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Small angle scattering of cell nuclei

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Abstract. Neutron and X-ray small angle scattering techniques have been applied to study chromatin structure inside different types of cell nuclei.

A. Scattering from genetically inactive chicken erythrocyte nuclei exhibits a maximum at $Q = 0.1 - 0.15\,\mathrm{nm}^{-1}$ which cannot be observed by studying isolated chromatin derived from the same kind of cells. In highly active transcribing rat liver nuclei such a nuclear pattern is absent.

B. The radius of gyration of isolated "superbeads" was determined. It is discussed whether the characteristic maximum of the nuclei originates from this superstructural organisation of chromatin.

C. Rat liver nuclei were fractionated on sucrose gradients in order to determine whether the absence of the extra maximum in scattering profiles of these nuclei is due to overlapping effects of different chromatin organisation in the various cell types of the liver. As compared to unfractionated nuclei no strong deviations in the scattering profiles of the fractions could be observed.

D. Erythrocyte nuclei were dialysed in buffers differing in the ionic strength of monovalent cations. The typical maximum from the nuclei is shifted from 60 nm (very low salt concentration) to about 35 nm (physiological ionic strength) and is linearly proportional to the decreasing radius of the nuclei.

In conclusion, chromatin structure inside the nucleus has a scattering maximum due to an ordered packing of the fibres which is absent in nuclei with high genetic activity.

Key words: Neutron small angle scattering, X-ray small angle scattering, chromatin structure, cell nuclei

Introduction

For the investigation of chromatin structure in solution the techniques of neutron and X-ray small angle scattering have successfully been applied to reveal structural parameters (Suau et al. 1979; Notbohm et al. 1979). While many details about the structure of the nucleosomes, which are arranged as beads on a DNA-string, have been elucidated there is still a controversial debate concerning the supranucleosomal level. X-ray scattering data of chromatin in low ionic strength solvents suggest a model having straightened DNA-linker strands following a helical path with an internucleosomal distance of about 20 nm. Increasing the ionic strength induces a compact solenoid with tightly packed nucleosomes (Perez-Grau et al. 1984; Bordas et al. 1986). Compaction can also be achieved with a hundred times lower concentration of divalent cations such as Mg^{2+} or Ca^{2+}. On the other hand, chromatin in about 40 m*M* monovalent salt sometimes has a discontinuous appearance (so-called superbeads or supranucleosomal particles; Renz et al. 1977).

Recently, chromatin structure has been studied within intact interphase nuclei or in chromosomes (Langmore and Paulson 1983; Ibel et al. 1983; Notbohm and Harbers 1981). Small angle scattering can hardly be applied to material of such high concentrations (300 mg/ml) without getting interparticle interference. Nonetheless, experiments of this kind revealed interesting results.

Maxima at 40–60 nm equivalent Bragg spacings could be demonstrated. In chicken erythrocyte nuclei these were interpreted as interfibre distances of parallel ordered chromatin (Langmore and Paulson 1983) while Ibel et al. (1983) considered the distance distribution function of rat liver nuclei in buffers of monovalent ionic strength below 20 m*M* as direct proof for a globular chromatin superstructure.

In this work new scattering data are presented for chicken erythrocyte nuclei as well as for different kinds of rat liver nuclei. These data are compared with scattering curves of isolated superbeads.

Various aspects of data evaluation are discussed in detail and a probable interpretation for the observed scattering maxima is given.

Apparently, the study of chromatin structure inside the nucleus by small angle scattering techniques offers a new approach for comparative descriptions of nuclei derived from different cell types.

The data obtained describe packing density and the degree of structural heterogeneity of chromatin fibres, especially in cells differing in genetic activity. Which type of radiation is advantageous when studying these problems is also discussed. Results from X-ray as well as neutron scattering of nuclei and chromatin are presented.

