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Nucleosome mono-, di-, tri-, and tetramers from chicken embryo chromatin

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ABSTRACT

The fractionation of gram quantities of nuclease digested chromatin from chicken embryos into nucleosome mono-, di-, tri-, and tetramers is described in detail. Each of these nucleosomal species contains a fraction soluble in 0.1 M KCl that decreases with increasing repeat number. Less histone H1 is associated with the nucleosome fractions soluble as compared to the respective fractions precipitated in 0.1 M KCl. Thermal denaturation profiles of the four nucleosomal species are monophasic. The same T_m of 78 °C has been determined for the KCl-soluble nucleosomes and for the KCl-insoluble monomer. The T_m of the KCl-insoluble oligomers is 79.8 °C. Multiphasic melting curves were recorded for nucleosomal material that was concentrated by lyophilisation or stored at 4 °C in 0.25 mM EDTA. Total nucleosome mono-, di-, tri-, and tetramers (consisting of both the fraction soluble and insoluble in 0.1 M KCl) have been analyzed concerning their sedimentation, diffusion, partial specific volume, and molecular weight and compared with the sedimentation and molecular weight data of KCl-soluble nucleosome mono- and tetramers.

INTRODUCTION

Initiated by the work of Hewish and Burgoyne[1], Sahasrabuddhe and Van Holde[2], Kornberg and Thomas[3], Olins and Olins[4], Noll[5], and Baldwin et al.[6] a great number of data on the nucleosome, the basic unit of chromatin structure, have now accumulated (for a summary see ref.7). Recently Weintraub et al.[8] have presented a detailed model of the nucleosome. Nevertheless, the mode of DNA folding in the histone complex proximity is still ambiguous, and a precise analysis, e.g. by x-ray crystallography, has not yet been performed. The higher order structure of nucleosomes appears to rest mainly on protein-protein interactions with the histone H1 playing a leading role in its constitution[9,10]. Electronmicroscopy[4,16-19], supported by biochemical studies[3,11-15], has established a picture of "beats on a string", i.e. nucleosomes inter-

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connected by DNA stretches. Although the average length of the DNA spacer between nucleosomes and its function in histone H1 binding could be determined, still more information is needed to decide finally wether, in its native state, chromatin is a compact structure of nucleosomes in tight contact or a more extended arrangement[17,18,20]. Within this context it occurred to us that one way to contribute to the resolution of the problem of spatial arrangement of nucleosomes and final folding of chromatin, would be to study lower order oligomers of nucleosomes, i.e. beside nucleosome monomers, chromatin segments contain 2, 3, or 4 consecutive nucleosomes. We report here large scale preparation techniques and physicochemical parameters of such nucleosomes isolated from decapitated and eviscerated 13-day-old chicken embryos which consist mostly of mucsle and skin. This material was chosen because of being a very active protein synthesizing system, which may also be suitable for the investigation of structural differences between actively transcribed and non transcribed inactive nucleosomes.

METHODS

Isolation of nuclei. Decapitated 13-day-old chicken embryos were bled for 1-2 min, freed of all viscera, and the distal parts of the legs were removed. The trunks were extensively rinsed in an ice-cold solution of 0.9% NaCl, 2.5 mM EDTA, 0.5 mM EGTA, and disrupted, by using a Waring blendor at low speed for 5", in an equal volume of 2.5 M sucrose in a buffer containing 2.5 mM EDTA, 0.5 mM EGTA, 0.15 mM spermine, 0.5 mM spermidine, 15 mM 2-mercaptoethanol, 0.5 mM phenylmethylsulfonylfluoride (PMSF), 15 mM Tris-HCl, pH 7.9[21,22,23]. The resulting slurry was homogenized by 2 strokes at 700 rpm in a Potter S homogenizer (B.Braun, Melsungen) with a loosely fitting Teflon pestle and then poured through two layers of gauze. After adjusting the sucrose concentration to 58% (w/v) by the addition of 2.5 M sucrose in buffer the homogenate was centrifuged at 45,000 x g in a Beckman B XIV zonal rotor (BXV for large volumes) for 90'. (The zonal rotors were used in the latter step as batch-type rotors. The open and empty rotor was filled with the homogenate, closed with the lid, and the run started. After centrifugation the rotor was removed and the supernatant sucked off. Then the rotorcore was carefully

drawn out and the nuclear pellet scraped off the rotor wall with a glass-spatula. The crude nuclear pellet was suspended in 0.25 M sucrose, 60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 0.25 mM EDTA, 20 mM Tris-HCl, pH 7.4[21], centrifuged at 5000 x g for 5', and the whitish upper layer of the pellet, containing the nuclei, was recovered, resuspended in the 0.25 M sucrose solution, and again centrifuged. The resulting soft nuclear pellet was suspended in the 0.25 M sucrose solution to give a final concentration of about $8x10^8$ nuclei/ml, deep-frozen and stored in liquid nitrogen.

