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ELECTRON DIFFRACTION OF FROZEN, HYDRATED PROTEIN CRYSTALS

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Kenneth A. Taylor (1)

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ABSTRACT

High resolution electron diffraction patterns have been obtained from frozen hydrated catalase crystals to demonstrate the feasibility of using a frozen specimen type hydration technique. The use of frozen specimens as a way to maintain hydration of complex biological structure has certain advantages over previously developed liquid hydration techniques.

There is great potential for the use of electron microscopy as an investigative tool in high resolution structural investigation of complex biological objects. However, if high resolution structure is to be observed, either by electron diffraction or by direct imaging, the specimen must be maintained in a hydrated condition in the high vacuum of the electron microscope. Until recently this has not been possible because techniques had not been available to maintain a necessary degree of specimen hydration, which is so vital to native biological structure. To overcome the hydration problem previous investigators have used two basic approaches; (a) the closed cell, thin window environmental chambers and (b) the differentially pumped hydration stages (1). A high degree of success has been achieved with the differentially pumped stage by Parsons and co-workers, who have obtained electron diffraction patterns from unstained, unfixed catalase crystals in the hydrated state (2), and electron diffraction patterns from wet, human erythrocyte membranes (3). Catalase crystals are a convenient specimen for testing hydration efficiency because the protein is both easily crystallizable as thin plates suitable for electron diffraction, and its diffraction pattern is sensitive to hydration effects (2, 4).

In this laboratory, we have been exploring the feasibility of using frozen specimens as an alternative method of maintaining specimen hydration. We have now obtained electron diffraction patterns from frozen, unstained and unfixed catalase crystals which were sandwiched between thin, hydrophilic support films, using the capillary action effect to obtain suitably thin specimens (5).

Catalase crystals suitable for electron diffraction were crystallized as described by Wrigley (6). The specimens for electron diffraction are mounted between thin hydrophilic support films and frozen as described in (5).

Frozen specimens are introduced into the microscope vacuum of the JEM 100B electron microscope using a combination cold sink and frost protector mounted in the airlock door. Selected area diffraction patterns were obtained using current densities at the specimen of approximately 10^{-5} A/cm^2, a diffraction camera length of 3.8 meters and an accelerating voltage of 100 keV. In these conditions the low angle diffraction pattern is easily visible on the fluorescent screen out to the third order of the 70 Å unit cell dimension. The diffraction patterns were recorded on Kodak electron image plates and developed in 1:2 Kodak HRP developer.

Figure 1 shows an electron diffraction pattern, obtained from a frozen catalase crystal, which extends to a resolution of 4.5Å. Electron diffraction patterns extending to resolutions of 3.4Å have been obtained on plates, but are difficult to reproduce photographically. Because of the relatively slow electron speed of the conventional electron image plates, specimen electron exposures of the order of 10^{-3} C/cm^2 were necessary in order to record the diffraction patterns. However, even after such doses the low resolution pattern remained unchanged. Quantitative measurements of the critical dose for loss of crystalline diffraction as a function of resolution are now in progress.

The demonstration that crystalline order is retained in protein crystals that are directly frozen in liquid nitrogen, without the use of cryoprotectants, is important for establishing the feasibility of using frozen specimens for maintaining hydration. There was ample reason to believe, both from results in freeze fracture and in frozen thin section specimen preparation techniques and from unpublished early X-ray diffraction experiments, that severe disorder of biological structure could occur during direct freezing in liquid nitrogen. Our results show that this need not necessarily be the case for specimens of the size and thickness used for electron microscopy.

There are several reasons for using the present technique of frozen specimens as the method of choice for maintaining hydration in the electron microscope. First of all, the frozen specimen technique does not require delicate regulation of water vapor pressure as is the case in liquid hydration conditions. This is the case because the vapor pressure of ice at temperatures below -100° C is negligible. Furthermore, the thin support films on both faces of the specimen greatly reduce the area available for sublimation. Another important factor in favor of using frozen specimens is their mechanical stability compared to fluid specimens. For example, colloidal particles can be expected to move quite rapidly due to Brownian motion in a liquid water hydration stage. Finally, instrument modifications are minimal for the use of frozen specimens. A liquid nitrogen cooled stage and suitable methodology for the introduction of a pre-frozen specimen into the microscope vacuum are all that is needed to use this method.

As techniques are now available for maintaining specimen hydration in the electron microscope, it is important to note the relative advantages and disadvantages which hydrated specimens have over stained, dried specimens. Contrast in hydrated specimens should be directly interpretable as structure rather than as stain (7). Fixation and drying artifacts are avoided. Hydrated protein crystals retain a high degree of periodicity while negatively stained protein crystals are rarely periodic to resolutions greater than 8Å. On the other hand, radiation damage will be expected to be much more severe in hydrated specimens than in their stained-dried counterparts.

There are two possibilities for overcoming the limitations which radiation damage imposes on working with hydrated specimens. One possibility is to utilize a theory for interpreting the diffraction intensities, which may be expected to be dynamical. Perhaps a more direct possibility is the use

of Statistically Noisy Averaged Pictures (8) or any equivalent method, which would utilize both the full, high resolution potential inherent in hydrated specimens and the imaging properties of the electron microscope. It is generally believed that the phase problem is more easily solved for the case of image intensities than for diffraction intensities, but the dynamical effect will be present in either case.