Experimental

A. Biochemical methods

Rat liver nuclei were prepared as described by Pogo et al. (1966). The preparation of chicken erythrocyte nuclei followed the procedure of Shaw et al. (1976), except that the Nonidet P-40 concentration was lowered to 0.2%.

Rat liver and chicken erythrocyte chromatin were prepared following the method described by Strätling and Klingholz (1981). The isolated chromatin was finally dialysed against 20 mono-buffer (13 m*M* KCl, 7 m*M* NaCl, 5 m*M* *Tris*-HCl, 0.4 m*M* $MgCl_2$ and 0.1 m*M* EGTA).

Chromatin fractionation was obtained by centrifugation, using an isokinetic sucrose gradient in 20 mono-buffer ($c_t = 5\%$, $c_r = 26.9\%$, $V_M = 9.4$ ml, particle density 1.51 cm^{-3}) and centrifugation in a Beckman SW40 rotor at 40,000 rpm at 4 °C for 1.5 h. Figure 1 a shows a typical fractionation of chicken erythrocyte chromatin and Fig. 1 b of rat liver chromatin. For electrophoretical analysis of DNA and proteins see Strätling and Klingholz (1981).

Fractionation of rat liver nuclei followed a procedure described by Albrecht (1969). The isolated nuclei were layered on a flat (5% – 10%) sucrose gradient and centrifuged for 5 min in a minifuge GL (Heraeus-Christ). The content of tubes was fractionated while simultaneously the UV-absorbance at 260 nm was monitored in a flow-through cell (ISCO) (Fig. 2).

B. Small angle scattering

The neutron scattering experiments were carried out using the SANS facility at the Danish National Institute of Physics in Risø. Measurements were done at $\lambda = 0.8$, 1.2 and 1.6 nm neutron wavelength, calibrated with a mechanical velocity selector, the

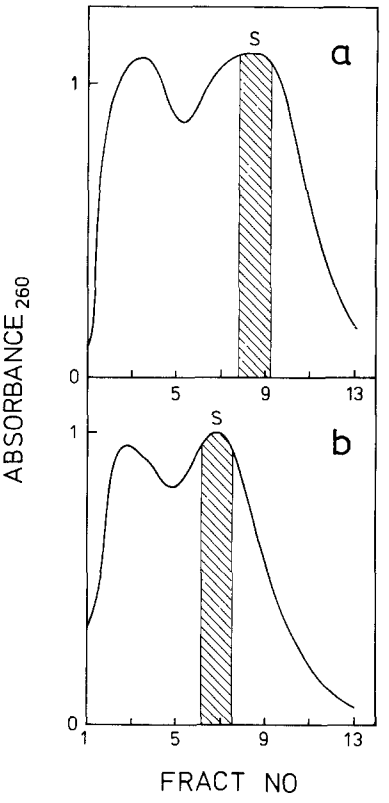


Fig. 1. Isolated chromatin fractionated in an isokinetic sucrose gradient 19% – 27% and 20 mono-buffer components. **a** Chicken erythrocyte chromatin; 30 min digestion with micrococcal nuclease (80 units/ml nuclear suspension at 37 °C). **b** Rat liver nuclei; 15 min digestion by endogenous nuclease at 37 °C. For neutron scattering studies fraction *S* (superbeads) was taken

