin recovered in the supernatant fraction amounted to 88% of the original nuclear chromatin at zero salt, 73% at 50 mM NaCl, 33% at 150 mM NaCl, and 66% at 350 mM NaCl. This fraction was experimentally defined as "soluble" under the conditions employed for its preparation. Variation of the experimental conditions showed that the mode of digestion, of nuclear lysis and of the low-speed centrifugation step, the concentration of nuclei and the temperature have a significant influence on the recovery of chromatin in the soluble fraction. Aliquots of the soluble chromatin preparations were then centrifuged for 9 h in isokinetic sucrose gradients containing the same NaCl concentrations as used during nuclear lysis. The 9-h centrifugation period was chosen because it resolved—at 150 mM NaCl—oligonucleosomes up to the hexamer, while oligonucleosomes of longer chain length were sedimented to the bottom of the centrifuge tube. The sedimentation profiles in Figure 1 show that the resolution of the oligonucleosomal peaks increased with increasing salt concentration up to about 150–200 mM NaCl and then rapidly decreased at higher ionic strength. The yield of original nuclear chromatin recovered in the mono- to hexanucleosomal fraction after centrifugation was 18% at zero salt, 16% at 50 mM NaCl, 9% at 150 mM NaCl, and 13% at 350 mM NaCl. Based on the assumption that mono- to hexanucleosomes are completely soluble at zero salt, the use of 150–200 mM NaCl reduced the recovery of oligonucleosomes to about one-half. The high resolution of the oligonucleosomal peaks in the sedimentation profile of gradients containing 150–200 mM NaCl suggested a high purity of the recovered fractions. The purity of these fractions was estimated by sizing the DNA fragments in 1.1% agarose gels. Figure 2 shows that a very good separation was obtained without appreciable contamination of adjacent oligonucleosomal chains. As will be shown later, the good separation resulted from two effects of the elevated salt concentration: (1) a dissociation of composite particles into single nucleosomal chains and (2) a maximal compaction of these chains.

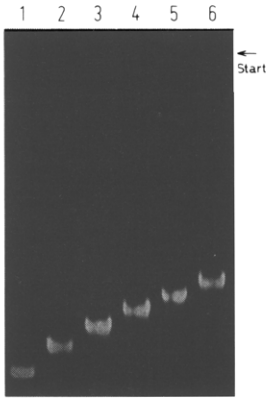

FIGURE 2: Gel electrophoresis of the DNA fragments contained in isolated oligonucleosomes. Oligonucleosomes were purified by centrifugation in isokinetic sucrose gradients containing 170 mM NaCl. The DNA was extracted from the various peak fractions and electrophoresed in 1.1% agarose slab gels. The numbers refer to mono- to hexanucleosomal chains.

