X-RAY PATTERNS FROM MICROTUBULES *

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Electron microscopy and x-ray diffraction look at structures under different conditions. In general, there has been good agreement in relating images obtained with these methods. In the case of microtubules, however, the interpretation of the x-ray diffraction patterns in terms of the image seen in the electron microscope has not been straightforward. The results from the two methods have led to the proposal of different arrangements of subunits (i.e., different surface lattices) making up the microtubule. In this paper we consider this problem in the light of new experimental results.

Most of our knowledge of microtubule structure has come from electron microscope observations. Microtubules from different systems appear to have certain common structural features, although they differ in stability. The tubule consists of a cylindrical shell about 250 Å in diameter, with a wall thickness of about 50 Å and a hollow center about 140 Å in width. The wall of the tubule is made up of 13 longitudinal filaments about 50 Å apart.[1, 2] The filaments are built from globular subunits about 40 Å in width which are revealed in negatively stained preparations. Fraying of the tubules into the filaments readily occurs.

The surface lattice of microtubules was first described by Grimstone and Klug [3] in 1966. They used optical diffraction patterns from negatively stained protozoan flagella. A basic feature of the pattern is a 40 Å layer line showing a strong maximum near the meridian. Klug and Grimstone were able to define the general features of the microtubule lattice, which was shown to be a low-pitch helix with a small vertical displacement between neighboring subunits (FIGURE 1a). A similar structure has been found in negatively stained preparations of a variety of ciliary and flagellar microtubules.[4]

* This work was supported in part by the United States Public Health Service Grants AM 17436 (C.C.), GM 21189 (D.D.), CA13202 (S.C.H.), and GM15500; National Institutes of Health Career Award GM70164 (R.E.S.); and National Institutes of Health Developmental Biology Training Grant T1-HD-22 (J.T.).

The first x-ray diffraction patterns from hydrated, oriented sea urchin flagellar microtubules were described by Cohen, et al.[5] in 1971. Although the specimens showed considerable disorientation, and were weakly diffracting, the x-ray photographs confirmed many of the electron microscope observations. The diffracting structure in the hydrated state was shown to be a cylindrical shell with a mean radius of about 110 Å. The rapid falloff of the equatorial intensity was in agreement with a thick-walled cylinder. The pattern also showed a 40 Å layer line and a 53 Å equatorial reflection, so that the dimensions of the unit cell in the surface lattice corresponded closely to those deduced from the electron microscope results. The x-ray data also indicated that there were either 12 or 13 filaments making up the tubule wall.

A striking difference between the x-ray pattern and the optical diffraction patterns was the intensity distribution on the 40 Å layer line. On the x-ray pattern the strong intensity was well off the meridian at an angle of about 40°; moreover there was a strong near-meridional reflection at 20 Å. The simplest explanation for the x-ray diffraction pattern was that the microtubule consisted

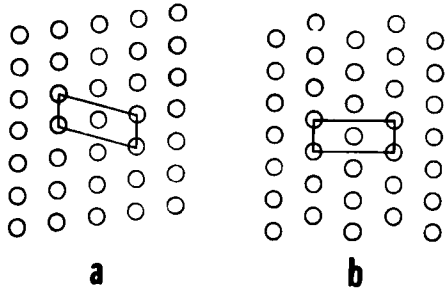

FIGURE 1. Surface lattices proposed for microtubules. (a) Surface lattice deduced from electron microscope data.[3] (b) Surface lattice deduced from earlier x-ray results,[5] showing half-staggered arrangement of filaments. Unit cells in both lattices are outlined.

of roughly spherical subunits arranged in filaments half staggered in relation to each other (FIGURE 1b). Thus there was a puzzle posed by these two interpretations using different methods. Either the two lattices were different—so that, as Cohen et al.[5] suggested at the time, the microtubule lattice might show different ordered states under different environmental conditions—or, as Klug suggested (personal communication), there might be differences in the appearance of the subunits as revealed by the two methods.

Since that time Erickson [6] and Amos and Klug [7] have shown that the strong near-meridional reflection on the optical diffraction pattern arises from a three-start helix. Amos and Klug have produced a three-dimensional reconstruction to a resolution of about 40 Å of the microtubule surface lattice. In this volume Erickson describes his results on higher resolution two-dimensional image processing from the electron micrographs.

