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Neutron diffraction from crystals of nucleosome core particles

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The nucleosome is the basic repeating unit of chromatin (see review by Kornberg (1977)). It is a complex of histone protein molecules with a length of DNA, which digestion studies with the enzyme micrococcal nuclease have shown to be often about 200 base pairs in length but with quite wide variations between different cell species. With sufficient digestion, however, a 'core' particle is produced, which for all species so far investigated contains close to 145 base pairs of DNA associated with an octamer of pairs of the histones H3, H4, H2A and H2B. The molecular mass of the core particle is about 200000, roughly equally divided between DNA and protein.

From an earlier low resolution study by X-ray diffraction and electron microscopy on crystals of nucleosome core particles from rat liver chromatin, it was concluded that the core particle was flat, of dimensions about 110 Å × 110 Å × 57 Å†, somewhat wedge-shaped and strongly divided into two layers (Finch *et al.* 1977). A model was proposed in which the DNA was wound into about $1\frac{3}{4}$ turns of a flat superhelix of pitch about 28 Å around the histone octamer. The core particles in these crystals were found to have the histone proteins partly proteolysed, but their physico-chemical properties remained very similar to those of intact particles. Crystals have since been grown in the Cambridge laboratory from intact nucleosome cores. The unit cell is smaller than that of the proteolysed material, but is closely related to it; the space group is the same and the *a* and *b* unit cell dimensions very similar, but the *c* dimension is reduced by a factor close to 3. The approximate packing arrangement of the particle in this smaller cell was deduced from a low resolution, three-dimensional Patterson, calculated from the low angle X-ray data (Finch *et al.* 1978). This did not unambiguously resolve the relation of the particles to the screw axes of the space group, but it was found possible to choose signs for the strong low angle $0kl$ reflexions, which resulted in a Fourier map showing units with a very similar appearance to that in the corresponding map from the earlier crystals and preserving the local arrangement of units along the 2_1 screw axes parallel to y in that map.

The approximately equal division between protein and DNA makes the nucleosome core particle a good subject for study by neutron scattering by using the method of contrast variation. Several groups have made such a study on solutions of the particles (see, for example, Richards *et al.* 1977; Hjelm *et al.* 1977) and the Searle group in particular have shown that a model similar to that described above is consistent with their solution scattering data (Pardon *et al.* 1978). However, solution scattering suffers from the great disadvantage of not showing the orientation relation in the particle of the different features which give rise to the diffraction

† 1 Å = 10^{-10} m = 10^{-1} nm.

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maxima. Moreover, the signal:noise ratio is very low for data at spacings smaller than about 35 Å. These disadvantages are largely overcome in crystal diffraction, and a low angle neutron diffraction study was therefore begun when sufficiently large crystals were produced.

The crystals of intact nucleosome cores have the space group $P2_12_12_1$, with unit cell dimensions a = 111 Å, b = 198 Å, c = 111 Å. The crystals used for this study had volumes 0.02–0.04 mm^3. The data were measured at the I.L.L. on the small angle scattering camera D17, which has an area detector of 64 cm × 64 cm (128 × 128 counting elements) and which was adapted for single crystal work and software developed to collect integrated intensities. By using a wavelength of 9.2 Å, $h0l$ and $0kl$ sets of data were collected out to about $(25\ Å)^{-1}$ for crystals soaked in mother liquors containing 0, 39, 65 and 90 % D_2O. The 0 % and 90 % data sets required 5 days each and the intermediate sets 10 days each.

After correction for geometrical factors, the data from the different crystals were scaled as follows. At low angles of diffraction the reflexions mainly relate to the particle shape, and their amplitudes depend on the difference between the scattering of the particle and that of the surrounding solution: there is a linear relation between the scattering amplitudes of centrosymmetric reflexions and the concentration of D_2O. Approximate scaling factors were first deduced from a few reflexions whose behaviours were clear, and more accurate factors calculated by linear regression by using all the data. The resulting scaled amplitudes showed a good linear dependence on D_2O concentration. This method of scaling is independent of the absolute signs of the reflexions at any one contrast (D_2O concentration), but if the signs for one contrast are known, the signs of the reflexions at all other contrasts can be deduced from the linear relation between structure factor and contrast.