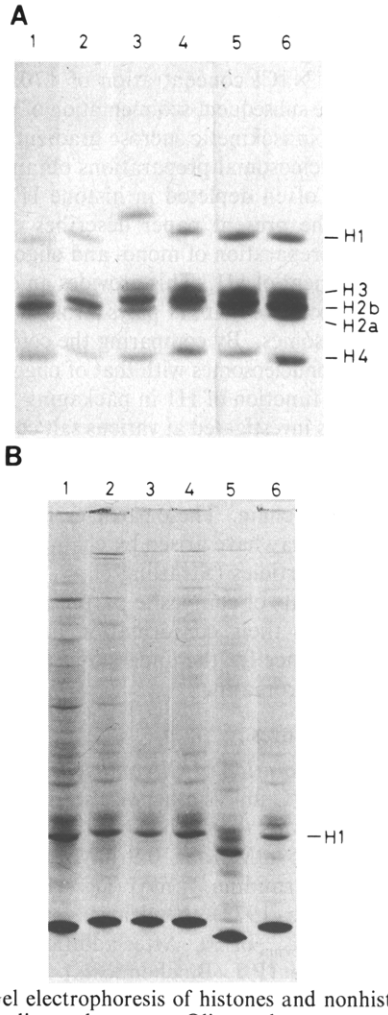

FIGURE 3: Gel electrophoresis of histones and nonhistone proteins from isolated oligonucleosomes. Oligonucleosomes were purified on sucrose gradients containing 50 (A) and 150 mM NaCl (B). The various peak fractions were dialyzed against 2 mM Tris-HCl, 0.2 mM EDTA, pH 7.5. (A) Histones were extracted with 0.4 N H_2SO_4 and electrophoresed on acid–urea gels. That the band for histone H2b is more pronounced is due to the different absorption spectrum of the histone H2b–dye complex. (B) Nonhistone proteins were resolved by electrophoresis on 8.75% polyacrylamide gels. The numbers in A and B refer to mono- to hexanucleosomal fractions.

The histone and nonhistone protein composition of fractionated oligonucleosomes was analyzed by polyacrylamide gel electrophoresis. The histone gels in Figure 3A were

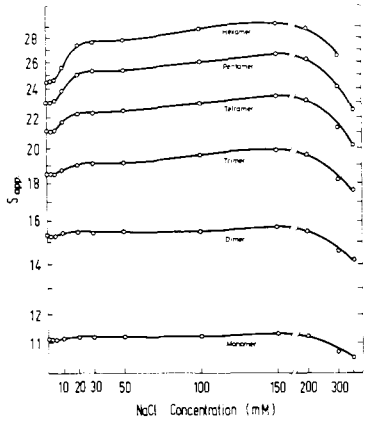

FIGURE 4: Effect of NaCl concentration on the conformation of isolated mono- to hexanucleosomes. Mono- to hexanucleosomes were isolated on isokinetic sucrose gradients containing 170 mM NaCl. They were then dialyzed against TE buffer containing various NaCl concentrations and centrifuged for 9 h in isokinetic sucrose gradients containing the NaCl concentrations used for dialysis.

scanned and the amount of each histone fraction was quantitated using isolated histones as standards. The results revealed that mono- to hexanucleosomes prepared in the presence of 50 mM NaCl contained the full complement of all five histones. In particular, no losses of histone H1 greater than 10% were observed. Similar results were obtained with oligonucleosomes separated in gradients containing 150 mM NaCl (data not shown). This indicated that a redistribution of H1 from short to long oligonucleosomes had not occurred under the conditions used. A comparison of the electrophoretic mobility in polyacrylamide gels of the DNA fragments contained in mononucleosomes with that of markers of known length demonstrated that the linker DNA segment in mononucleosomes had not been degraded to a significant extent (data not shown). Thus by choosing mild conditions of nuclease digestion degradation of linker DNA and thereby release of histone H1 from the nucleosome could be avoided.

Nonhistone proteins from oligonucleosomes separated in gradients containing 150 mM NaCl resolved electrophoretically into a complex pattern. The band pattern of tri- to hexanucleosomes was very uniform suggesting that these proteins represented integral components. The patterns of mono- and dinucleosomes differed in two respects. First, they showed bands which were not present in higher order oligonucleosomes. Second, some of those bands, found in larger oligonucleosomes, were not or only weakly expressed in mono- and dinucleosomes. It is possible that those bands only found in mono- and dinucleosomes derived from high molecular weight nucleoplasmic proteins and/or from contaminating ribonucleoprotein particles. Ribonucleoprotein particles are known to have a high protein content (Pederson, 1974); therefore, a slight contamination by these particles may result in an appreciable change of the nonhistone protein pattern.

Sedimentation Behavior of Oligonucleosomes as a Function of Ionic Strength. The availability of homogeneous preparations of mono- to hexanucleosomes offered the possibility to investigate the sensitivity of oligonucleosomal conformation to changes in salt concentration in greater detail. Mono- to hexanucleosomes prepared from a limited nuclease digest at 170 mM NaCl were recentrifuged after dialysis in isokinetic sucrose gradients containing NaCl concentrations between 0 and 350 mM. Figure 4 shows that a minimal NaCl concentration of 5–10 mM was required for an increase in the sedimentation coefficients of oligonucleosomes to be observed. When the NaCl concentration was raised further, the sedimentation coefficients continued to increase over a very small change of the NaCl concentration to reach a plateau at about 30 mM NaCl. These results indicated that at a critical salt concentration oligonucleosomes underwent a cooperative transition from a slowly sedimenting to a fast sedimenting form. The sedimentation coefficients showed a further slight increase at higher NaCl concentrations reaching a maximal value at about 150 mM NaCl. From there on they declined progressively.