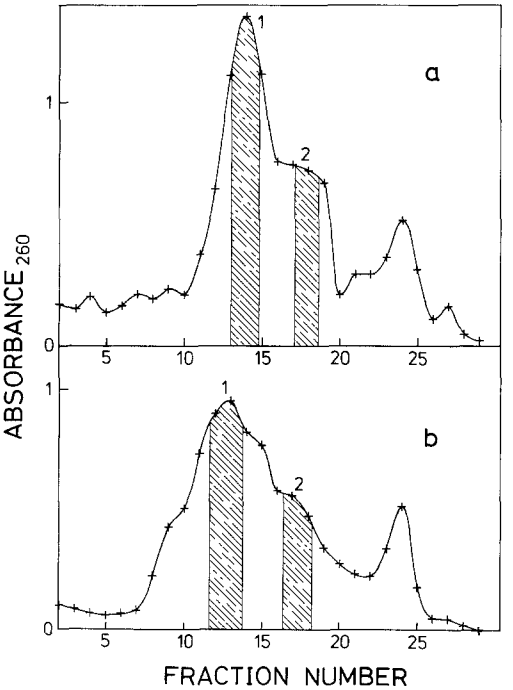


Fig. 2. Fractionation of rat liver nuclei. **a** adult rats, **b** 3 week old rats. Fractionation in linear sucrose gradients (0.5 – 1.0 m). According to the electronmicrographs only fraction 1 and 2 could be considered as populations of identical nuclei

spectral property of which is $\lambda/\Delta\lambda = 0.19$ at peak position of the cold neutron source, decreasing considerably at higher wavelengths.

The momentum transfer, Q, and the normalized scattering angle, h, are defined as $Q = h = 2\pi/\lambda \cdot (2\vartheta)$, λ = wavelength, 2ϑ = full scattering angle; Bragg spacing $d = 1/(2\pi \cdot Q)$.

Normalization of the area sensitive counter was performed with water, a homogeneous scattering medium for neutrons. The statistical error increases for higher angles on the area sensitive counter, because the exponential decrease of scattering intensity is not compensated by the detector geometry. Multiple scattering is negligible.

For X-ray scattering a Kratky camera was used with a sample detector distance of 210 mm, $\lambda =$ 0.154 nm, entrance slit 40 µm with a step scanning device (Paar KG, Austria). The Cu K_α-line was electronically discriminated and filtered for K_β-content with nickel. Every measured point had a statistical error of $\pm 1\%$. The raw scattering data had to be numerically desmeared for slit height and width interference effects using a computer program provided by Glatter (1974). Because of background scattering, the data in Fig. 7a using the plot $\lg (I \cdot Q^2)$ versus Q, gave a positive slope.

Results

Neutron scattering studies of chicken erythrocyte and rat liver nuclei were performed in solvents of different ionic strength which always contained 60% glycerol in order to eliminate fast sedimentation during measurements and to stabilize the nuclei. Comparing erythrocyte nuclei and nuclei from whole rat liver in a Guinier plot (Fig. 3) a difference in chromatin structure inside these nuclei is obvious. The scattering curve of rat liver chromatin is flatter, it has no straight Guinier region. It is not possible to compute a radius of gyration from this data because interference due to dense packing in the nucleus diminishes the scattering intensity profile at the smallest angles observed. Nonetheless, the difference in scattering reflects structural variations of chromatin structure or its packing in the nucleus. Using a different way of plotting the data, in order to enhance the maxima $Q/\lg (I \cdot Q^2)$ (this is a correction for the peak intensity which decreases in proportion to Q^2 for disordered samples) another difference can be demonstrated – the maximum at about $Q = 0.1 - 0.15\,\mathrm{nm}^{-1}$ (it can be achieved most clearly in buffers with less than 20 mM monovalent salt concentration). At this angle range rat liver nuclei also have scattering maxima, but these are much flatter and two or more of these maxima can, in fact, be observed (Fig. 4).

In addition, isolated and fractionated chromatin (Fig. 1) was studied with neutron scattering. The radius of gyration of the whole particle, derived from Guinier plots of fraction S (superbeads) of chicken erythrocyte chromatin, is $R = 13$ nm, while the corresponding value for the same rat liver fraction S is $R = 10 - 11$ nm (Fig. 5) (standard deviation from repeated measurements and from linear regression of the Guinier region from which the radius of gyration was about $\pm$ 0.5 nm).

