Studies on the structure of isolated chromatin in three different solvents

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ABSTRACT

Properties of calf thymus chromatin, prepared by mild procedures, have been studied in various solvents. In 0.2 mM EDTA *s*-values ranged from 20 to 30 S and intrinsic viscosities from 5 to 24 dl/g. Dialysis against 0.15 M NaCl or 0.2 mM MgCl₂ changed these values to 80 to 100 S and 0.2 to 5 dl/g, respectively, indicating an essentially more compact structure. In 0.2 mM EDTA X-ray scattering yielded a cross section diameter of 9 nm, which is associated with the tertiary structure of chromatin fiber (*M/L* = 21200 Dalton/nm). By dialysis against 0.15 M NaCl or 0.2 mM MgCl₂ part of the material spontaneously formed quarternary structures (cross section diameters 25-29 nm). The rest of the material with cross section diameters <9 nm is supposed to be more strongly sheared tertiary structure which seems to be unable to form quarternary structure due to artificial conformational changes.

INTRODUCTION

During the last decade the structure of chromatin has been investigated using a variety of methods. For all of these studies chromatin first has to be isolated. However, chromatin can only be obtained as a fragmentary material; therefore, the method of preparing chromatin is of decisive importance when evaluating studies on its structure.

A "classical" property of isolated chromatin seemed to be its insolubility in isotonic NaCl (1). A partial precipitation occurs in 0.15 M NaCl, even when the material has been prepared according to Zubay and Doty (2), a widely used "mild" procedure. Noll et al. (3) introduced a new technique: the isolated nuclei were incubated briefly with a micrococcal nuclease and subsequently lysed in 0.2 mM EDTA. A year earlier, Rees et al. (4, 5) had obtained chromatin in a similar manner and observed very

high sedimentation coefficients combined with fairly low intrinsic viscosities. An unusual property of their material was its solubility in 0.15 M buffers; this solubility was lost, however, after the chromatin had been sheared. Thus, precipitation of chromatin in 0.15 M salt solutions must be due to artificial structural changes in the fragmentary molecules.

The goal of the experiments reported in this paper is to get some information about structural changes of isolated chromatin in dependence of the applied solvent. They are also based on electronmicroscopic studies by Finch and Klug (6) who observed superhelical quarterny structures of isolated chromatin fibers in 0.2 mM MgCl_2 . We deduce our conclusions from measurements of sedimentation and intrinsic viscosity, supplemented by low angle X-ray scattering studies. More detailed data of chromatin structure are presented in a subsequent paper (7). These were derived by comparison of experimental X-ray scattering curves with those simulated by a computer.

MATERIALS AND METHODS

Preparation

Chromatin was prepared from calf thymus essentially as described by Noll (3) and dialysed for 16 h at 4°C against 0.2 mM EDTA, 0.15 M NaCl or 0.2 mM MgCl_2 . For low angle X-ray scattering studies these solutions (concentration 1 to 10 mg/ml) were concentrated 2 - 3 times further using Milliporefilters (membrane type Pellicon PTGC).

Sedimentation analysis

Sedimentation coefficients of chromatin in the different solvents were determined using standard techniques at 20°C in a MSE analytical ultracentrifuge (Mark II) equipped with an ultraviolet absorption optical system and scanner [Schachman (8)]. The measured s-values were corrected for water as solvent.

Viscosity measurements

Viscosity measurements were carried out at 20°C in a (modified) rotating cylinder viscometer according to Zimm and Crothers (9). Relative viscosities η_{rel} were measured at dif-

ferent chromatin concentrations. The concentrations were calculated from absorption at 260 nm (chromatin concentration of 1 mg/ml corresponds to $A_{260} = 10.4$). $\eta_{red} = (\eta_{rel} - 1)/c$ and $(\ln \eta_{rel})/c$ were plotted against concentration c (Fig. 1). From the linear regression on these data, intrinsic viscosity $[\eta]$ was obtained as an average value of the two intercepts on the y-axis.