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REFERENCES

1. Two recent reviews are now in print covering the development of the closed cell, thin window environmental chambers and the differentially pumped hydration stages. Parsons, D. F., V. R. Matricardi, R. C. Moretz, and J. N. Turner, Advan. Biol. Med. Phys. 15, 162 (1974); Joy, R. T., Advan. Opt. Electron Microsc. 5, 297 (1973).
2. Matricardi, V. R., R. C. Moretz and D. F. Parsons, Science 177, 268 (1972).
3. Hui, S. W. and D. F. Parsons, Science 184, 77 (1974).
4. Longley, W., J. Mol. Biol. 30, 323 (1967).
5. Taylor, K. A. and R. M. Glaeser, Rev. Sci. Instrum. 44, 1596 (1973).
6. Wrigley, N. C., J. Ultrastruct. Res. 24, 454 (1968).
7. This point has been previously made by D. F. Parsons in his discussions of the usefulness of hydration chambers. However, the possibility has been suggested by P. N. T. Unwin (J. Mol. Biol., in press) that the combined use of a partial beam stop and electrostatic phase plate results in images of the protein structure and suppression of contrast due to the negative stain.
8. Glaeser, R. M., I. Kuo and T. F. Budinger, Proceedings, Electron Microscopy Society of America, 29th Annual Meeting. C. J. Arceneaux, Ed. (Claitors, Baton Rouge, 1971) p. 466.
9. This investigation was supported by USPHS Training Grant No. 5 TO1 GM00829 from the National Institute of General Medical Sciences and the U.S. Atomic Energy Commission.

FIGURE CAPTION

Figure 1. Electron diffraction pattern of a catalase crystal which has been frozen in liquid nitrogen and observed on a liquid nitrogen cooled specimen stage. The photographic reproduction extends to a resolution of 4.5Å although the diffraction pattern on the original plate extended to 3.4Å resolution.

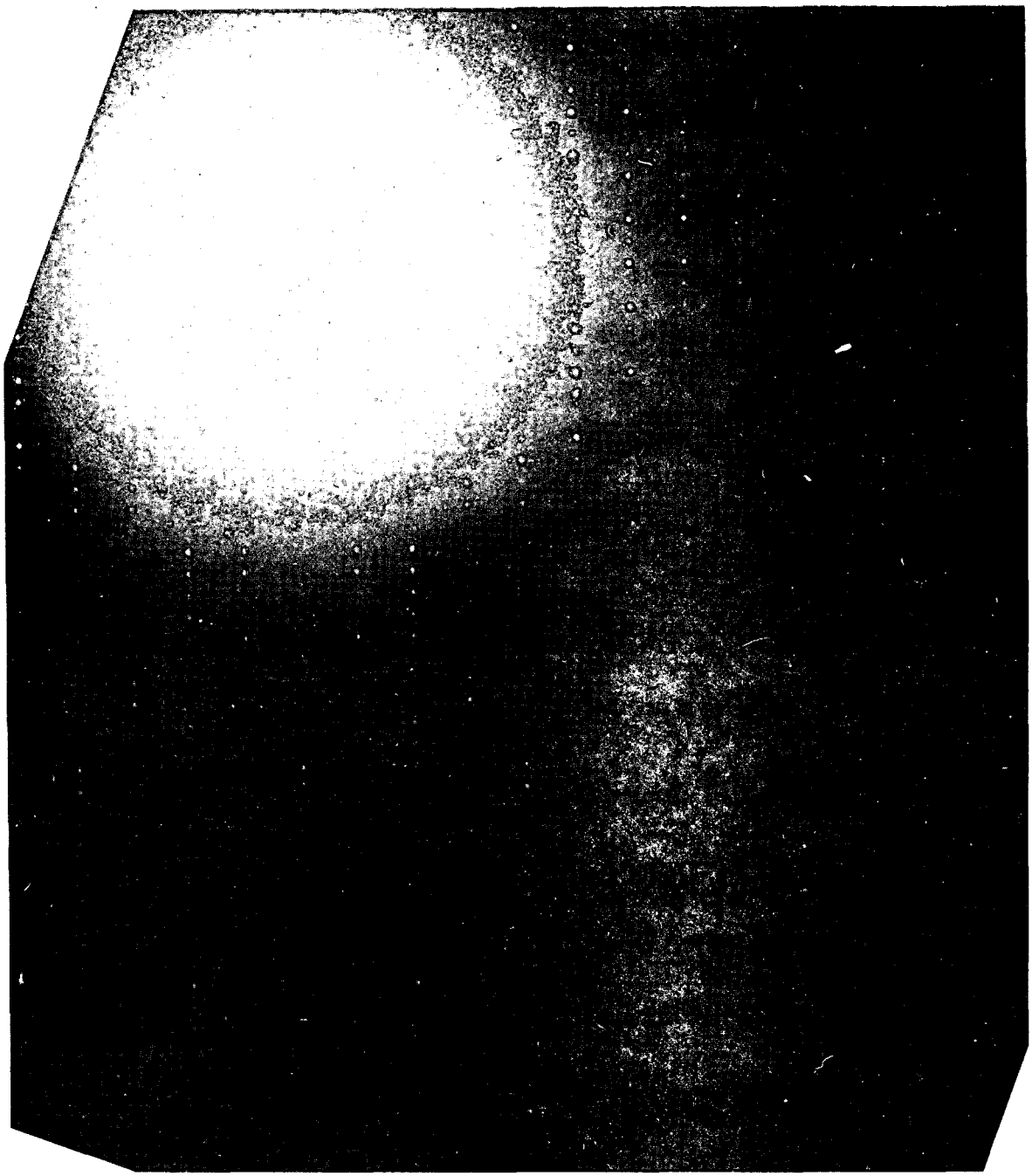

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Figure 1.

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