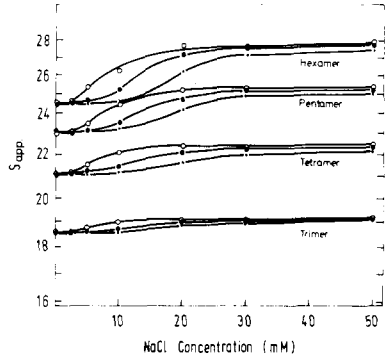

FIGURE 5: Effect of chromatin concentration on the conformational transition of oligonucleosomes. Aliquots of a limited nuclease digest were adjusted to 0.75 (•), 1.5 (●), and 2.5 (○) mg/mL of chromatin DNA, respectively, and dialyzed against TE buffer containing various NaCl concentrations (no salt, 2.5, 5, 10, 20, 30, and 50 mM NaCl). The solubilized chromatin was centrifuged in isokinetic sucrose gradients containing the NaCl concentrations used for dialysis.

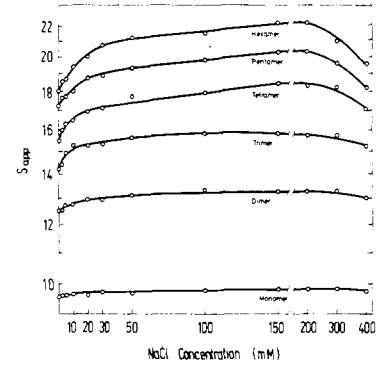

FIGURE 6: Effect of NaCl concentration on the conformation of H1-stripped mono- to hexanucleosomes. Isolated mono- to hexanucleosomes were stripped of histone H1, dialyzed into various NaCl concentrations (in TE buffer), and centrifuged for 9 h in isokinetic sucrose gradients containing the NaCl concentrations used for dialysis.

Figure 5 shows that the salt concentration of the midpoint of the cooperative transition was inversely related to the chromatin concentration in the limited digest loaded onto the gradient. When the chromatin concentration was adjusted to 2.5 mg/mL of DNA, the transition was observed at about 8 mM NaCl. A decrease in chromatin concentration to 1.5 and 0.75 mg/mL of DNA shifted the transition midpoint to 13 and 18 mM NaCl, respectively. As will be shown later, oligonucleosomal chains were packaged into composite particles above the transition, but not below the transition. This result supports the explanation that the chromatin concentration dependence of the transition midpoint was caused (in part) by an interaction of oligonucleosomal chains.

To determine the function of histone H1 in the salt-induced conformational changes shown above, H1 was removed from isolated mono- to hexanucleosomes by centrifugation in sucrose gradients containing 0.5 M NaCl. Gels of acid-extracted histones showed that histone H1 was completely removed without any losses of the core histones H2a, H2b, H3, and H4 (data not shown). Figure 6 shows that in TE buffer alone