Digestion of chromatin with staphylococcal nuclease. Nuclei were thawed and diluted with 0.25 mM EDTA, 20 mM Tris-HCl, pH 7.9, to a concentration of $1-2x10^8$ nuclei/ml. The digestion was carried out in batches of 200 ml. After preincubation at 37 °C in a shaking water bath for 5', 1 mM $CaCl_2$ (final concentration) was added, and then digestion started by the addition of staphylococcal nuclease (Sigma, München, grade IV) to a final concentration of 5.5 U/10^8 nuclei. After 10' at 37 °C the digest was transferred into a 3-fold volume of ice-cold 0.5 mM EDTA, 20 mM Tris-HCl, pH 7.0, the nuclei lysed by 3 strokes in a tightly fitting Dounce homogenizer, and the solution cleared from nuclear debris by centrifugation at 2000 x g for 10'. Acid-soluble material was measured according to Sollner-Webb and Felsenfeld[11].

RNase-digestion and Mg^{2+}-precipitation of chromatin. RNase A (Serva, Heidelberg) was added to the supernatant to a final concentration of 10 µg/ml, incubation was at room temperature while slowly stirring for 30'. $MgCl_2$ was added to 5 mM and the chromatin allowed to precipitate while standing at 0 °C for 3 h. The chromatin was recovered by centrifugation at 30,000 x g in a Sorvall GSA-rotor for 30' and the pellet resuspended in 10 mM EDTA, 5 mM Tris-HCl, pH 7.0, by 3 strokes in a tightly fitting Dounce homogenizer. The turbid solution was dialyzed against 5 l of 10 mM EDTA, 5 mM Tris-HCl, pH 7.0, and twice against 5 l of 1 mM EDTA , 20 mM KCl, 5 mM Tris-HCl, pH 7.0.

Large scale sucrose gradient zonal centrifugation. All data refer to the titanium zonal rotor B XIV (Beckman Instruments, München), those for the B XV rotor will be given in paranthesis.

For the present purpose a special non-linear 6-30% (w/w)

sucrose gradient in 20 mM KCl, 1 mM EDTA, 5 mM Tris-HCl, pH 7.0, was applied (see Fig.1) with a total volume of 600 ml (1535 ml). The sample contained 600 mg (1.35 g) of digested chromatin (assuming that 1 mg of chromatin is represented by 10 A_{260}-units). The sample volume was adjusted to 30 ml (80 ml), the overlay was 20 ml (50ml). Centrifugation was at 45,000 rpm (34,000 rpm) for 21 h (34 h) at 1 °C. Fractions of 5 ml (10 ml) were collected. Fractions corresponding to nucleosome monomers, dimers, trimers, and tetramers were pooled. At least five fractions between each pool were discarded to minimize cross contamination. Pools were diluted approximately 3-fold with 20 mM KCl, 1 mM EDTA, 5 mM Tris-HCl, pH 7.0, and pelleted by centrifugation at 250,000 x g in Ti-60 rotors (Beckman Instruments,München) for 24 h. Pellets were resuspended and dialyzed against the buffer required.

DNA extraction, histone analysis, gel electrophoresis.

DNA was extracted with 1 M NaCl, 5 mM EDTA, 1% (w/v) SDS by shaking with an equal volume of chloroform/isoamyl alcohol (24:1, v/v)[24], incubated with Proteinase K (Merck, Darmstadt) (50 µg/ml) at 37 °C for 30', and finally extracted with phenol/chloroform/isoamyl alcohol (50:50:1, v/v/v)[25]. DNA was precipitated with ethanol and dissolved in 10^{-1} diluted electrophoresis buffer.

Electrophoresis of DNA fragments was carried out in 0.2x20x40 cm slab-gels using the Tris-borate-EDTA buffer system (44.5 mM Tris, 44.5 mM borate, 0.125 mM EDTA) of Maniatis et al.[26] and 6% polyacrylamid in the presence of 7 M urea. Electrophoresis was at 500 V, 40 mA until the xylene cyanole marker had travelled 12 cm (ca. 3 h), gels were stained with ethidium bromide (1 µg/ml) and photographed using the Polaroid Type 107 C Film and UV transillumination (Chromato-Viu C-62 Ultra violet Prod., Inc., Calif.).

Histones were extracted with 0.25 N HCl[27] and subjected to electrophoresis in 18% polyacrylamid slab-gels (0.2x20.25 cm) in the presence of 0.2% SDS[28], stained with amidoblack and destained in the presence of some free dye[29]. Slab-gels were cut into strips (1x25 cm) and scanned at 570 nm in a Gilford spectrophotometer 2400-S equipped with a linear transport. In the range employed there was a linear relationship between the area under the histone H4 peak (as measured by the paper weight of the cut-

out densitometric tracing[29]) and the amount of total histone-extract loaded onto the gel.