Cohen et al.[5] had previously examined specimens of sea urchin sperm tail outer fibers, either as intact doublets, or A-subfiber specimens prepared by thermally depolymerizing the B-tubules. They also examined gels of scallop

gill cilia,[8] which are more stable structures (unpublished results). All the x-ray specimens were prepared by centrifuging suspensions of the microtubules in low ionic strength buffer to produce a birefringent pellet, which was sealed in a quartz capillary to maintain the humidity of the sample.

In this paper we report improved x-ray diffraction photographs from oriented neurotubules. To prepare neurotubules we used a variation of the methods of Weisenberg[9] and Shelanski et al.,[10] and Weisenberg (personal communication). Neurotubules were extracted in the cold from calf brain as tubulin dimers and polymerized at 35° C in buffer containing 25% glycerol. The neurotubules tend to polymerize into very long fibers that are difficult to orient. The polymerized tubules were therefore homogenized before centrifugation. They were spun at 4° C to form a viscous pellet which—in contrast to pellets of outer fibers or cilia—showed very weak birefringence. This is probably due to the glycerol matching out the form birefringence of the specimen. Diffraction patterns were obtained both at ambient temperature and with cooling, using a focusing monochromator or a focusing double mirror camera, with an Elliott rotating anode x-ray source.

The best x-ray diffraction patterns obtained from neurotubules are more highly oriented and detailed than from any previous microtubule system. The intensity distribution is very similar to that reported for outer fibers[5] (FIGURE 2a). The pattern shows a set of near-meridional layer lines with a spacing of 40 Å. The intensity profile on the 40 Å layer line is complex: on and near the meridian there are two weak reflections, and the most prominent reflection is well off the meridian. Additional weaker peaks are also seen at higher angles. The second, third, fourth, and fifth layer lines show significantly arced on- or near-meridional reflections. On very strongly exposed photographs, traces of a set of very faint meridional reflections can be seen between the layer lines of the main repeat, indicating a larger axial period of 80 Å. The equator of the pattern shows a very strong diffraction maximum at 192 Å, three other maxima at 104 Å, 68 Å and 54 Å, and relatively diffuse diffraction at 25 Å.

At this stage in the analysis we cannot present a definitive structure based on these photographs. Rather, we will indicate the approach to the solution of the structure, and certain critical parameters in the arguments. We consider only major features of the pattern and assume it is possible to interpret the diagram on the basis of a single diffracting system. The first step in the structure determination is the analysis of the equatorial intensity. This gives the boundaries of the microtubule wall. A reliable estimate of the wall thickness is needed to index the diffraction maxima on the 40 Å layer lines. This indexing, in turn, determines the arrangement of the subunits in the microtubule.

The equatorial intensity shows that the diffracting structure is a thick-walled cylindrical shell. The positions of the first three maxima and the intervening zeros in the transform can be approximated by a zero-order Bessel function. This fixes the mean radius of the diffracting cylinder as about 110 Å. The wall thickness is best displayed in a plot of the microtubule density as a function of radius. The radial density distribution calculated from the first three peaks of the equatorial diffraction pattern is sensitive to the relative intensity of the diffraction maxima. An accurate determination of the thickness therefore depends on a careful analysis of this scattering profile. This analysis, which is in progress, should give the inner and outer boundaries of the microtubule wall.

The equatorial peak at 54 Å would not arise from a uniform tubule wall. Its position gives the symmetry of the structure in projection. Electron micro-