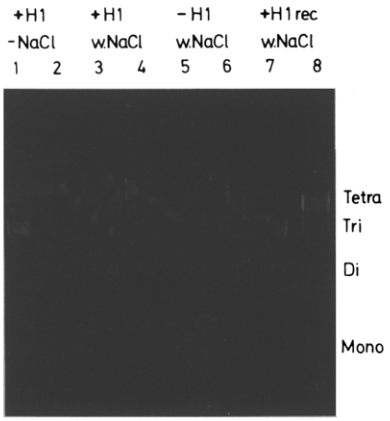

FIGURE 7: Chain length heterogeneity of trimer and tetramer particles as measured by gel electrophoresis. Trimer (slots 1, 3, 5, and 7) and tetramer (slots 2, 4, 6, and 8) particles were recovered from isokinetic sucrose gradients after various pretreatments. (Slots 1 and 2) A limited nuclease digest of rat liver nuclei was dialyzed in TE buffer containing no salt. The soluble chromatin fraction was centrifuged in isokinetic sucrose gradients under the same salt conditions. (Slots 3 and 4) Nuclear lysis and gradient centrifugation was performed in the presence of 50 mM NaCl. (Slots 5 and 6) H1 was removed from the nuclease digest prior to dialysis in 50 mM NaCl–TE buffer, as described in the Experimental Procedures. (Slots 7 and 8) Chromatin was reconstituted from an H1-deprived digest and histone H1 and dialyzed in 50 mM NaCl–TE buffer. DNA fragments were extracted from the peak fractions of the trimer (slots 1, 3, 5, and 7) and tetramer peak (slots 2, 4, 6, and 8) of each gradient and electrophoresed in a 3.5% polyacrylamide slab gel. "Mono", "Di", "Tri", and "Tetra" refer to the position of DNA bands from mono-, di-, tri-, and tetranucleosomes.

H1-stripped oligonucleosomes sedimented more slowly than the respective H1-containing particles (compare with Figure 4). When the salt concentration was raised, a steep, continuous increase in the sedimentation coefficients was observed up to a NaCl concentration of about 30 mM NaCl. Between 30 and 150 mM NaCl the increase in the sedimentation coefficients was slower, reaching maximal values between 150 and 200 mM NaCl. The decrease in sedimentation coefficients at higher salt concentrations (300 and 400 mM NaCl) was most clearly seen with oligonucleosomes of longer chain length; it was less pronounced in monomers, dimers, and trimers. The conformational changes of H1-stripped oligonucleosomes, in response to changes in salt concentration, differed from those of H1-containing oligonucleosomes in two respects (compare Figure 4 with Figure 6). First, the cooperative transition at low salt was only observed in H1-containing nucleosomes. Second, above 200 mM NaCl, the sedimentation coefficients of H1-containing oligonucleosomes declined much more drastically than those of oligonucleosomes lacking H1. Thus, the sedimentation properties of H1-containing and H1-lacking particles approximate each other progressively.

Role of H1 in Packaging Oligonucleosomal Chains into Composite Particles. At low salt concentrations, H1-containing oligonucleosomes underwent a cooperative transition from an extended to a compact form, which was not observed with H1-stripped oligonucleosomes. This observation prompted me to study the role of H1 in packaging oligonucleosomal chains into composite particles at salt concentrations above and below the conformational transition. As performed to demonstrate the gradient profile in Figure 1B, a limited nuclease digest was centrifuged in isokinetic sucrose gradients containing 50 mM NaCl (above the transition). The chain length heterogeneity of trimer and tetramer particles recovered from the peak fractions of this gradient was investigated by sizing the extracted DNA fragments in 1.1% agarose gels. Slots 3 and 4 in Figure 7 show that trimers