The usefulness and limits of resolution of the small angle scattering data on overall structural entities inside whole cell nuclei and the influence of

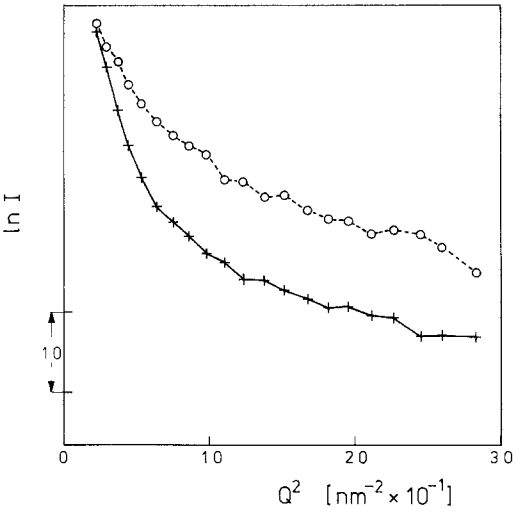


Fig. 3. Guinier plot of neutron scattering data: rat liver nuclei (-○—○—○-), chicken erythrocyte nuclei (-+—+—+-). Nuclei were suspended in a 10 mM monovalent salt buffer, 60% glycerol

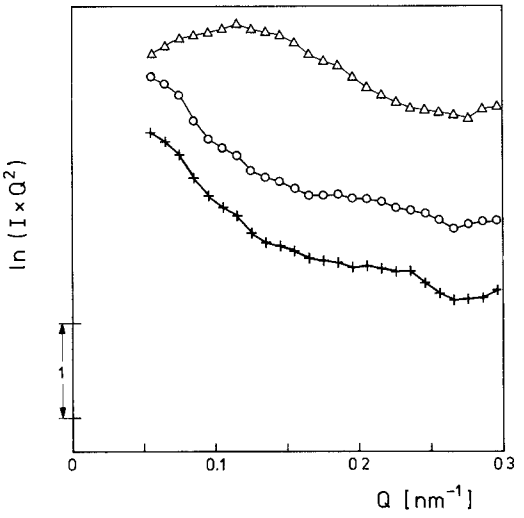


Fig. 4. Neutron small angle scattering of chicken erythrocyte nuclei (-△—△—△-), rat liver nuclei from adult rats (-○—○—○-), and from 3 weeks old rats (-+—+—+-). The method of plotting Q versus $\lg (I \cdot Q^2)$ corrects the maximum intensity, which decreases proportionally with Q^2

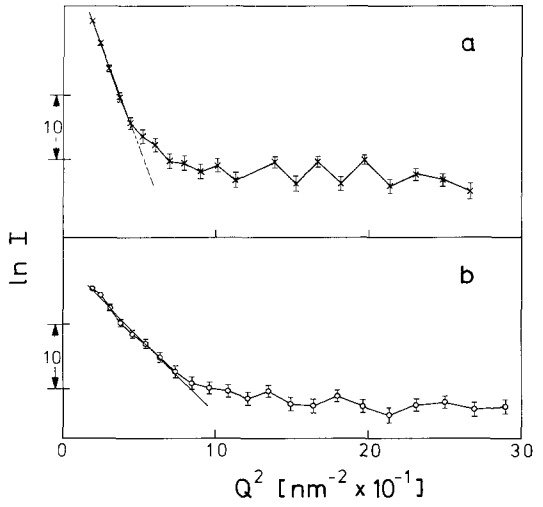


Fig. 5. Guinier plot of isolated chromatin (superbeads) from chicken erythrocyte **a** and rat liver **b** (fraction *S* from Fig. 1). Optical density of the sample, $A_{260} = 2$