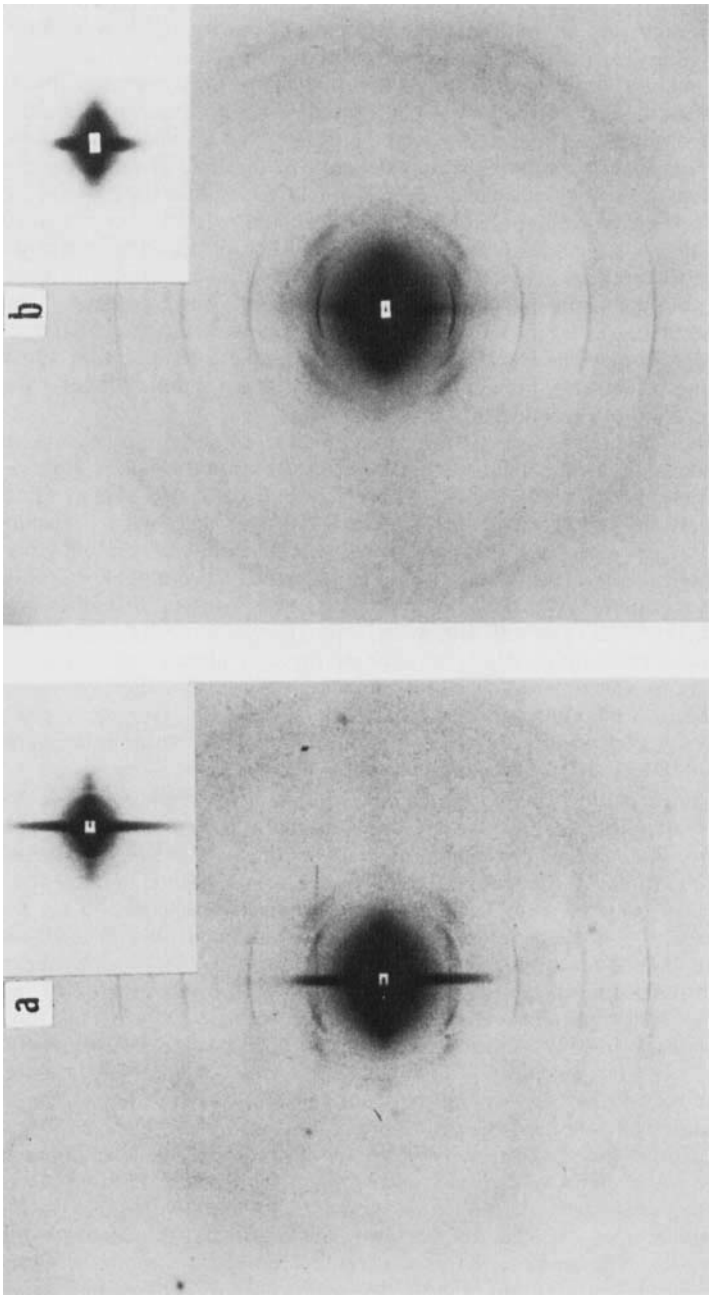

FIGURE 2. (a) X-ray diffraction pattern from gels of neurotubules centrifuged from low ionic strength buffers containing 25% glycerol. Layer lines of a 40 Å axial repeat are clearly visible. Scatter from monochromator produces vertical streak. Equatorial reflections are shown in inset. Specimen-to-film distance, 123 mm. (b) X-ray diffraction pattern from specimen similar to that in (a), but partially dried. There is now strong intensity near the meridian at 40 Å. Specimen-to-film distance 170 mm.

scope results [2] have now established that there are 13 longitudinal filaments making up the tubule wall. The peak at 54 Å (corresponding to a thirteenth-order Bessel function) arises from the grooving of the wall between the filaments. The grooves have an average radius of about 130 Å. On strongly exposed x-ray photographs, there are two additional very weak subsidiary maxima further out on the equator, followed by a peak at 25 Å. Erickson first discovered the corresponding 25 Å reflection on optical diffraction patterns of electron micrographs of flattened sheets of microtubules and intact tubules.[6] In his two-dimensional reconstruction this reflection gives rise to a longitudinal division of the microtubule filament. We have not analyzed the equatorial intensity profile in sufficient detail to determine whether this reflection on the x-ray pattern is strong enough to correspond to this kind of structural feature. In this connection it should be noted that a number of factors would tend to decrease the intensity of this spot on the x-ray photographs: one of them is simply the difference in scattering between a flat and a cylindrical arrangement of filaments. The scattering from a particular feature in a cylindrical object is spread out over a larger area, and tends to fall off more rapidly than that from the corresponding feature in a two-dimensional array.

The complete determination of the symmetry of the structure, i.e., the surface lattice, depends on the analysis of the *positions* of the strong intensity maxima on the 40 Å layer line, and the radius of the diffracting structure. In the previous publication on the analysis of the x-ray diagram from outer fibers [5] the position of the strongest maximum on this layer line was measured at about 70 Å. This corresponded to Bessel functions of orders 6–8, taking a permitted range of microtubule wall radii from 80–120 Å as determined by electron microscope observations. The reflection at 70 Å was then indexed as a sixth- and/or seventh-order Bessel function, leading to a half-staggered model for the surface lattice of the microtubule (FIGURE 1b).