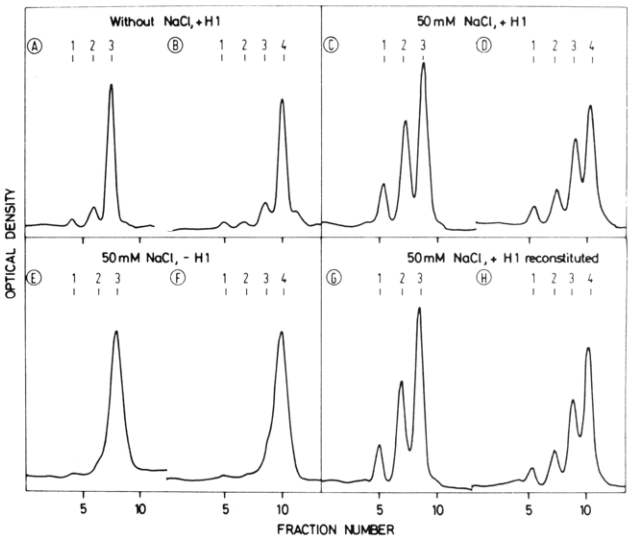

FIGURE 8: Chain length heterogeneity of trimer and tetramer particles as measured by resedimentation at 170 mM NaCl. Trimer (A, C, E, and G) and tetramer (B, D, F, and H) particles were obtained from isokinetic sucrose gradients as described in Figure 7. They were redialyzed in TE buffer containing 170 mM NaCl and then recentrifuged for 9 h in isokinetic sucrose gradients at the same NaCl concentration. (A and B) Trimer and tetramer peak fractions from an isokinetic sucrose gradient fractionating a nuclease digest in the absence of salt. (C and D) Trimer and tetramer peak fractions from a gradient containing 50 mM NaCl. (E and F) Trimer and tetramer peak fractions from a gradient fractionating an H1-stripped nuclease digest at 50 mM NaCl. (G and H) Trimer and tetramer peak fractions from a gradient in 50 mM NaCl buffer separating chromatin reconstituted from an H1-deprived nuclease digest and histone H1. The numbers 1–4 refer to the position of true-mono- to true-tetranucleosomes.

contained appreciable amounts of monomeric and dimeric chains, and tetramers contained significant amounts of monomeric, dimeric, and trimeric chains. As DNA fragments from longer chains were completely absent, the presence of shorter chains in trimer and tetramer particles could not result from an incomplete separation from adjacent nucleosomal peaks. This indicated that the population of trimers prepared under these conditions contained two types of particles: first, true-trimers in which nucleosomes were covalently linked by DNA; second, pseudo-trimers in which part of the nucleosomes were linked only by physical bonds. Similarly, the population of tetramer particles consisted of true-tetramers and pseudo-tetramers.

The agarose gels in Figure 2 demonstrated that, by the use of 170 mM NaCl during nuclear lysis and gradient centrifugation, oligonucleosomes were obtained which were homogeneous with respect to chain length. This effect of the use of 170 mM NaCl was exploited to investigate, by a second method, the chain length heterogeneity of trimers and tetramers recovered from a gradient centrifuged at 50 mM NaCl. Recovered trimer and tetramer particles were dialyzed against 170 mM NaCl and recentrifuged in isokinetic sucrose gradients containing this salt concentration. The sedimentation profiles in Figure 8C,D show that both trimers and tetramers dissociate at 170 mM NaCl into a spectrum of oligonucleosomal chains which were resolved by sedimentation. From the area under each peak, it was determined that trimers contained 25% monomeric, 37% dimeric, and 38% trimeric chains. Tetramers contained 15% monomeric, 20% dimeric, 32% trimeric, and 33% tetrameric chains. From these numbers it could be calculated that about 39% of the "trimer population" represented pseudo-trimers composed of one monomeric and one dimeric chain. A similar calculation

revealed that about 25% of the "tetramer population" represented pseudo-tetramers composed of one monomeric and one trimeric chain, and about 17% represented pseudo-tetramers composed of two dimeric chains.

In order to study the effect of H1 removal on the aggregation of oligonucleosomal chains, the experiment described above was repeated with an H1-stripped limited digest. Figure 7 (slots 5 and 6) shows that nearly all trimer and tetramer particles were composed of trimeric and tetrameric chains, respectively. This conclusion was supported by recentrifugation of H1-stripped trimers and tetramers in isokinetic sucrose gradients containing 170 mM NaCl (Figure 8E,F). The result showed again that after removal of H1 nearly all trimer and tetramer particles represented true-trimers and true-tetramers, respectively. This indicated that, at 50 mM NaCl, histone H1 mediated packaging of oligonucleosomal chains into composite particles.