Thermal denaturation curves. DNA and total nucleosome samples (i.e. nucleosome mono-, di-, tri-, and tetramers consisting of both the KCl-soluble and insoluble fraction) were dialyzed extensively against 0.25 mM EDTA; when fractions soluble and insoluble had to be compared, the insoluble material was recovered by centrifugation and solubilized by dialysis against 0.25 mM EDTA. The last dialysate was used as reference in all experiments. Thermal denaturation curves were recorded at 268 nm with a Gilford spectrophotometer 2400-S equipped with an electronic temperature programmer and a digital print out. The heating rate was 0.25 ^{o}C/ min, about 200 absorption values were recorded for the transition region of each sample. Concentrations of A_{260}= 1.0 to 1.2 were employed.

Hydrodynamic experiments. For the determination of the partial specific volume nucleosome samples were dialyzed extensively against 0.25 mM EDTA, 5 mM Tris-HCl, pH 7.0; the last dialysate was used as reference. The apparent partial specific volume was calculated from density measurements on 1 ml samples with a Sartorius ultramicro-electrobalance (Type 4120, Sartorius, Göttingen) equipped with a plummet. Measurements at 10, 5 2.5, and 1 mg/ml were carried out for each nucleosome size class. Since no concentration dependence was observed the values obtained represent the partial specific volume $\bar{v}$. From dry-weight measurements an average extinction coefficient for nucleosome mono- and oligomers of $E^{1\%}_{260}$ = 95.4 $\pm$ 0.8 was obtained. Variations with respect to the nucleosome size class were within experimental error.

A Beckman Model E analytical ultracentrifuge equipped with the photoelectric scanning system was used for the determination of sedimentation and diffusion coefficients and for the sedimentation equilibrium experiments, absorbance was recorded at 265 nm. Boundary sedimentation was measured at 28,000 rpm and 20 ^{o}C. Boundary spreading was measured at 4400 rpm in a capillary type synthetic boundary cell at 20 ^{o}C. Total nucleosome mono-, di-, tri-, and tetramers were analyzed in 0.25 mM EDTA, 5 mM Tris-HCl, pH 7.0. With total nucleosome monomers and tetramers five runs each were performed at concentrations from A_{260}=0.3 to 1.8, for

total dimers and trimers three runs each at A_{260}=0.8 were evaluated. For the analyses of the respective fractions soluble in 0.1 M KCl, the insoluble material was removed by centrifugation and the supernatant dialyzed against 0.1 M KCl, 0.25 mM EDTA, 5 mM Tris-HCl, pH 7.0, or against 0.25 mM EDTA, 5 mM Tris-HCl, pH 7.0. Three runs each were performed (A_{260}=0.6 to 0.8) with the KCl-soluble nucleosomes in both solvents. The meniscus depletion method[30] was used for the determination of molecular weights by sedimentation equilibrium. Equilibrium was established with speed settings on 13,000, 9,000, 7,200, and 6,000 rpm for nucleosome mono-, di-, tri-, and tetramers, respectively. The temperature was maintained between 18 and 20 °C, the temperature control unit was turned off. Sample concentration was in the range from A_{260}=0.6 to 0.8.

RESULTS

Preparation and purification of nucleosome mono- and oligomers. A systematic investigation of several parameters relevant in zonal sucrose gradient centrifugation enabled us to choose such a set of conditions that, with our technique, it was possible to fractionate between 0.5 and 1.3 g (depending on rotor type) of nuclease digested chromatin and achieve complete separation of nucleosome mono- to tetramers (Fig.1). To obtain this result it was prerequisitory to change a number of conditions in preparing the chromatin digest as compared to methods currently in use[12,24,31]. First, in preparing the nuclei, we combined the buffer system introduced by Hewish and Burgoyne[1] with a high sucrose concentration[23] and PMSF[32] in order to prevent nuclear proteins from leaking out and to inhibit protease action. Such nuclei were stored deep-frozen until use. Second, we did not collect the digested chromatin by sedimentation of the treated nuclei, but, in order to maximize recovery, completely lysed the nuclei, cleared the digest from debris, and isolated the nucleosomal material by precipitation with Mg^{2+}. The convential procedure of collecting treated nuclei leads up to 50% loss of nucleosomes in the supernatant, inspite of the fact that, under the phase-contrast microscope, the thawed nuclei appear to be intact. Third, and most important, the resolution of the nucleosome mono- and oligomers is heavily obscured by the existence of considera-