We can now examine this part of the diagram in the light of the new x-ray patterns from neurotubules, and of the surface lattice recently determined from the optical diffraction of electron micrographs.[6, 7] The x-ray patterns with improved orientation show a more complicated intensity distribution on the 40 Å layer line; there are two weak reflections on and near the meridian, but the strongest reflection is off the meridian at 70 Å. From the x-ray pattern alone, it is difficult to make an unambiguous assignment of Bessel function orders to these peaks. We can, however, attempt to interpret the x-ray pattern using the surface lattice derived from electron microscope results. Both Erickson [6] and Amos and Klug [7] have shown that the two prominent reflections on this layer line correspond to a J_3 and a J_{10} Bessel function. On this basis, we can assign the weak near-meridional intensity as the J_3 and the strong reflection would then be the J_{10}. A J_{10} Bessel function assigned to this reflection has a radius of about 130 Å; that is, diffraction would be coming from a feature of the structure near the outer surface of the tubule wall. Since the 54 Å equatorial reflection would correspond to a J_{13} with a mean radius of about 130 Å for the longitudinal grooves, this is a plausible assignment.

Although the positions of the diffraction maxima in the optical diffraction and x-ray patterns may correspond, their intensities differ. The strongest reflection on the optical diffraction pattern is near the meridian, and the other peak further out is somewhat weaker. This kind of intensity distribution could arise from roughly spherical subunits. The x-ray pattern, on the other hand, has very weak intensity near the meridian and the strong peak further out on

the layer line. This diffracting feature, interpreted by means of the lattice from electron microscopy, would indicate that the microtubule subunits are not spherical and differ in some way from the subunits seen in the electron micrographs. Thus, if the x-ray and optical diffraction patterns do arise from the same surface lattice, there are still differences in the subunits which may be due to the distribution of electron stain or even to changes in the subunits.

The interpretation of the meridional or near-meridional intensity on the 40 Å layer line as a J_3 Bessel function is not clear-cut if a second phase is present. Cohen et al.[5] had previously reported that dried oriented microtubules had a different pattern from the hydrated samples, and gave a strong meridional or near-meridional reflection at 40 Å. They interpreted this feature to indicate that the lateral coherence of the surface lattice was destroyed by drying and that the structure diffracts as simple linear arrays of protein subunits. This conclusion was in agreement with electron microscope findings indicating that the lateral bonds between units are weak, and that the strongest interactions are axial along the filaments. We have also obtained a diffraction pattern from a hydrated neurotubule sample which had undergone partial drying in the capillary. Although showing more disorientation, the pattern is very similar to that of the hydrated sample, but there is a striking change in the intensity distribution on the 40 Å layer line (FIGURE 2b). The strong intensity is now near or on the meridian. This may well correspond to partial breakdown of some of the microtubules to form linear arrays, so that, in effect, we have a two-phase system and a mixture of two patterns. Hence, the sharp but weak meridional reflection on the 40 Å layer line in patterns from the *hydrated* sample may also arise from some frayed microtubules in the sample diffracting as linear arrays. It is also possible, however, that the microtubules in the x-ray samples are intact, and that the near-meridional intensity is not the result of scattering by filaments. By partially drying the sample we have in fact obtained an x-ray pattern which more closely resembles the optical diffraction patterns; i.e., the strong intensity on the 40 Å layer line is now nearer the meridian. Thus, the dried microtubules may more closely correspond in appearance to the microtubule subunits as seen in the electron microscope.

One other aspect of microtubule structure we may refer to here concerns the tubulin dimer. Amos and Klug[7] have interpreted a strong 80 Å layer line in optical diffraction patterns from flagella as arising from a radial staggering of alternate subunits, probably corresponding to the two subunits of the tubulin dimer. Samples from sea urchin sperm outer fibers showed this period in the x-ray patterns, but rather weakly, as did patterns from scallop gill cilia. The neurotubules also show faint traces of a series of reflections with this axial spacing. This feature could be due to a periodic perturbation which is less regular or less pronounced in the x-ray samples than in those examined by Klug and his colleagues, or there may be additional material in some of these microtubule systems enhancing these periods, as Erickson[6] has suggested.

To summarize, there is considerable agreement between the structures of microtubules seen in the x-ray diffraction patterns and in the electron microscope. The surface lattice deduced from the electron micrographs may account for major features of the x-ray diagram. The appearance of the subunits revealed by electron microscopy is somewhat different from that revealed by the x-ray studies. The improved x-ray patterns from neurotubules reported here should allow us to determine the structure to a resolution sufficient to define the subunit shape and interactions.

Acknowledgments

We thank Dr. Walter Phillips and Dr. Peter Vibert for aid in obtaining the x-ray photographs.

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