The $0kl$ data have been used most extensively for sign determination so far, since it is known from the X-ray work that the particles in the crystal are arranged in approximately hexagonally packed columns, and seen in the direction of the x-axis of the crystal there is little overlap between the projections of neighbouring columns (Finch *et al.* 1978); this projection thus yields the clearest picture of individual core particles. As implied above, the problem in the analysis is to get a starting set of signs for one contrast: for this we turned to the X-ray work. For the crystal in 0 % D_2O, the relative scattering of neutrons by protein and DNA should not be all that different from that of X-rays. Thus the signs used to calculate the X-ray Fourier map (Finch *et al.* 1978) were used to calculate the corresponding neutron map in 0 % D_2O, and were changed as indicated by the contrast variation series to calculate the maps for other contrasts. Of the resulting maps, that corresponding to the 39 % D_2O data was particularly disappointing. At this concentration, the scattering of D_2O is close to that of protein and so the diffraction pattern is mainly due to DNA for which there was the well defined model described above. However, the positive density in that map was not continuous and did not always overlap positive density in the 0 % map, and there were also unrealistically strong negative regions. A trial-and-error method was therefore adopted and by this it was found possible to choose signs for the strong reflexions of the 39 % data that produced a Fourier map strikingly similar to the projection of about $1\frac{3}{4}$ turns of a superhelix (figure 1).

At a concentration of 65 %, the scattering density of D_2O closely matches that of DNA and thus the crystal scattering is dominated by that of the protein. By using the signs corresponding to this data set predicted from those used in figure 1 together with other strong reflexions of appropriate sign, the Fourier map shown in figure 2c was calculated. It shows the expected columns of protein, and the heart shape is very similar to a projection of the three-dimensional

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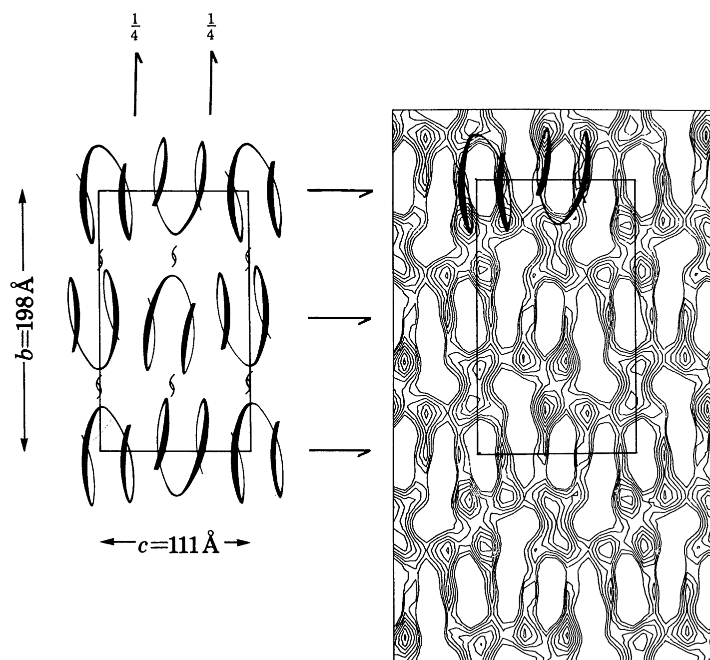

FIGURE 1. A Fourier map (right) calculated from the $0kl$ neutron scattering data from a nucleosome core crystal soaked in 39% D_2O. At this concentration, the scattering of D_2O closely matches that of protein and so virtually the DNA alone is seen in contrast. With the signs chosen for the reflexions here, the positive density in the map correlates strikingly with the projection of about $1\frac{3}{4}$ turns of a superhelix arranged as indicated in the diagram of the x-projection of the unit cell on the left.

