High-Angle Electron Diffraction of Frozen Hydrated Collagen

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By using the techniques developed by Taylor et al. [(1975) J. Mol. Biol. 92, 165–167] (freezing of the hydrated specimen before its insertion into the electron microscope and keeping it frozen throughout the diffraction experiment), it was possible to obtain a high-angle electron-diffraction pattern from collagen fibrils. This pattern is in good agreement with that obtained by high-angle X-ray diffraction. Electron diffraction will be very useful to study collagen, because the diffraction pattern from a carefully selected area of one fibril is now feasible.

X-ray-diffraction techniques have been widely used in recent years for the investigation of collagen structure (Fraser & MacRae, 1974). However, the information recorded by using X-ray diffraction is statistical, and it corresponds to a rather large area of the specimen and includes many fibrils. With a fibrillar specimen such as collagen it would be expected that electron diffraction would allow a record of the diffraction data to be obtained from a carefully selected area of one or a few fibrils.

So far, all reported attempts to record electron-diffraction patterns of collagen fibrils have been unsuccessful, except for the low-angle data recorded on dried stained or unstained collagen fibrils (Murray & Ferrier, 1968). The lack of high-angle diffraction data is linked to the fact that collagen dehydrates readily inside the vacuum of the electron microscope. This departure of the structural water is accompanied by decrystallization and disappearance of the diffraction pattern.

Techniques have been developed recently to overcome the dehydration of biopolymers in the vacuum of the electron microscope. Thus good results were obtained by using a hydration chamber (Parsons, 1974), or alternatively by freezing the hydrated specimen before its insertion into the electron microscope and keeping it frozen throughout the diffraction experiment (Taylor & Glaeser, 1974; Taylor et al., 1975). The former method requires sophisticated apparatus, whereas the latter may be performed with any electron microscope fitted with a cooling sample holder. This latter method was applied to collagen and the results are reported below.

Experimental

Successful results were obtained with three samples of collagen, obtained from fresh mouse tail tendon, freeze-dried rat tail tendon (kindly provided by Dr. D. H. Reneker, National Bureau of Standards, Washington, D.C., U.S.A.) and reconstituted calf skin collagen. Tail tendon collagen was teased in water and small fragments were mounted on carbon-coated copper grids. With freeze-dried collagen the mounted specimen was further hydrated by overnight storage in an atmosphere of 95% relative humidity at normal pressure. For the reconstituted collagen, obtained by dialysis of a solution of collagen in acetic acid against 0.02M-sodium phosphate, a drop of the suspension was deposited on the carbon-coated grid and excess of water was removed with a filter paper. Each specimen was treated as previously described (Taylor et al., 1975), i.e. mounted on a Philips EM300 cooling sample holder, quenched in isopentane kept close to liquid-N₂ temperature and maintained cold during insertion and observation inside the electron microscope.

Results and Discussion

Electron-diffraction experiments were performed on selected areas of the collagen fibrils obtained from the three different sources described above. All three samples gave similar results. Plate 1(a) illustrates a typical view of the unstained mouse tail tendon, and the electron-diffraction pattern from the circled area is shown in Plate 1(b). For a better understanding a
schematic diffraction diagram is presented in Plate 1(c).

This pattern is characterized by a strong meridional arc at 0.291 ± 0.001 nm and an equatorial reflexion at 1.17 ± 0.1 nm. In addition to these two well-defined diffraction spots, more diffuse reflexions are observed at 0.45 nm on the equator and near-meridional arcs at 0.4 nm spacing. These reflexions are just visible above the background on the original negative and are difficult to reproduce in Plate 1(b). These results are in good agreement with those obtained by high-angle X-ray diffraction. For example, the 0.291 nm meridional arc is the same as that recorded by X-ray diffraction (Dickerson, 1964; Herbage et al., 1972). The variation in the spacing of the 1.17 nm equatorial reflexion between different specimens appears to be related to the difficulty in the precise control of hydration of the cooled specimen inside the electron microscope.

Nevertheless electron-diffraction information is obtainable for hydrated collagen and it is believed that this technique will be very useful in future work. For instance, the possibility exists of following a fibril of collagen in the diffraction mode in order to obtain information on the local orientation of the molecules and therefore obtain a better understanding of unexplained details such as the crimp morphology (Diamant et al., 1972). To obtain images of collagen fibrils by diffraction-contrast techniques such as dark-field or lattice imaging also seems feasible and should give information on the organized and disorganized domains of the fibrils, as shown elsewhere with other fibrillar biopolymers (Bourret et al., 1972). Finally, the identification of minute amounts of collagen may be possible by using selected-area electron-diffraction on ultra-thin sections of tissues or on dispersions of extracted products.

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References


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EXPLANATION OF PLATE I

High-angle electron diffraction of frozen hydrated collagen

(a) Unstained specimen of mouse tail tendon collagen with the area selected for the electron diffraction (circle). (b) Electron-diffraction pattern of a collagen fibril obtained from mouse tail tendon and corresponding to the circled area in (a); the axis of the fibre is vertical. (c) Schematic diagram corresponding to the electron-diffraction pattern shown in (b).

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