Small angle X-ray scattering

The X-ray scattering measurements were done with a highly stabilized X-ray generator (Siemens, Kristalloflex IV) using a copper tube (35 kV, 32 mA, Ni-filter). For collimation of the X-ray beam, a Kratky camera (10, 11) was employed. The chromatin solutions were placed in Mark capillaries (diameter about 0.7 mm) and irradiated at a temperature of 5°C. A proportional counter, equipped with a pulse height discriminator as detector for the Cu K_{α} -line, recorded the scattered intensities. The electronically programmed step-scanning-device (12) allowed automatic operations and repeated scanning of the scattering curves. Each curve was corrected by subtracting the blank scattering curve (that of the pure solvent). In such corrected scattering curves (range of angles 1.25 to 18.2 mrad, 35 points per curve, width of entrance slit 60 μ m) elimination of the collimation effect,

Figure 1: Determination of intrinsic viscosity $[\eta]$: The measured values η_{red} and $(\ln \eta_{rel})/c$, respectively, were plotted against concentration c of chromatin and extrapolated to $c = 0$.

so called "desmearing", was effected using a computer program (from the Graz Institut für Physikalische Chemie, Austria) according to the method of Glatter (13). To determine the mass per unit length, one needs the absolute values of scattered intensities. These were obtained by using a Lupolen standard sample (14, 15) which had been calibrated at the Graz Institut für Physikalische Chemie.

DATA ANALYSIS AND RESULTS

The results of sedimentation analysis and viscosity measurements on chromatin of different preparations are summarized in Fig. 2. The double logarithm plots of s against $[\eta]$ yield two straight lines, one for 0.2 mM EDTA, and one for either 0.15 M NaCl or 0.2 mM MgCl_2 . Each pair of points of the two curves belongs to separate preparations of the same kind. The wide range of $[\eta]$ -values at the abscissa axis apparently resulted from differences in shearing, unavoidable when preparing chromatin and receiving fragments of different lengths. In Fig. 3 (desmeared) scattering curves (Guinier plots for cross section) are represented. In the linear regions of the curves (2.25 - 4.25 mrad, 5 points and 7.25 - 14.25 mrad, 15 points resp.) where

Figure 2: Sedimentation coefficients (Svedberg units) as a function of intrinsic viscosities (units dl/g) for several preparations in different solvents.

Figure 3: Guinier-plots (cross section) of desmeared small angle X-ray scattering curves for chromatin in different solvents.

linear regression was done, the relation $h \cdot R_g \approx 1$ (with $h = \frac{2\pi}{\lambda} \cdot 2\theta$) is sufficiently satisfied. The slope of the lower curve (chromatin in 0.2 mM EDTA) indicates a cross section radius of gyration of 3.2 nm, i.e. a cylinder with a 9 nm diameter. Analysis of the two remaining curves is more difficult. In case of 0.15 M NaCl or 0.2 mM MgCl_2 , each curve contains two clearly distinguishable ranges of straight line slopes. Note that for the 0.2 mM EDTA curve, the first slope (small angles) is nonexistent. After evaluation of the curves for MgCl_2 and NaCl according to a method of Kratky (15), we postulate the existence of two kinds of rodlike particles with different cross sections. For separating the two portions an approximating Gaussian course for each of them was used. So a relation is deduced between the cross section radii of gyration of both kinds of particles R_1 (smaller cross section, outer part of scattering curve) and R_2 (larger cross section), and the apparent cross section radius of

gyration R_g determined from the inner part of the curve:

$$R_g^2 = k \cdot R_1^2 + (1 - k) \cdot R_2^2,$$

k is the ratio of the scattering of the small cross section $(I \cdot 2\theta)_{01}$ to total scattering $(I \cdot 2\theta)_{0s}$, both at zero angles. According to this method our curves yielded the following cross section radii of gyration: for chromatin in 0.15 M NaCl 10.9 nm, and in 0.2 mM $MgCl_2$ 9.5 nm. For the outer slopes the corresponding values are 2.8 nm (0.15 M NaCl) and 3.2 nm (0.2 mM $MgCl_2$), respectively. In a subsequent paper (7) these relatively simple calculations are extended by the application of another method which leads to more accurate data of chromatin structure (comparision of experimental X-ray scattering curves and those simulated by a computer). Using the solenoidal model approximated by a hollow cylinder for quarterny structure (6) as a basis for further analysis (wall thickness of 9 nm equal to the fiber diameter in 0.2 mM EDTA) outer diameters (hollow cylinder) of 28.9 nm (0.15 M NaCl) and 25.7 nm (0.2 mM $MgCl_2$) are obtained. From the outer slopes of curves in Fig. 3 the following cylinder diameters are evaluated: 7.9 nm (0.15 M NaCl) and 9.0 nm (0.2 mM $MgCl_2$). Comparing different preparations the diameters have a maximal variation of about ± 10 percent. In Table I average values for NaCl and $MgCl_2$ are listed from 6 or 2 preparations, respectively.

Table I: Experimental results for chromatin in different solvents. The left and right side of column 4 correspond to the inner and outer part of scattering curves, resp.

Solvent	s_{20} (S)	$[\eta]$ (dl/g)	diameter (cross sect.) with standard errors (nm)	M/L (Dalton/nm)
0.2 mM EDTA	20 - 30	5 - 24	9.0 $\pm$ 1.0	21 200
0.2 mM $MgCl_2$	80 - 100	} 0.2 - 5	25.6 $\pm$ 0.3 8.3 $\pm$ 1.0	-
0.15 NaCl	80 - 100		27.8 $\pm$ 1.6 7.5 $\pm$ 0.2	-

Additionally, we could determine the mass per unit length for a chromatin preparation in 0.2 mM EDTA by measuring the absolute X-ray intensities. With the values $\bar{V} = 0.668 \text{ cm}^3/\text{g}$ (partial specific volume) (16), $z_1 = 0.53$ (number of mols of electrons per gram of the solute) (17) and the obtained data using the well-known evaluation method (15), the result was $M/L = 21\ 200 \text{ Dalton/nm}$.

DISCUSSION

The obtained results are summarized in Table I, Fig. 2 and Fig. 3. With 0.2 mM EDTA as a solvent, the relatively low s-values (20 - 30 S) are associated with high intrinsic viscosities (5 - 24 dl/g) (Fig. 2). Moreover, dialysis not only against 0.15 M NaCl, but also against 0.2 mM MgCl_2 , leads to higher s-values (80 - 100 S) and lower $[\eta]$ -values (0.2 - 5 dl/g), i.e. to a significantly more compact particle structure. The flatness, especially of the upper line, is remarkable. It indicates, that with increasing $[\eta]$, i.e. with effective particle volume, there is only a slight alteration of the s-values in both cases. Such behaviour can theoretically be expected not only for rodlike, but also for more flexible particles, as long as they are very anisometric and the radius of curvature is sufficiently large [Schachman (8), p. 244 ff]. For such particles the sedimentation coefficient is an approximate measure for the mass per unit length and hence the thickness or diameter. From these observations it can be concluded, that in 0.2 mM EDTA solutions isolated chromatin exists in the form of thin long particles, part of which condenses into rodlike particles with larger diameters when dialysed against 0.15 M NaCl or 0.2 mM MgCl_2 . The two types of structure should correspond to tertiary structure (fibers of chromatin) and superhelical quarternary structure of chromatin as described by Finch and Klug (6). This conclusion is supported by low angle X-ray scattering studies.

The X-ray data can be summarized in the following way: In 0.2 mM MgCl_2 and 0.15 M NaCl thicker rodlike particles are obtained with diameters ranging from 25 to 29 nm. Obviously, these structures were spontaneously formed during dialysis from particles with cross section diameters of 9 nm, i.e. those which

exclusively were observed in 0.2 mM EDTA. There is additionally a small amount of thinner particles with cross sections in general distinctly less than 9 nm.

