THE MASS PER UNIT LENGTH OF CHROMATIN BY LOW-ANGLE X-RAY SCATTERING

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1. Introduction

Recent biochemical work has shown that chromatin is made of repeating units which contain an octamer of the four major histones and about 200 base pairs of DNA [1–3]. This biochemically well-defined subunit is associated with bead-like structures in electron micrographs of chromatin [4–7], and two different models of the chromatin fibre, each incorporating the notion of a bead-like subunit, have been proposed (fig.1). A summary of the reasoning which led to these models is found in [7]. Although the repeating unit has the same chemical composition in both types of model, the packing ratio of the DNA (ratio of extended length of DNA to chromatin fibre length), and therefore the average mass per unit length of the chromatin fibre, are strikingly different. In models of type I (e.g. [8,9]), a mass of 260 000 daltons (the mol. wt. of the repeating unit [10] plus one molecule of HI) is associated with a length of 200 to 300 Å of chromatin fibre, giving a packing ratio of 2.5–3.5:1 and a mass per unit length of 850–1300 daltons/Å. In contrast, the packing ratio of Model II ir roughly 7:1 and the mass per unit length is 2600 daltons/Å.

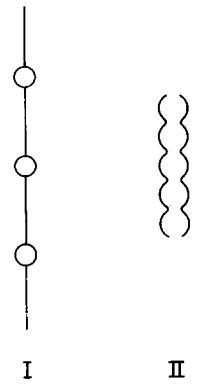

Fig.1. (adapted from [7]) (I) In models of this type the chromatin consists of beads 70–100 Å in diameter containing all of the protein and part of the DNA of the repeating unit. The beads are separated by the rest of the DNA, in extended form [5,8]. (II) In this model the chromatin is a chain of 100 Å beads in contact, each bead containing the 8 histones and 200 base pairs of DNA in the repeating unit [10].

We report here a determination of the mass per unit length of chromatin fibres in solution by low-angle X-ray scattering which provides evidence in support of Model II. We find a value twice as great as values previously determined by the same technique [11,12] and attribute this difference to the fact that the earlier methods of preparing chromatin involved a shearing step which pulled out the DNA, giving the chromatin an extended form of the type represented in Model I.

2. Materials and methods

Chromatin was isolated from rat liver nuclei by the procedure of Noll, Thomas and Kornberg [13]. Chromatin of average length 40 repeating units (range 15–150 repeating units), the longest material which can be solubilised without shear, was made by

very brief micrococcal nuclease digestion and lysis of the nuclei into ½ or ¼ volume of 0.2 mM EDTA. Solutions of chromatin were clarified by spinning for 5 to 10 min at 3000 rev/min, and contained between 4 and 8 mg/ml of chromatin; vacuum dialysis was used to prepare more concentrated solutions. Concentration was determined by measuring the A_{260} in water with a Cary 14 Recording Spectrophotometer, taking a value of $1\,A_{260} = 0.1$ mg/ml of chromatin.

The X-ray measurements were made at 20°C immediately after the preparation of the sample. The X-ray camera and the experimental procedures are described elsewhere [14,15]. The electron mass per unit length μ and the axial radius of gyration R_c of chromatin are calculated using equation (1) which gives the expression for the normalised scattered intensity near the origin of a solution of cylindrical rods [16]:

$$2sI(s)/\upsilon\eta E_o = (1-\rho_o\psi)^2\, c_e\, \mu\, (1-2\pi^2 R_c^2 s^2) \qquad (1)$$

where:

$s = 2 \sin\theta/\lambda$. 2θ is the scattering angle, λ the wavelength ($\lambda = 1.54$ Å).

$I(s)$ is the experimental intensity curve, corrected for background and collimation distortions.

E_o, η υ are the energy of the incident beam, the thickness of the sample (electrons/cm^2) and a physical constant $\upsilon = \lambda^2 \times 7.9\cdot10^{-26}$.

ψ is the partial electronic volume. A value of 2.09 Å^3/electron (standard deviation: 0.45%) has been determined for chromatin prepared as above (P. J. G. Butler and L. S. unpublished).

c_e is the concentration in number of electrons of solute per number of electrons of solution.

ρ_o is the electron density of the solvent (electrons/Å^3).