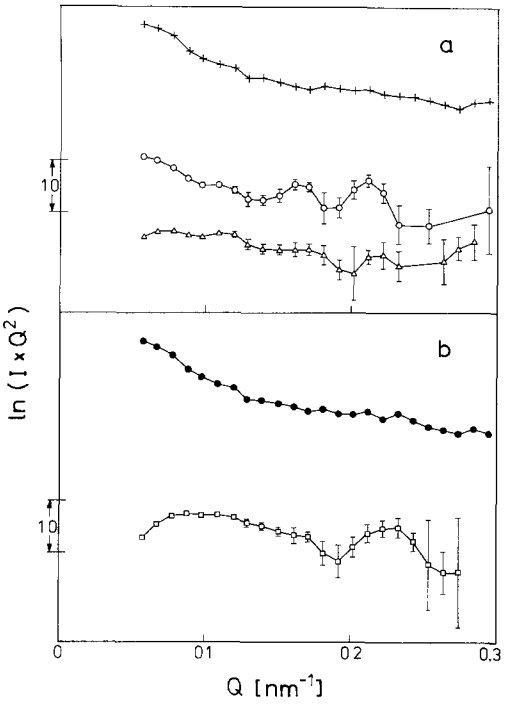
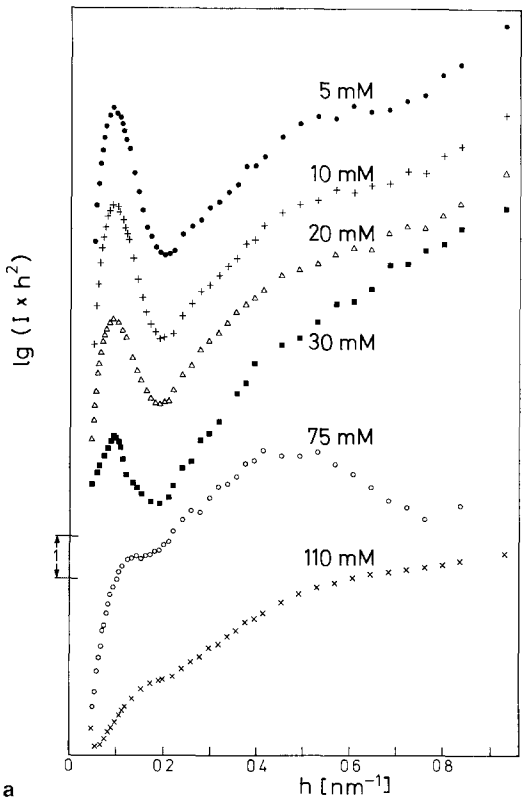


Fig. 6. Neutron scattering of the fractions described in Fig. 2. **a** 3 weeks old rats: unfractionated (–+—+—+–), fraction 1 (–○—○—○–), and fraction 2 (–△—△—△–). **b** Adult rats: unfractionated (–●—●—●–) and fraction 1 (–□—□—□–)



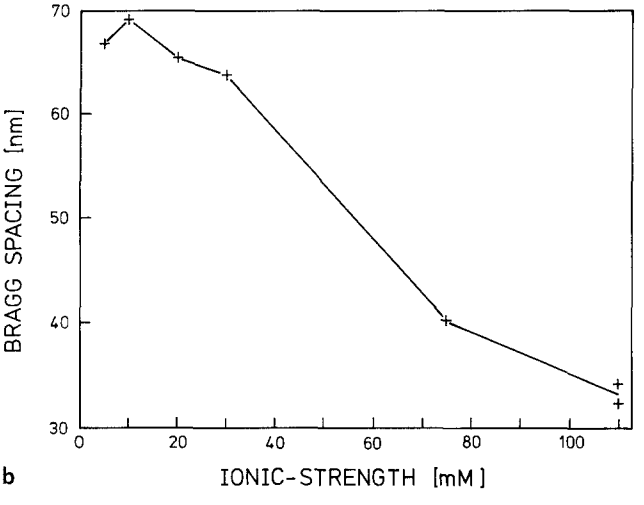


Fig. 7. a X-ray scattering curves of chicken erythrocyte nuclei at various monovalent salt concentrations as indicated in the graph. Further buffer components were 5 m*M* *Tris*-HCl, 1 m*M* PMSF, 0.9 m*M* $CaCl_2$, pH 7.5. **b** The position of the maxima at very low scattering angles from Fig. 7a as equivalent Bragg-spacings plotted against the ionic strength in the buffer