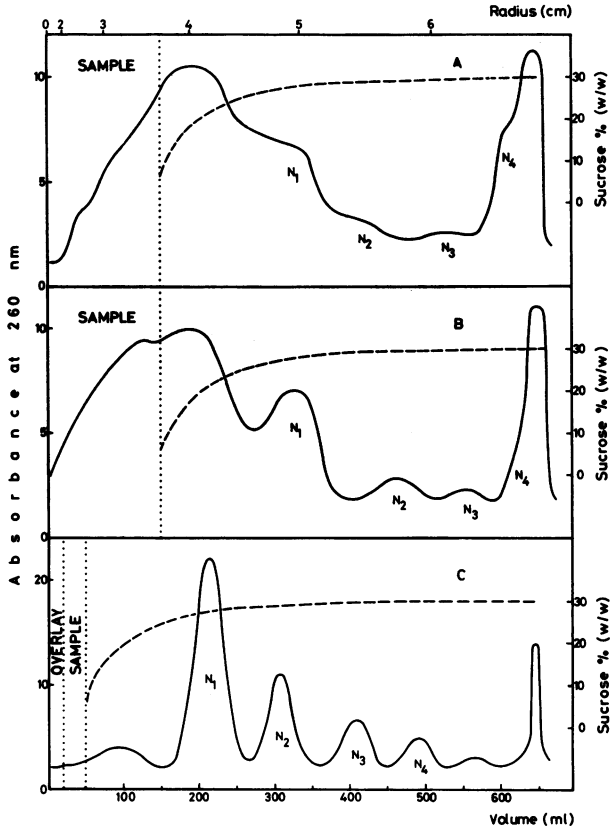

Fig. 1. Large scale sucrose gradient zonal centrifugation of staphylococcal nuclease digested chromatin. (A) Digested chromatin (2500 A_{260}-units) not incubated with RNase and not precipitated with $MgCl_2$. (B) Digested chromatin (3000 A_{260}-units) incubated with RNase. (C) Digested chromatin (5700 A_{260}-units) after incubation with RNase and precipitation with $MgCl_2$. N_1, N_2, N_3, N_4 denote nucleosome mono-, di-, tri-, and tetramers, resp. (———) absorbance at 260 nm; (------) sucrose concentration.

ble amounts of RNA (Figs.1A and 2). By the treatment of nuclease digested chromatin with RNase an improvement of oligomer resolution becomes apparent (Fig.1B). Complete separation was achieved when in addition the sample was applied in a small volume (Fig.1C). As Fig.2 clearly shows the RNase treatment does not alter the DNA extractable from the nucleosome mono- and oligomers.

Employing our finally adapted procedure the following overall yields were obtained: Nuclease digestion renders 20% of the total

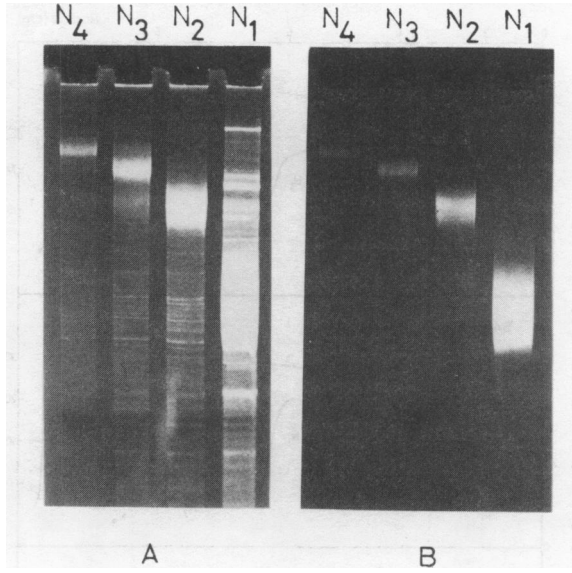

Fig.2. Polyacrylamid slab-gel electrophoresis in the presence of 7 M urea of DNA extracted from nucleosomes. DNA extracted from fractionated total nucleosomes not incubated (A) and incubated (B) with RNase prior to fractionation. N_1, N_2, N_3, N_4 correspond to the denotation of Fig.1.

DNA acid-soluble. From a batch of $4x10^{10}$ nuclei a sample of nucleosomal material corresponding to 1200 A_{260}-units may be obtained for zonal centrifugation. This is about 60% of the DNA present in the original nuclei. Nucleosome mono- to tetramers (in a ratio of 20:11:5:4) amount to 50% of the chromatin loaded into the rotor, the rest being precipitated material, pelleted higher class nucleosome oligomers (40%) and intermediate fractions discarded for the sake of purity. For further experimentation only freshly prepared nucleosome mono- to tetramers were used, since upon freezing and thawing (inspite of unaltered DNA-size, histone-content, thermal denaturation profiles, and hydrodynamic properties) about 20% of the material was constantly rendered insoluble by unspecific aggregation.