In order to further investigate the function of H1 as a packaging element between different oligonucleosomal chains, histone H1 was added back to an H1-stripped nuclease digest at a ratio of 0.2:1 (H1:DNA) on a w/w basis. After dialysis of the mixture in 50 mM NaCl–TE buffer overnight, the reconstituted chromatin was centrifuged in an isokinetic sucrose gradient (containing 50 mM NaCl). Sizing of the DNA fragments in trimers and tetramers particles showed that chains shorter than tri- and tetranucleosomes, respectively, were present in appreciable amounts, while longer chains were not observed (Figure 7, slots 7 and 8). This conclusion was supported by recentrifugation of trimers and tetramers under nonaggregating conditions (170 mM NaCl). Figure 8G,H shows that trimers contained 23% monomeric, 34% dimeric, and 34% trimeric chains. Tetramers contained 13% monomeric, 17% dimeric, 29% trimeric, and 29% tetrameric chains. From these numbers it could be calculated that about 35% of the "trimer population" respresented pseudo-trimers composed of one monomeric and one dimeric chain; about 22% of the "tetramer population" represented pseudo-tetramers composed of one monomeric chain and one trimeric chain, and about 14% pseudo-tetramers composed of two dimeric chains. These results showed that, at 50 mM NaCl, the chain length heterogeneity of trimer and tetramer particles obtained from reconstituted chromatin was very similar to that of the corresponding particles obtained from native chromatin. Furthermore, these results identified histone H1 as the structural component which induced packaging of different oligonucleosomal chains into composite particles.

As concluded from sedimentation studies of isolated oligonucleosomes (see Figure 4), H1-containing oligonucleosomes existed in an extended conformation at very low ionic strength. This observation prompted me to investigate the packaging of oligonucleosomal chains into composite particles in the absence of salt (below the cooperative transition) in comparison with 50 mM NaCl. The chain length composition of trimers and tetramers obtained from gradients containing TE buffer (see Figure 1A) was analyzed by agarose gel electrophoresis and by recentrifugation at 170 mM NaCl. The agarose gel in Figure 7 (slots 1 and 2) shows that trimeric and tetrameric chains represented the major component in trimer and tetramer particles, respectively. When trimers and tetramers were recentrifuged at 170 mM NaCl (Figure 8A,B), the sedimentation profiles indicated that trimers contained 5% monomeric, 17% dimeric, and 78% trimeric chains, and tetramers 7% monomeric, 4% dimeric, 16% trimeric, and 72% tetrameric chains. These numbers have to be considered as upper limits, as trimers and tetramers are likely to be contaminated by neighboring oligonucleosomal peaks due to the lower sedimentation velocities at zero salt compared with 50 mM NaCl. Thus, at zero salt concentration, trimers contained at most 6% pseudo-trimers with one cut between nucleosomes, and tetramers contained at most 7% pseudo-tetramers composed of one monomeric and one trimeric chain and at most 3% pseudo-tetramers composed of two dimeric chains. This result indicated that packaging of oligonucleosomal chains into pseudo-particles is heavily impaired at very low ionic strength.

Discussion

Isolation of Homogeneous Oligonucleosomes. This paper describes a simple procedure for the isolation of oligonucleosomes—homogeneous with respect to chain length—from a limited nuclease digest of rat liver nuclei. The procedure used a salt concentration of about 170 mM NaCl during nuclear lysis and subsequent centrifugation in isokinetic sucrose gradients. Although the recovery of short chain oligonucleosomes was reduced to about one-half at this salt concentration, the use of 150–170 mM NaCl was advantageous over that of 50 mM or no salt because the isolated oligonucleosomal fractions were homogeneous with respect to chain length. This resulted mainly from the dissociation of composite particles (pseudo-particles) into single nucleosomal chains (see Figures 7 and 8). In addition, the degree of compactness of oligonucleosomal chains reached a maximum at 150–200 mM NaCl. The observation of a higher resolution of a nuclease digest in sucrose gradients containing 100 mM NaCl compared with zero salt (and 500 mM NaCl) has been made already earlier by Whitlock & Simpson (1976) but interpreted in a different way.