heterogeneous cell populations were tested with fractionated nuclei from rat liver which are known to occur as cells of different type and degree of ploidy. The nuclei had been separated in a sucrose gradient at low speed as described in materials and methods. The distribution of these nuclei in the gradient is shown in Fig. 2. From electronmicrographs of thin sections of embedded nuclei one could not deduce the cell type the nuclei were derived from. DNA mass per nucleus is found to be doubled in the heavier nuclei. Apparently, nuclei having different degrees of ploidy had been obtained while the cell types could not be determined. Differences in the scattering curves are demonstrat-

ed in Fig. 6 using the plot Q versus $\lg(I \cdot Q^2)$. Minima and maxima are more pronounced in fractionated nuclei. Thus, it seems probable that the smooth appearance of the scattering curve from unfractionated nuclei is the result of an overlap of different maxima and minima due to the interference of various types of nuclei and differences in their higher-order chromatin structure organisation. On the other hand, scattering of each fraction never exhibits a strong maximum in the region $0.1-0.2$ nm^{-1}, as observed in chicken erythrocyte nuclei.

Furthermore, the position of the $0.1-0.2\,nm^{-1}$ maximum was tested upon swelling of the nuclei. This was performed while carefully lowering the ionic strength of the buffer medium. Because these experiments were carried out with the Kratky camera using X-ray scattering, no glycerol was added to the buffer (otherwise chromatin would be contrast-matched by the solution). The swelling was controlled by taking light micrographs from the nuclei and deducing an ellipsoidal volume from the measured axes. Upon increasing the ionic strength, the maximum at about $0.1\,nm^{-1}$ changed position to higher angles. The inverted angular maximum positions (Bragg-spacings) from Fig. 7a are plotted as a function of ionic strength in Fig. 7b. Model calculations show that the decrease of Bragg spacings is linearly proportional to the decrease in the equivalent spherical radius of the nucleus caused by osmosis.

Discussion

Two very different types of cell nuclei have been compared using neutron scattering. In chicken erythrocyte nuclei, in particular, a small scattering maximum at $Q = 0.1\,nm^{-1}$ appeared, this has been viewed in different ways.

First, the morphological data of nuclear structural composition should be discussed. Nuclei from rat liver and chicken erythrocyte have different amounts of densely packed chromatin. Furthermore, rat liver nuclei are transcriptionally active while erythrocyte nuclei have only residual genetic activity in chicken older than 3 months.

Rat liver has dense chromatin close to the nuclear membrane, in the surrounding of the nucleolus and in smaller heterogeneously scattered dense areas. In electronmicroscopic investigations Everid et al. (1970) found parallel ordering of chromatin fibres, especially in chicken erythrocytes close to the nuclear membrane, using tangential sectioning of fixed nuclei. The authors observed parallel fibres arranged in wide circles, which they named "arcs", located around nuclear pore complexes. In rat liver this phenomenon can also be observed with electronmicroscopic techniques, but quantitatively the amount of dense material is much smaller (Zentgraf, personal communication). Erythrocyte chromatin is very homogeneous in structure. Because of its genetic passivity, it is almost completely organized at the quarternary structural level in the form of solenoids or superbeads (Zentgraf 1980).

On the other hand, rat liver chromatin is organized much more heterogeneously. One can find compact long solenoids as well as knobby structures. Sometimes even naked DNA is visible, although this may be an artifact due to fixation or spreading techniques.

Besides this morphological background, X-ray and neutron scattering results have provided some new data on chromatin structure. The fibre diameter of condensed chromatin in chicken erythrocytes was found to be up to 40 nm (Bordas et al. 1986), while rat liver chromatin fibres have a diameter of $30-35$ nm (Notbohm et al. 1979). The maximum at about $Q = 0.1\,nm^{-1}$ is a unique feature of the chromatin structure in cell nuclei. Until now controversial interpretations about the structural origin of this maximum exist, as was mentioned in the introduction.

