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Electron Diffraction of Frozen, Hydrated Protein Crystals

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

There is great potential for the use of electron microscopy to investigate the structures of complex biological objects at high resolution. 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 was not possible because techniques were not available for maintaining the hydration of the native biological structure. To overcome the hydration problem, previous investigators have used (i) closed cell, thin window environmental chambers and (ii) differentially pumped hydration stages (*1*). A high degree of success has been achieved with differentially pumped stages by Parsons and co-workers, who obtained electron diffraction patterns from unstained, unfixed catalase crystals in the hydrated state (*2*) and from wet, human erythrocyte membranes (*3*). Catalase crystals are convenient specimens for testing hydration efficiency because the protein is easily crystallizable as thin plates suitable for electron diffraction, and its diffraction pattern is sensitive to hydration effects (*2*, *4*).

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, unfixed catalase crystals sandwiched between thin, hydrophilic support films.

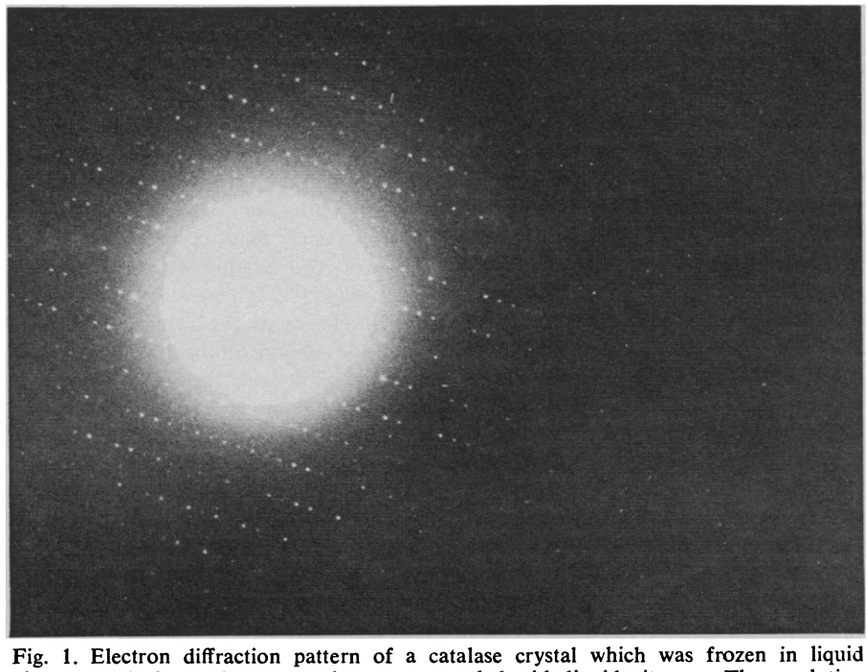

Fig. 1. Electron diffraction pattern of a catalase crystal which was frozen in liquid nitrogen and observed on a specimen stage cooled with liquid nitrogen. The resolution of the photographic reproduction is 4.5 Å, although that of the diffraction pattern on the original plate was 3.4 Å.

Catalase crystals suitable for electron diffraction were prepared as described by Wrigley (*5*). Specimens for electron diffraction were prepared from suspensions of the very thin plate crystals, which were placed between thin, hydrophilic support films by the capillary action effect; the specimens were frozen as described in (*6*). Frozen specimens were introduced into the microscope vacuum of a JEM 100B electron microscope by using a combined cold sink and frost protector mounted in the airlock door. Selected area diffraction patterns were obtained with current densities at the specimen of approximately 10^{-5} ampere/cm^2, a diffraction camera length of 3.8 m, 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 Kodak HRP developer mixed with water (1 : 2).

Figure 1 shows an electron diffraction pattern obtained from a frozen catalase crystal; the resolution in the photograph is 4.5 Å. Electron diffraction patterns with resolutions of 3.4 Å 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} coulomb/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 have been made (*7*).