Solubility and homogeneity of nucleosome mono- and oligomers. The presence of monovalent cations is favourable in hydrodynamic studies to reduce electrostatic interactions. But detailed investigation of appropriate solvent conditions revealed that at KCl-

concentrations higher than 30 mM a considerable amount of nucleosomes precipitates; this fraction increases with increasing repeat number (Fig.3). An analysis of the histones of each of the KCl-soluble and insoluble nucleosome species showed that histone H1 is present in both, but reduced in the KCl-soluble nucleosome mono-, di-, tri-, and tetramers (Fig.4). Compairing the amount of stain adsorbed to histone H1 relative to H4, KCl-soluble mono- and tetramers contain about 20% and 40%, resp., the amount of H1 extractable from KCl-insoluble nucleosomes (Table 1). The H1/H4 ratios of the KCl-insoluble mono- and oligomers are constant within experimental error and approach the value generally accepted[33]. Two additional protein bands were always found in the histone extracts from our nucleosomal preparations. One of them, present only in the KCl-insoluble fractions, indicates a slight contamination with histone H5, the other, denoted U in Fig.4, has not yet been identified.

Melting properties of nucleosome mono- and oligomers. Thermal denaturation profiles were recorded for mono-, di-, tri-, and tetramers, in each case employing separately the respective KCl-soluble and insoluble fraction and total nucleosomes (i.e nucleosomes consisting of both the KCl-soluble and insoluble fraction) in one and the same heating experiment. Melting curves of the DNA extracted from total nucleosome mono-, di-, tri-, and tetramers, resp. have been recorded in separate experiments. As demonstrated in Fig.5 the melting curves of freshly prepared KCl-soluble and insoluble nucleosomes are mainly monophasic. No differences in T_m depending on the nucleosome repeat number were observed. The T_m of the KCl-soluble nucleosomes is 78 $\pm$ 0.3 °C, whereas the transition-midpoint of the KCl-insoluble nucleosomes is at 79.8 $\pm$ 0.3 °C. Melting curves of freshly prepared total nucleosomes (not shown) are also monophasic with a somewhat broader transition caused most probably by the two included fractions i.e. KCl-soluble and insoluble the T_m is 79.2 $\pm$0.4 °C.

When however total nucleosomes were stored at 4 °C in 0.25 mM EDTA for longer than 3-5 days or concentrated by lyophilization and then subjected to thermal denaturation, the melting curves recorded are multiphasic and exhibit peaks at 48 °C, 57 °C, and 68 °C in addition to the main transition (Fig.5, curves de-

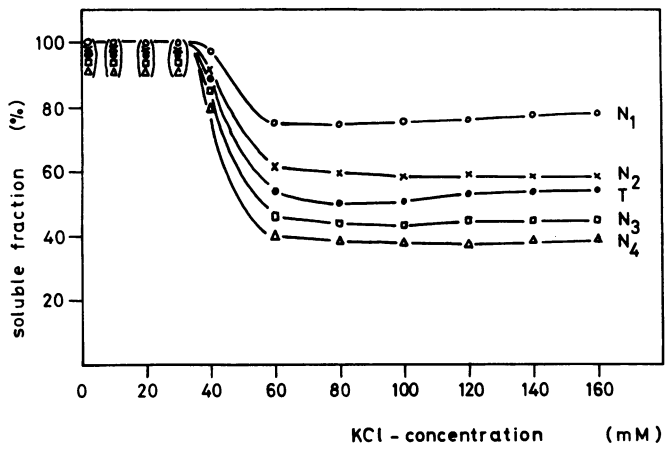

Fig.3. Solubility of nucleosome mono-, di-, tri-, and tetramers as a function of KCl-concentration. 0.8 to 1.0 A_{260}-units of each of the nucleosome fractions in 0.25 mM EDTA, 5 mM Tris-HCl,pH 7.0, were adjusted to the respective KCl-concentration in a total volume of 1 ml and allowed to stand overnight at 1 °C. Solutions were centrifuged for 10' at 1800 x g, A_{260} was measured in the supernatant. N_1, N_2, N_3, N_4 refer to nucleosome mono-, di-, tri-, and tetramers. T denotes chromatin digested with staphylococcal nuclease, incubated with RNase, precipitated with $MgCl_2$, and finally dialyzed against 0.25 mMEDTA, 5 mM Tris-HCl, pH 7.0. Points in brackets are identical with filled circles.