Oligonucleosomes prepared at 170 mM NaCl constitute only 9% of the total chromatin. Its salt solubility and H1 content seemingly contradict the finding that H1-containing particles are insoluble at this salt concentration (Olins et al., 1976; Wittig & Wittig, 1977). This may indicate that one deals with a special portion of chromatin. On the other hand, there are reports on salt-soluble oligonucleosomes (Itzhaki et al., 1978; Campbell & Cotter, 1977; Wittig & Wittig, 1977; Whitlock & Simpson, 1976; Renz et al., 1977; Lewis et al., 1976; Strätling et al., 1978a,b). Also, the "solubility" of oligonucleosomes depends on the chromatin concentration and the chain length (Campbell & Cotter, 1977; Wittig & Wittig, 1977). In addition, a variety of other experimental conditions have great influence on the "solubility" of oligonucleosomes, such as the temperature, the stringency of the centrifugation steps, and whether the oligonucleosomes leaking the nuclei after digestion are discarded (Wittig & Wittig, 1977). Thus, authors who reported that "almost all" of the oligonucleosomes precipitate at moderate ionic strength may have selected optimal conditions for precipitation (Whittaker & Blanchard, 1977). All this makes it obvious that it cannot be stringently concluded that one deals with a chromatin fraction of special properties. It may be excluded, however, that this fraction would be the actively transcribed portion of chromatin. Hybridization studies with cDNA transcribed from liver polysomal mRNA have shown that the fraction of chromatin most readily digestible with micrococcal nuclease is not enriched in active chromatin (Itzhaki et al., 1978). Secondly, the isolated oligonucleosomes were not remarkable with respect to protein composition (cf. Figure 3A,B). The full complement of all five histones and a heterogeneous spectrum of nonhistone proteins were found in all six oligonucleosomal fractions. Preliminary studies indicated that mono- to hexanucleosomes contained high mobility group proteins. These proteins have been found also by other investigators as integral components

in mono- and oligonucleosomes (Vidali et al., 1977; Goodwin et al., 1977). Important for the upcoming discussion is the fact that all oligonucleosomal fractions seem to have stoichiometric amounts of H1.

Function of H1 in the Salt-Dependent Conformation of Oligonucleosomes. The present paper investigated the involvement of histone H1 in the two processes: first, compaction of single oligonucleosomal chains; second, packaging of oligonucleosomal chains into composite particles. At low salt concentrations, H1-containing oligonucleosomes exhibited a cooperative transition from an extended to a compact form. Such a transition was also described by Renz et al. (1977) for a soluble chromatin preparation with an average chain length of about 30 nucleosomes. In contrast, the sedimentation response curve of H1-stripped oligonucleosomes showed a continuous increase in apparent sedimentation coefficient with increasing salt concentration. From these results it may be hypothesized that H1 mediates packaging of nucleosomes into a compact form. Analysis of the chain length heterogeneity revealed that, above the transition, 39% of the trimer particles and 37% of the tetramer particles represented pseudo-trimers and pseudo-tetramers, respectively. The degree of chain length heterogeneity was similar in trimer and tetramer particles from reconstituted chromatin. However, the contribution of pseudo-particles in trimers and tetramers at salt concentrations below the transition was found to be very low. Also, trimers and tetramers obtained from an H1-stripped digest were found to lack any pseudo-particles under packaging salt conditions (50 mM NaCl). The simplest explanation for these observations is that, at 50 mM NaCl, histone H1 mediates cross-linking of oligonucleosomal chains into composite particles. Other candidates among the variety of proteins in oligonucleosomes are excluded by the reconstitution experiment using purified H1. Electron microscopy has been used by other investigators to analyze the structure of chromatin as a function of ionic strength (Renz et al., 1977; Müller et al., 1978). Very low ionic strength produces an extended beads-on-a-string appearance. At elevated salt concentrations nuclear chromatin is organized in a 250-Å thick fiber, whereas SV40 chromatin is observed as a 350-Å thick particle. These studies lend support to the interpretation that moderate salt concentrations (50 mM NaCl) induce oligonucleosomal chains to be packaged into a compact structure.

