19.6. Electron cryomicroscopy

BY T. S. BAKER AND R. HENDERSON

19.6.1. Abbreviations used

0D	Zero-dimensional (single particles)
1D	One-dimensional (helical)
2D	Two-dimensional
3D	Three-dimensional
EM	Electron microscope/microscopy
Cryo EM	Electron cryomicroscopy
FEG	Field-emission gun
CTF	Contrast-transfer function
CCD	Charge-coupled device (slow-scan TV detector)

19.6.2. The role of electron microscopy in macromolecular structure determination

Diffraction and imaging techniques are one manifestation of the use of scattering of beams or wavefronts by objects to analyse the structure of that object (Fig. 19.6.2.1). Such methods can be used in general for the examination of structures of any size, ranging from elementary subnuclear particles all the way up to the structure of the earth's core. For macromolecular structure determination, there are two main differences between the use of electrons and X-rays to probe structure. The most important is that the scattering cross section is about 10^5 times greater for electrons than it is for X-rays, so significant scattering using electrons is obtained for crystals or other specimens that are 1 to 10 nm thick, whereas scattering or absorption of a similar fraction of an illuminating X-ray beam requires crystals that are 100 to 500 μm thick. The second main difference is that electrons are much more easily focused than X-rays since they are charged particles that can be deflected by electric or magnetic fields. As a result, electron lenses are much superior to X-ray lenses and can be used to produce a magnified image of an object as easily as a diffraction pattern. This then allows the electron microscope to be switched back and forth instantly between imaging and diffraction modes so that the image of a single molecule at any magnification can be obtained as conveniently as the electron diffraction pattern of a thin crystal. By contrast, X-ray microscopy has been much less valuable than X-ray diffraction, but may be useful for imaging at the cellular level.

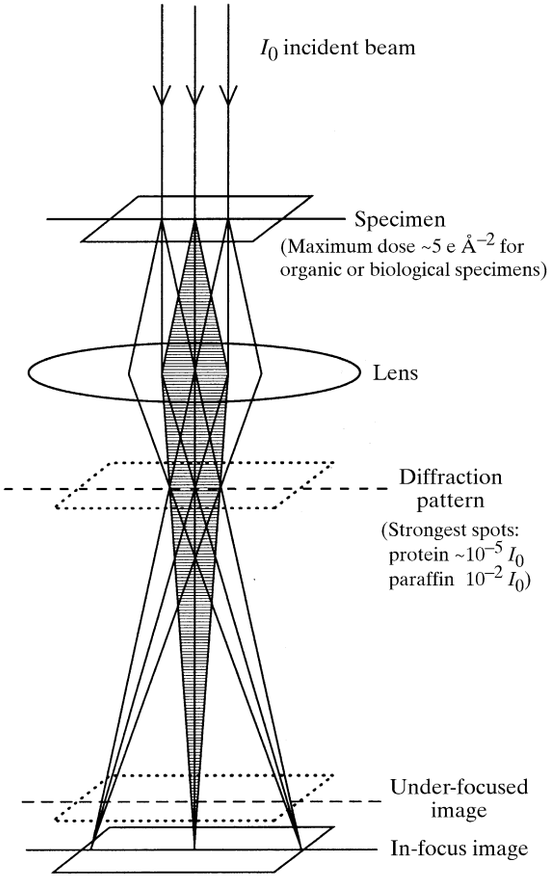

Fig. 19.6.2.1. Schematic diagram showing the principle of image formation and diffraction in the transmission electron microscope. The incident beam, I_0, illuminates the specimen. Scattered and unscattered electrons are collected by the objective lens and focused back to form first an electron-diffraction pattern and then an image. For a 2D or 3D crystal, the electron-diffraction pattern would show a lattice of spots, each of whose intensity is a small fraction of that of the incident beam. In practice, an in-focus image has virtually no contrast, so images are recorded with the objective lens slightly defocused to take advantage of the out-of-focus phase-contrast mechanism.

In the early years of electron microscopy of macromolecules, electron micrographs of molecules embedded in a thin film of heavy-atom stains (Brenner & Horne, 1959; Huxley & Zubay, 1960) were used to produce pictures which were interpreted directly. Beginning with the work of Klug (Klug & Berger, 1964), a more rigorous approach to image analysis led first to the interpretation of the two-dimensional (2D) images as the projected density summed along the direction of view and then to the ability to reconstruct the three-dimensional (3D) object from which the images arose (DeRosier & Klug, 1968; Hoppe *et al.*, 1968), with subsequent more sophisticated treatment of image contrast transfer (Erickson & Klug, 1971).

Later, macromolecules were examined by electron diffraction and imaging without the use of heavy-atom stains by embedding the specimens in either a thin film of glucose (Unwin & Henderson, 1975) or in a thin film of rapidly (Dubochet, Lepault *et al.*, 1982; Dubochet *et al.*, 1988) or slowly (Taylor & Glaeser, 1974) frozen water, which required the specimen to be cooled while it was examined in the electron microscope. This use of unstained specimens thus led to the structure determination of the molecules themselves, rather than the structure of a 'negative stain' excluding volume, and has created the burgeoning field of 3D electron microscopy of macromolecules. Many of the image-analysis techniques now used for studying unstained specimens originated from those used in the analysis of negatively stained samples.

At this point in year 2000, hundreds of medium-resolution structures of macromolecular assemblies (*e.g.* ribosomes), spherical and helical viruses, and larger protein molecules have been determined by electron cryomicroscopy in ice. Three atomic resolution structures have been obtained by electron cryomicroscopy of thin 2D crystals embedded in glucose, trehalose or tannic acid (Henderson *et al.*, 1990; Kühlbrandt *et al.*, 1994; Nogales *et al.*, 1998), where specimen cooling reduced the effect of radiation damage. The medium-resolution density distributions can often be interpreted in terms of the chemistry of the structure if a high-resolution model of one or more of the component pieces has already been obtained by X-ray, electron microscopy, or NMR methods. As a result, electron microscopy is being transformed from a niche methodology into a powerful technique for which, in some cases, no alternative approach is possible. This article outlines the key aspects of electron cryomicroscopy (cryo EM) and 3D image reconstruction. Further information can be obtained from several reviews (*e.g.* Amos *et al.*, 1982; Glaeser, 1985; Chiu, 1986;

references