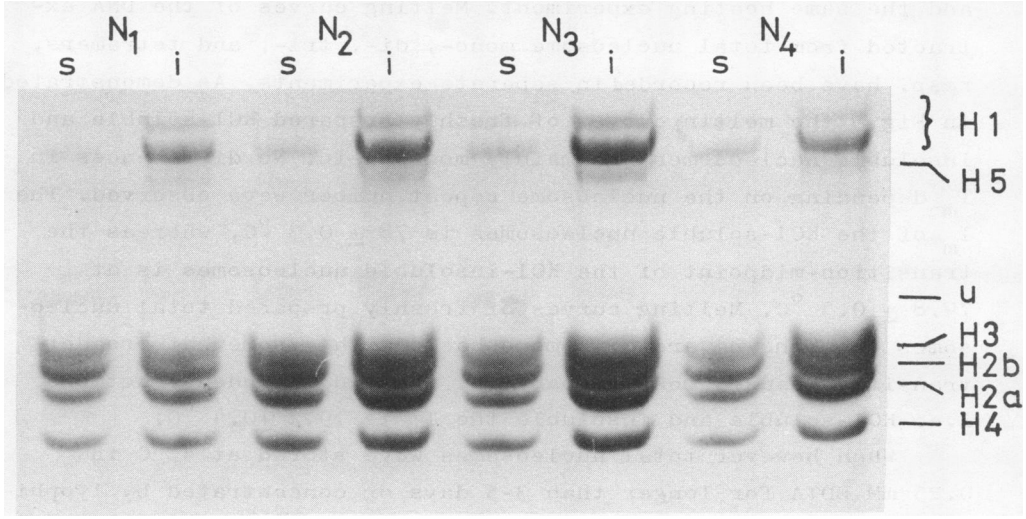

Fig.4. Histone composition of nucleosome mono-, di-, tri-, and tetramers. Histones extracted from nucleosomes insoluble (i) and soluble (s) in 0.1 M KCl. Individual histones were identified using calf thymus histones as references. N_1,N_2,N_3,N_4 as in Fig.1.

noted t). The T_m of the main transitions of these nucleosome mono- to tetramers increase from 77.5 °C to 79.8 °C. A corresponding

Table 1. Amount of histone H1 relative to histone H4.

	Nucleosomes			
	monomer	dimer	trimer	tetramer
Fraction soluble in 0.1 M KCl	0.12 ± 0.01	0.14 ± 0.01	0.17 ± 0.02	0.23 ± 0.03
Fraction insoluble in 0.1 M KCl	0.57 ± 0.05	0.59 ± 0.10	0.60 ± 0.07	0.58 ± 0.08

The respective values were obtained from 5 separate nucleosome preparations analysed on a single gel each. To correct for the molar staining ratio of H1 which is about two times that of H4, the values given have been divided by two.

Table 2. Hydrodynamic data of mono-, di-, tri-, tetranucleosomes.

	Nucleosomes			
	monomer (N_1)	dimer (N_2)	trimer (N_3)	tetramer (N_4)
$\bar{v}$	a) 0.664 ±0.006	0.671 ±0.005	0.674 ±0.006	0.678 ±0.007
$s^o_{20,w} \times 10^{13}$	a) 10.85 ± 0.15	15.06 ± 0.18	18.47 ± 0.18	21.30 ± 0.16
	b) 11.32 ± 0.21	15.87 ± 0.24	19.62 ± 0.23	23.46 ± 0.32
	c) 11.28 ± 0.23	—	—	21.28 ± 0.26
$D^o_{20,w} \times 10^7$	a) 3.39 ±0.09	2.57 ±0.11	2.13 ±0.10	1.89 ±0.12
M (s,D)	a) 231670 ± 13370	442270 ± 31330	646210 ± 48490	853820 ± 79240
M (equil.)	a) 235560 232140	463150 456910	694445 680275	931180 894080
	b) 218850 215730	—	—	920455 886365
	c) 221515 218005	—	—	926015 891345

(a) Total nucleosomes (i.e. consisting of both the fraction soluble and insoluble in 0.1 M KCl) in 0.25 mM EDTA, 5 mM Tris-HCl, pH 7.0; (b) KCl-soluble fraction in 0.1 M KCl, 0.25 mM EDTA, 5 mM Tris-HCl, pH 7.0; (c) KCl-soluble fraction in 0.25 mM EDTA, 5 mM Tris-HCl, pH 7.0. M (s,D) was calculated using the Svedberg-equation, deviations of M (s,D) were obtained using the extreme values of $\bar{v}$, $s^o_{20,w}$, and $D^o_{20,w}$. M (equil.) denotes molecular weights measured by sedimentation equilibrium (meniscus depletion method).

increase of T_m from 37.5 °C to 39.8 °C is found with DNA extracted from total monomers to tetramers.