The interpretation of sedimentation studies of polyelectrolytes (like DNA) at very low ionic strength is limited by the so-called "primary charge effect" leading to a slowing of the sedimentation rate. In chromatin about half of the phosphate charges are neutralized by histone charges. Therefore, the primary charge effect should be smaller than with DNA, but cannot be excluded. However, this effect should underly the sedimentation response curve of H1-containing as well as H1-lacking oligonucleosomes, as the removal of H1 does not grossly change the charge distribution of oligonucleosomes. The interpretation that H1-containing particles underwent a cooperative transition from an extended to a compact conformation was obtained from a comparison of the sedimentation response curves of H1-containing and H1-lacking oligonucleosomes. Therefore, a contribution the primary charge effect does not argue against the above interpretation. Secondly, electron microscopy has been used by other investigators (Renz et al., 1977; Müller et al., 1978) as an independent method to gain support for the salt-dependent transition of chromatin from an extended to a compact form.

At more elevated salt concentrations (150 mM NaCl) oligonucleosomes occurred in a slightly more compact conformation than at 50 mM NaCl, as judged from the small increase in sedimentation velocity (cf. Figure 4). On the other hand, a packaging of different oligonucleosomal chains into composite particles was not observed at 150 mM NaCl anymore. This may be indicative of a different nature of cross-linking of different chains and of compaction of single nucleosomal chains. The first process is mediated by H1 and is chromatin concentration dependent. Compaction of nucleosomal chains at low salt concentration is also mediated by histone H1. But at higher ionic strength this process is progressively a function of the salt concentration. Above about 80 mM NaCl, the shape of the sedimentation response curve of H1-containing oligonucleosomes is somewhat similar to that of H1-lacking oligonucleosomes, though shifted to higher sedimentation coefficients. This indicates that the constituents of the nucleosomal cores contribute to the compaction process. Above 150–200 mM NaCl the sedimentation response curves of H1-containing and H1-lacking particles approximate each other suggesting a progressive loss of the influence of H1 on the degree of compaction. At about 400 mM NaCl, finally, H1 starts to dissociate (Ohlenbusch et al., 1967). This implies that the packaging function of H1 is affected at much lower salt concentrations than the physical binding of H1 to chromatin.

Presently, it is not known how H1 fullfills its role both as a packaging and cross-linking element in the structure of oligonucleosomes. However, various pieces of evidence are available. First, H1 has been shown to have a binding site for the spacer DNA between nucleosomal cores (Varshavsky et al., 1976; Noll & Kornberg, 1977). At elevated salt concentrations, H1 may modify the conformation of the spacer such that nucleosomal cores come closer together. Second, at elevated salt concentrations H1 may form a cross-link between neighboring nucleosomal cores and/or spacer sequences, thereby contracting nucleosomes into a more compact particle. This model receives support from physical studies which indicate that, at physiological NaCl concentrations, H1 assumes a conformation consisting of a globular head and two free tails (Hartman et al., 1977). Third, elevated salt concentrations may induce neighboring H1 molecules to interact with each other and thereby constrain oligonucleosomal chains into a more compact particle. This model is supported by studies investigating the association of histones by cross-linking reagents; histone H1 was found predominantly as H1 polymer (Hardison et al., 1977).

In conclusion, H1-containing oligonucleosomes occur in four discrete conformations, depending on the surrounding ionic strength. First, below 10 mM NaCl oligonucleosomes exist in an extended conformation, and packaging of oligonucleosomal chains into composite particles is barely observed. Second, above a cooperative transition (i.e., at 50 mM NaCl), oligonucleosomes assume a compact conformation. Cross-linking of different chains into composite particles is mediated by H1. Third, at 150 mM NaCl, single oligonucleosomal chains exhibit a conformation slightly more compact than at 50 mM NaCl. However, cross-linking of different chains does not occur. Fourth, above 200 mM NaCl, oligonucleosomes appear increasingly in an extended conformation again.

Added in Proof

After submission of this manuscript a paper by Bloom & Anderson (1978) appeared reporting that after limited micrococcal nuclease digestion of hen oviduct nuclei the mononucleosomes released in digestion buffer are enriched in the actively transcribed ovalbumin gene. This report raises again the possibility that the soluble oligonucleosomes studied in the

present paper are preferentially cleaved from the transcriptionally active region of chromatin.

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