Based on the various measurements, it is concluded that chromatin in 0.2 mM EDTA, prepared by using Noll's very mild procedure, essentially consists of tertiary structure fiber fragments. Part of these fragments change their configuration into quarternary structure in the solvents 0.15 M NaCl and 0.2 mM MgCl_2 . Li et al. (18) recently demonstrated that such structural changes of chromatin occur already in $2 \cdot 10^{-3}$ M NaCl or $1 \cdot 10^{-5}$ M MgCl_2 . Our 0.2 mM EDTA prepared chromatin showed cross sections of 9 nm and a mass per unit length of 21 200 Dalton/nm. These values are somewhat smaller than the X-ray results of Sperling and Tardieu (16), who, using similar methods, obtained 10.7 nm and 23 400 Dalton/nm, respectively. We feel this to be the consequence of stronger shearing of our material, thus getting a higher portion of partially "unwound" beads. Recently, Baudy and Bram (19) deduced from their measurements - which were done under quite different conditions - a cross section diameter of 14 nm which is considerably higher than the values of other authors (16), including our own. A possible explanation might be that fragments of quarternary structure were involved in the measurements of Baudy and Bram. In fact, we could observe such an effect in our scattering curves just in the angle range which has been used by Baudy and Bram for their analysis. Due to our experimental M/L value together with the well-known molecular weight for nucleosomes of 230 000 Dalton (20) an average distance of 10.8 nm [see also (21)] between chromatin subunits is required. So according to our measurements we get for chromatin in 0.2 mM EDTA an average cross section diameter of 9 nm and a nucleosomal distance of 10.8 nm. This distance of nucleosomes is an average value, which results from our experimental M/L-data; it does not exclude a locally more dense packing of nucleosomes [see (7)].

The obviously more compact structure of particles in the solvents 0.15 M NaCl and 0.2 mM MgCl_2 can be explained as a mixture of quarternary structure fragments and (partially damaged) fibers of tertiary structure, corresponding to the inner or the outer straight parts of scattering curves in Fig. 3. In this

manner evaluated cross section diameters of 25 to 29 nm for quarternary structure agree well with electronmicroscopic and neutron or X-ray scattering data of other authors (6, 22, 23, 24, 25, 26, 27, 28, 29). The diameters obtained from the outer slopes of the scattering curves (Table I, column 4, side right) in general are less than 9 nm. We suppose these to be sheared tertiary structure fragments as a consequence of the isolation procedure. Portions of these structure may be connected partially with quarternary structures. The compact structures are formed spontaneously from fibers of tertiary structure in the solvents 0.15 M NaCl and 0.2 mM MgCl_2 . It cannot be excluded that in this process several shorter tertiary structure fragments also might participate, so in this process the number of particles has not necessarily to remain constant. In this connection it is referred to the electronmicroscopic studies of

Figure 4: Simplified scheme to demonstrate the influence of the isolation procedure on structure and solubility of chromatin fragments in 0.15 M NaCl. Left side: Formerly used methods. Right side: Modern preparation techniques, including nuclease treatment of nuclei.

Finch and Klug (6), who observed an association of individual nucleosomes to products, which seemed to be similar to super-helical quarterny structure.

With respect to the solubility of "mildly prepared" chromatin in 0.15 M NaCl the following explanation is suggested (Fig. 4). Applying mild conditions - including brief incubation with nucleases - single strand breaks are produced in the DNA of chromatin. During subsequent lysis of nuclei in 0.2 mM EDTA chromatin fragments evolve with less damage due to shearing, most of which apparently remain unchanged with respect to their tertiary structure. In a physiological 0.15 M environment only such fragments with intact tertiary structure are able to form soluble quarterny structure fragments; however, the more damaged parts of chromatin fragments precipitate when attempting to develop a normal quarterny structure.

Discussing problems of chromatin structure one has to keep in mind that so far all the quarterny structures - examined with isolated chromatin - represent reconstituted material. Isolation of chromatin requires lysis of cell nuclei. In general, this is done using hypotonic solvents, as a consequence of which quarterny structure gets lost. As yet one cannot decide whether the reconstituted quarterny structure of chromatin is exactly the same as that in intact nuclei, or whether there might be certain differences.

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