Hydrodynamic properties of nucleosome mono- and oligomers. The hydrodynamic properties of nucleosome mono-, di-, tri-, and tetramers are summarized in Table 2. Since the measured sedimentation

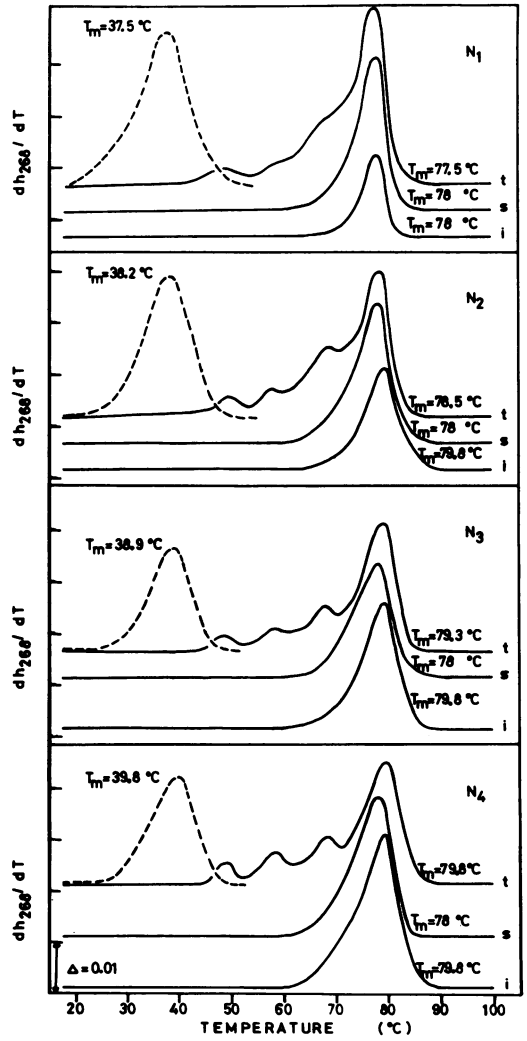

Fig.5. Thermal denaturation curves of nucleosome mono- (N_1), di- (N_2), tri- (N_3), and tetramers (N_4) and their extracted DNA. (-----) free DNA extracted from total N_1, N_2, N_3, N_4, resp.; (——— i) fraction insoluble in 0.1 M KCl; (——— s) fraction soluble in 0.1 M KCl; (——— t) total nucleosomes i.e. consisting of both the soluble and the insoluble fraction. For clearer display curves have been displaced on the vertical axis.

and diffusion coefficients of total nucleosome mono- and tetramers had exhibited no concentration dependence over the range employed, the respective values for total dimers and trimers and the KCl-soluble mono- and oligomers were measured at one concentration only. The plateau region remained flat and the boundary was symmetrical in all sedimentation velocity runs, as indicated by the absorbance tracing, regardless of nucleosomes being analysed in high salt or in low salt buffer. Only two runs each were performed for the equilibrium sedimentation studies within the concentration range where the sedimentation and diffusion coefficients exhibited no concentration dependence. The graphs of the logarithm of the absorbance, as measured by the photoelectric scanner, versus r^2 were linear for all nucleosome species.

DISCUSSION

The observed solubility behaviour of nucleosome mono-, di-, tri-, and tetramers from chicken embryos together with the reduced histone H1 content in the KCl-soluble fraction, has recently been described similarly for erythrocyte mononucleosomes by Olins et al.[34]. It is generally accepted, that histone H1 is removed from chromatin simultaneously with the digestion of DNA to the 140 basepairs in core particles[10,12]. This mechanism seems hardly acceptable for the creation of such a reduced H1/H4 ratio in nucleosome di-, tri-, and tetramers (Fig.4, Table 1). However, Renz et al.[35] suggested that upon nuclease digestion of chromatin, H1 is redistributed among nucleosomes preferentially to the larger oligomers. Besides being an explanation for the removal of H1 from oligomers, this is also consistent with our observation that the H1 content of the KCl-soluble fraction is increasing with the nucleosome repeat number.

Melting curves of nucleosome mono- and oligomers have been published by several authors[36,37,38]. Woodcock and Frado[36] found melting curves to be monophasic for monomers and biphasic for higher order oligomers, the T_m of 77 °C being independent from particle size. Mandel and Fasman[37] reported multiphasic thermal denaturation profiles for mono- and oligomers and T_m increasing from 73.5 °C for monomers to 79 °C for pentamers. Contrary to these data but in good agreement with our work Lawrence et al.[38]

recorded monophasic melting curves for nucleosome mono- and oligomers with a T_m of 79 °C; this is also the value we determined for the chicken embryo total nucleosome mono-, di-, tri-, and tetramers (79.2 ± o.4). We found a difference in T_m between the KCl-soluble and insoluble fractions with the nucleosome oligomers only but not with the monomer particles (Fig.5), which signifies that the two fractions might differ in their nucleosome-nucleosome interactions. We demonstrate here (Fig.5) that chicken embryo nucleosomes that have been subjected to conditions like storage at 4 °C in 0.25 mM EDTA or lyophilization, exhibit multiphasic thermal denaturation profiles, with additional peaks at about 48, 57, and 68 °C. Since Whitlock and Simpson[31] observed multiphasic melting curves of nucleosome core particles only in the presence of urea, we feel confirmed with our suggestion that such multiphasic profiles originate from in some way denatured nucleosomal material. The increase of T_m with the repeat number of such "denatured" nucleosomes seems to be a function of DNA size, since a corresponding increas is found with free nucleosomal DNA. The T_m of DNA is clearly size dependent at these low molecular weights[39].

