

19.2. ELECTRON DIFFRACTION OF PROTEIN CRYSTALS

map can be judged by the figure of merit of the phases, computed from the phase probability distribution function of the observed reflections.

19.2.4.3. 3D map

The three-dimensional (3D) map is computed from the amplitudes and phases at the resolution defined by the data (Henderson & Unwin, 1975). The resolution reported for the structure is defined by the observed reflections in the images. Owing to the missing data at high tilt angles, the reconstruction normally has a lower resolution in the direction of the electron beam than in the direction normal to it. As a result, many of the initial low-resolution structures appear stretched out along the vertical direction. The interpretation of the 3D map derived from electron crystallography is similar to that of X-ray crystallography. Often, the initial map is reported at about 7 Å, where some of the α -helices can be interpreted. With an improved map of about 3.5 Å, the polypeptide backbone is traced and some of the bulky side chains are recognized. Fig. 19.2.4.3 shows a chain tracing of a tubulin crystal (Nogales *et al.*, 1998).

19.2.4.4. Refinement

In order to arrive at a correct mechanistic model for the protein, an accurate atomic structure is needed. So far, in electron crystallography only bacteriorhodopsin has been refined (Grigorieff *et al.*, 1996). A common criterion used in X-ray crystallography to evaluate the progress of refinement is based on the free R factor, which measures the agreement between the model and a part of the experimental data not included in the refinement process. In electron crystallography, the phases are measured independently from images and hence are not refined. Therefore, they can be used as a 'free phase residual,' which is analogous to the free R factor, to assess the progress of refinement. The refined structure would result in improved peptide geometry, increased accuracy of the coordinates of the polypeptide backbone and of the amino-acid side chain residues, and improved temperature factors of the residues.

19.2.5. Future development

Electron crystallography has proven to be a high-resolution structural tool for two-dimensional protein crystals, to the point

where the polypeptide backbone can be traced and atomic coordinates derived. Needless to say, there is still much to be learned about how to make highly ordered two-dimensional crystals from either membrane or soluble proteins. Research in this direction is critical for the growth of electron crystallography. Recent results have promoted optimism; there has been an increase in the number of membrane proteins crystallized into two-dimensional arrays from which at least 6 to 8 Å structures can be obtained (Walz *et al.*, 1997; Auer *et al.*, 1998; Zhang *et al.*, 1998; Unger *et al.*, 1999).

In the most recent high-resolution structural study of tubulin, a 3.7 Å map was obtained from 100 electron diffraction patterns and 150 electron images. Effectively, this structure was the result of a computational average of about one million tubulin dimers. It took six years to determine the structure from the time when the first high-resolution crystal structure was reported (Downing & Jontes, 1992; Nogales *et al.*, 1998). All the experimental and computational procedures were basically the same as those developed for bacteriorhodopsin (Henderson *et al.*, 1990). An obvious future development in protein electron crystallography would be aimed at improving the throughput of the structural determination. This entails a search for better solutions to some of the technical problems mentioned above as well as the introduction of automation in both data collection and processing.

Finally, another potentially exciting aspect of electron crystallography is the ability to detect charged residues from the high scattering differences between neutral and charged atoms. This physical property may make electron crystallography a unique method for detecting the ionization state of the amino-acid residues in proteins (Mitsuoka *et al.*, 1999). Furthermore, there is also a good prospect of extending the structure close to 2 Å resolution, as the next generation of electron cryomicroscope will be equipped with a field emission gun operated at 300 keV, a liquid helium cryo-specimen stage and an energy filter. This combination of instrumental features is likely to bring electron crystallography a step closer to its ultimate potential for structural biology research at the atomic level.

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