For a string of beads the same equation can be shown to apply, at least to a first approximation at low angles. In this case, μ and R_c refer to the electron density averaged along the rod axis.

3. Results and discussion

The experimental scattering curves of chromatin in 0.2 mM EDTA were recorded over the range $2.5\cdot10^{-3}$ Å$^{-1} \leqslant s \leqslant 5\cdot10^{-2}$ Å^{-1}. The concentration dependence of the experimental curves is negligible within the range examined (2 mg/ml to 10 mg/ml). A Guinier plot of the experimental points is shown in fig.2 and is in agreement with what would be expected for a solution of rigid rods; a straight line can be drawn which defines the values of R_c and of s I(s) at the origin. The axial radius of gyration R_c is found to be 38 Å. The electronic mass per unit length is found to be 1240 electrons/Å with an uncertainty of about 5% arising from uncertainty in the concentration. Such an electronic mass per unit length is equivalent to 2340 daltons/Å and a DNA packing ratio of about 6.5:1. Therefore a unit of mol. wt. 260 000 would repeat approx. every 110 Å along the chromatin fibre, which is consistent with model II (fig.1). Moreover the observed value of R_c (38 Å) would correspond to an equivalent uniform rod 108 Å in diameter, which is also consistent with model II.

In table 1, our results are compared with earlier determinations of the electronic mass per unit length and the axial radius of gyration of chromatin by the same technique and under similar ionic conditions (i.e. very low ionic strength).

Our value for μ is larger than the others by a factor of 2, and R_c is correspondingly larger by a factor of about $\sqrt{2}$, as would be expected for internally consistent sets of measurements. An explanation for the

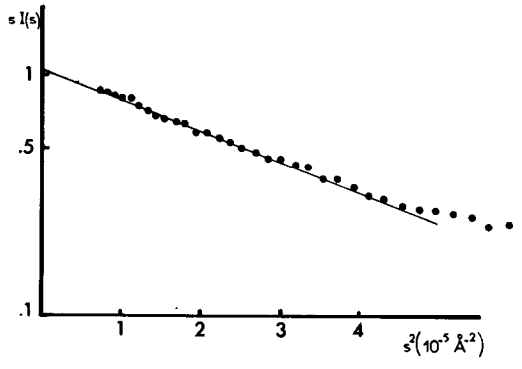

Fig.2. Guinier-type plot of the scattered intensity. For a straight rod a plot of s I(s) against s^2 should give a straight line, as is observed.

Table 1

	Sperling and Tardieu	Bram and Ris [12]	Luzzati and Nicolaieff [11]
R_c	38 Å	30 Å	26 Å
μ	1240 e/Å	580 e/Å	755 e/Å

discrepancy is found in the method of chromatin preparation. Both previous measurements were made on chromatin prepared according to the method of Zubay and Doty [17]. This method relies upon extensive shearing in a Waring blender in order to remove the chromatin from the nuclei and solubilise it. Recently it was shown that material prepared by shearing no longer contains the regular repeating structure of chromatin in nuclei, or chromatin in a solution prepared without shear, as judged by failure to give the highly characteristic pattern of DNA fragments on digestion with micrococcal nuclease [13]. Electron micrographs of sheared chromatin [12] show fibres containing some beads 70 to 110 Å in diameter separated by fibres of variable length about 25 Å in diameter (presumably naked, extended DNA). Electron micrographs of unsheared chromatin prepared as above and stained with uranyl acetate show fibres 100 Å in diameter, sometimes with a beaded appearance; there are almost no regions of extended DNA (J. T. Finch, unpublished. Also see [7] for pictures of very short lengths of unsheared chromatin).

We thus conclude that the low values of mass per unit length reported in the literature reflect the fact that the measurements were performed on sheared chromatin composed of partially 'unwound' beads and (pulled-off) naked DNA, which gave a value for μ intermediate between that for DNA fibres (105 e/Å) [11] and the value for unsheared chromatin. Our measurements on 'native' chromatin [13] show that the DNA packing ratio is about 7:1 (cf. [18]), and are consistent with a structure for chromatin, in solutions of very low ionic strength, in which 100 Å beads (repeating units) are arranged in a densely packed linear array (Model II), as originally proposed by Kornberg [10].

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