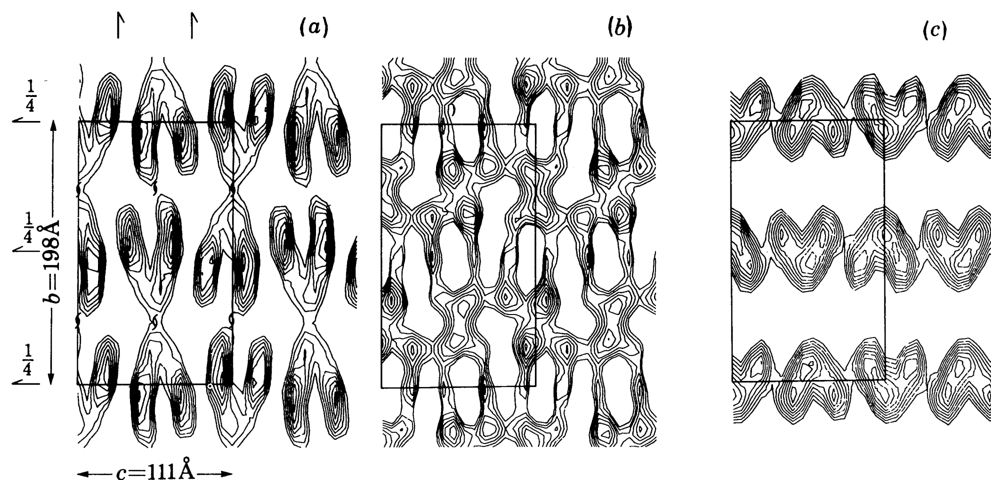

FIGURE 2. Fourier maps calculated from $0kl$ neutron scattering data to a resolution of 25 Å, from crystals soaked in (*a*) 0% D_2O, in which both DNA and protein are contrasted relative to the mother liquor, (*b*) 39% D_2O, in which DNA shows dominant contrast, and (*c*) 65% D_2O in which protein is dominantly contrasted. The structure factors used in (*b*) (figure 1) were changed in amplitude and sign as indicated by the contrast variation series for calculation of the maps in (*a*) and (*c*). The three maps show the self-consistency of the sign combination used, and indicate a feasible relation between the histone octamer (*c*) and the DNA superhelix (*b*). The map at 90% D_2O is not shown but is roughly midway between maps (*a*) and (*c*), as one would expect from the relative contrasts of protein and DNA at this D_2O concentration.

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image reconstruction from electron micrographs of the isolated histone octamer carried out in Cambridge (Klug *et al.* 1980). Furthermore, this reconstruction has a twofold axis which in the Fourier map lies a few degress away from the direction of the y-axis, more or less coincident with the twofold axis of the corresponding DNA superhelix (figure 1). Thus, to this resolution at least, the core particle has an overall twofold axis.

The corresponding map at 0% D_2O (figure 2*a*) shows units with a similar wedge-shaped, bifurcated appearance to that obtained in the earlier X-ray work from the large unit cell (cf. fig. 6 of Finch *et al.* 1977). The positions of the units with respect to each other are very similar to those in the earlier interpretation of the X-ray data from the small cell (Finch *et al.* 1978) but they are located differently with respect to the screw axes of the unit cell. The distinction between these two arrangements was not obvious in the three-dimensional Patterson map of the low resolution data. However, more recent Patterson maps, which include higher angle X-ray data, clearly favour the arrangement found here and also confirm the existence of the particle dyad lying a few degress from the y-axis (Finch *et al.* 1980).

Although one must always bear in mind the dangers of the trial-and-error method, the results described above do give confidence in the signs adopted for the neutron scattering data for this projection. They result in a set of Fourier maps which are self consistent for all contrasts. Moreover, the maps at 39% and 65% D_2O show a striking correlation both with the DNA superhelix of the proposed model and also with the histone octamer structure obtained from the three-dimensional image reconstruction which is not only consistent with the twofold symmetry of each. The result also defines the relative orientations of the two components about the common dyad in a way which in three dimensions is highly plausible (Klug *et al.* 1980). Thus, in addition to confirming the model, the neutron data provide a basis for further interpretation of the interaction between the protein and the DNA.

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