Perez-Grau et al. (1984) have demonstrated that this maximum can also be produced with isolated chromatin, upon concentrating the chromatin solution on a membrane. It leads to close packing of chromatin and thereby creates an interference maximum. Thus, these maxima should originate from dense packing of chromatin in the interphase cell nucleus. The shift of the $0.1\,nm^{-1}$ maximum with increasing ionic strength is also consistent with this interpretation, because the decrease of ionic strength is linearly proportional to the increasing radius of the swelling nucleus. This allows larger chromatin interfibre distances and therefore a shift of the diffraction peak to smaller angles.

Alternatively, the maxima at $0.1\,nm^{-1}$ may reflect a regular supranucleosomal structure (Ibel et al. 1983). Superbeads are known to occur preferentially in chicken erythrocyte nuclei (Zentgraf et al. 1980) which would be in agreement with the strong maximum found there. But, as our results demonstrate, the superbead in solution has a radius of gyration of about 13 nm for chicken erythrocyte chromatin (Fig. 5a). A globe of this size would produce a first subsidiary maximum at $Q = 0.3-0.35\,nm^{-1}$, which corresponds to $18-20$ nm equivalent Bragg-spacing. So, if superbeads are responsible for the maximum at $0.1\,nm^{-1}$, it can only be considered as an inter-superbead distance. But this structural feature should be observable by small angle scattering curves of isolated chromatin of considerable size in dilute solutions.

Furthermore, how can the movement of the maximum at 0.1 nm^{-1} with varying ionic strength be interpreted as a structural change in superbeads; in this case intrastructural alterations of chromatin due to changing salt conditions (e.g. a relaxed coil) could be an explanation for a shift of the maximum. However, the radius of gyration of the cross-section of chromatin in solution decreases when lowering the salt concentration of the buffer. This then should lead to a shift of the maximum to higher scattering angles, but the opposite occurs.

In summary, the results obtained favour the hypothesis, that the unique feature of X-ray or neutron scattering of chromatin in cell nuclei, the maximum at about 0.1 nm^{-1}, is as a consequence of dense fibre packing. This conclusion is also consistent with the idea that the structure of chromatin fibres at the quaternary level forms domains. These structures are thought to be organized in loops comparable to the structures observed in electronmicrographs of metaphase chromosomes. In interphase nuclei the loops are obviously attached to the nuclear matrix and the lamina (Lebkowsky and Laemli 1981; Cook et al. 1984). The 40–60 nm reflection could then be accounted for as a side by side fibre distance of a double-helically backfolded loop of nucleosomes.

The absence of such a strong maximum in rat liver nuclei might be due to longer interfibre distances of the euchromatic chromatin, which is much more abundant in these nuclei. A maximum would then be located at scattering angles which are not in the range of todays small angle scattering devices. It is also possible that the distance is not such a constant parameter as in highly inactive nuclei, leading to a broad distribution of unresolvable interference maxima. Additionally, the heterogeneity of cells in the liver having different genetic activities, polyploidy and possibly different chromatin structures due to varying linker lengths may contribute to this effect.

Comparing both scattering techniques, one is tempted to choose X-ray scattering in favour of neutron small angle scattering when working with cell nuclei, the interference effects originating from the densely packed material already flatten the maxima. The neutron scattering equipment used in our experiments provided a point focused beam. A rather poor selection of wavelength was allowed for, as the incident neutron flux was low, especially if one is measuring at very long wavelengths. There is an advantage in the use viscous buffers which eliminate fast sedimentation during the measurement (no mechanical stress due to centrifugation) and which also stabilize the nuclear shape.

Further research on small angle scattering of cell nuclei should therefore be done using synchrotron radiation at very low angle and short exposure times which avoid some of these weak points.

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