The hydrodynamic data indicate that total nucleosome monomers have a higher molecular weight and a more extended sructure than the monomers of the KCl-soluble fraction. Although the measured values do agree with those published by Olins et al.[34], we were surprised that the KCl-soluble fracti n, which amounts to only 20% in our total monomer preparation (Fig.3), seems to exhibit such a remarkable influence on the measured parameters. Since we never observed any heterogeneity in this sample, neither in the sedimentation velocity studies nor with the sedimentation equilibrium experiments, we suggest that electrostatic interactions at the low ionic strength in the buffer employed might have influenced these measurements. Unfortunately, it is not possible to analyse the total nucleosome mono-, di-, tri-, and tetramer fractions, as a control, at a high salt concentration. A comparison, however, of the data obtained for KCl-soluble nucleosome mono- and tetramers at high and low ionic strength reveals that the error might not be too serious.

A comparison of derived hydrodynamic quantities with values

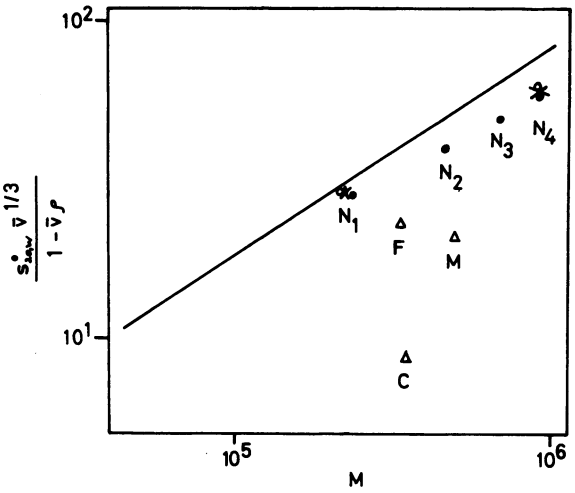

Fig.6. Van Holde plot for nucleosome mono-, di-, tri-, and tetramers. Filled circles: total nucleosomes (i.e. consisting of both the fraction soluble and insoluble in 0.1 M KCl); open circles: KCl-soluble fraction in 0.1 M KCl, 0.25 mM EDTA, 5 mM Tris-HCl, pH 7.0; X: KCl-soluble fraction in 0.25 mM EDTA, 5 mM Tris-HCl, pH 7.0; F, fibrinogen; M, myosin; C, collagen. N_1, N_2, N_3, N_4 denote nucleosome mono-, di-, tri-, and tetramers, resp. The drawn line is empirically deduced[40] for globular proteins and follows the equation $s^o_{20,w}\,\bar{v}^{1/3} / (1 - \bar{v}\rho) = (9.2 \times 10^{-3})\, M^{2/3}$ when $s^o_{20,w}$ is in Svedbergs.

calculated from small angle neutron scattering studies[41] corroborates the validity of the data measured for nucleosome monomers. Hjelm et al.[41] reported a radius of 5.2 nm and a particle volume of 249 nm^3. Our respective values calculated from $\bar{v}$, S, and D by standard methods[42] are 6.4 nm for the radius of the equivalent hydrated sphere and 256 nm^3 for the volume of the unhydrous monomer particle.

A correlation of the hydrodynamic data is given in Fig. 6 by the Van Holde plot[40]. It shows that the corresponding values of total nucleosome mono- to tetramers do deviate from those of "globular" proteins (drawn line), but not to a drastic extend (as, e.g., the values for myosin and fibrinogen do), and that they form a linear relationship. This indicates, as already proposed by Hjelm et al.[41], an ellipsoid rather than a sphere for the outer shape of the nucleosome monomer. The di-, tri-, and tetramers seem to display a compact structure rather than a rod

like extended form. Noll and Kornberg[10] recently found a marked reduction in sedimentation velocity when they removed H1 completely from nucleosome oligomers. No such difference was observed between total nucleosome tetramers and KCl-soluble tetramers (Fig.6), despite the low histone H1 content in the latter. Thus small amounts of H1 seem to be sufficient to stabilize the compact structure of nucleosome oligomers.

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