The demonstration that crystalline order is retained in protein crystals 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 with freeze fracture and with techniques for preparing frozen thin sections 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 is not necessarily the case for specimens of the size and thickness used for electron microscopy.

There are several reasons for using

the technique described here as the method of choice for maintaining hydration in the electron microscope. (i) Unlike liquid hydration methods, the frozen-specimen technique does not require delicate regulation of water vapor pressure, because the vapor pressure of ice at temperatures below −100°C is negligible. (ii) The thin support films on both faces of the specimen greatly reduce the area available for sublimation. (iii) Frozen specimens are mechanically stable 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. (iv) Instrument modifications are minimal for the use of frozen specimens. A stage cooled with liquid nitrogen and suitable methodology for introducing a frozen specimen into the microscope vacuum are all that are needed to use this method.

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

There are two possible ways to overcome the limitations that radiation damage imposes on working with hydrated specimens. One way is to use a theory for interpreting the diffraction intensities, which are expected to be dynamical. Perhaps a more direct way is to use spatial averaging of statistically noisy pictures (*9*) 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 image intensities than for diffraction intensities, but the dynamical effect will be present in either case.

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Sunday and Workday Variations in Photochemical Air Pollutants in New Jersey and New York

Abstract. *Concentration distributions of air contaminants and meteorological variables in New Jersey and New York for workdays (Mondays through Fridays, omitting holidays) and Sundays are compared by means of quantile-quantile plots. The ozone distributions are slightly higher on Sundays, and the primary pollutant distributions are lower. These results raise serious questions about the validity of current concepts underlying ozone reduction in urban atmospheres.*

Bruntz *et al.* have reported (*1*) that O_3 diurnal patterns in New Jersey and New York show only minor differences on weekdays and Sundays, despite markedly different traffic patterns. The situation appears to be similar in the Los Angeles Basin (*2*), even though the pollutant characteristics of the two regions differ substantially in many respects. In this report we present more detailed statistical evidence for the ozone "Sunday effect" and for the workday-Sunday behavior of related atmospheric variables.

Sunday and workday (Mondays through Fridays, omitting holidays) data have been studied for measurements (*3*) of the following pollutants and meteorological variables in New Jersey and New York for the "photochemical seasons" May through September of 1972 and 1973 (the number in brackets indicates the number of investigated sites): NO [7], NO_2 [7], SO_2 [8], aldehydes [4], CO [9], total hydrocarbons [5], CH_4 [1], nonmethane hydrocarbons [1], aerosols [2], O_3 [9], visible solar radiation [1], ultraviolet solar radiation [3], wind speed [8], wind direction [5], mixing height [1], temperature [1], standard deviation of the vertical wind direction (vertical sigma) [2], standard deviation of the horizontal wind direction (horizontal sigma) [1], and precipitation [6]. For all wind and solar radiation variables and for all air quality measurements except O_3 the average value from 5 a.m. to 1 p.m. was used since this interval includes the time of major O_3 production and early morning primary pollutant injection. For O_3 the maximum hourly average from 11 a.m. to 6 p.m. and the average from 7 a.m. to 8 p.m. were used; for temperature, the daily maximum, minimum, and average; for precipitation, the daily total; and for mixing height, the 7 a.m. reading.

The goal of the analysis is to compare the distribution of the Sunday values of a measured variable at a particular site with the distribution of workday values. One procedure is to compare the arithmetic mean of all the Sunday values with the arithmetic mean of all the workday values. However, the arithmetic mean is only one facet of the distribution and does not necessarily characterize the entire behavior. (For example, the three sets of numbers {10,20,30,40,50}, {28,29,30,31,32}, and {21,22,23,24,60} differ substantially but have the same arithmetic mean.) In this analysis the entire distribution of the Sunday values was compared with the entire distribution of the workday values via a statistical technique called a quantile-quantile (*Q-Q*) plot (*4*). The quantile of order p of a set of data is defined to be a value such that a fraction p of the data is less than or equal to the value. For instance, the median would be a quantile of order ½. On the *Q-Q* plot the quantiles of one set of data (in this case the Sunday values)