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RADIATION DAMAGE IN STAINED CATALASE AT LOW TEMPERATURE

Running Title: Radiation Damage

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SUMMARY

Radiation damage, which occurs in the beam of the electron microscope, has been studied in uranyl acetate stained catalase crystals. Damage is observed in terms of the disappearance of higher orders of the electron diffraction pattern. In this study it has been found that the damage at very low specimen temperatures proceeds both more rapidly and to a greater final degree than is the case at room temperature.

INTRODUCTION

Radiation damage, in specimens suitable for electron microscopy, has been investigated at temperatures lower than 20°K for a variety of organic materials, including some of biological interest. Siegel (1972) has found the existence of a "latent dose", during which the low order diffraction pattern of crystalline organic materials exhibits minimal changes. However, the exposure needed to totally destroy crystalline structure at very low temperature is increased by only a factor of three or less for saturated-bond type molecules and only a factor of four or less for aromatic molecules (cf. the review of Glaeser, 1974).

In spite of the limited nature of any low temperature effect for unstained organic materials, it seemed worthwhile to investigate the matter for stained biological specimens. Mass loss associated with the radiolytic production of low molecular weight organic fragments should be effectively eliminated. It seemed possible therefore that

the organic stain might be more nearly constrained to its original position in the specimen than has been found to be the case at room temperature. However, the outcome of the experiments reported now has been that the loss of crystalline diffraction in uranyl acetate stained catalase is markedly more severe at low temperature than at room temperature.

EXPERIMENTAL METHODS

The low temperature stage used in this investigation was developed by Hobbs (1973, 1974) for the Siemens Elmiskop I, and has been used for studies on radiation damage in alkali halides at low temperature (Hobbs, 1973; Hobbs et al. 1973). Specimen cooling is achieved by circulation of helium gas through a heat exchanger, which is in thermal contact with the stage. Specimen temperatures lower than 10°K are attainable, as demonstrated by the condensation of neon when the gas is intentionally introduced into the column. In the present experiments, a stage temperature of less than 15°K was maintained, and specimen temperature below 30°K was realized as evidenced by condensation of nitrogen gas on the specimen itself.

Catalase was recrystallized by the method of Wrigley (1968) in order to produce thin laths. Specimens either were mounted on formvar and then coated with carbon or were mounted directly on 30 Å thick carbon films that were prepared by the method of Williams & Glaeser (1972).

Specimen staining was carried out in the following way. A

dilute drop of crystals in suspension was placed directly on the support film. A similar sized drop of two percent uranyl acetate was added, and the mixture was allowed to stand for two minutes so that some of the crystals would settle and become attached to the substrate. The liquid was then drained from the grid with filter paper, and the specimen was allowed to dry at room temperature. The uranyl acetate solution was one that had been titrated with NaOH to a point just before that which caused precipitation. Preparation of a stable stock of stain by this method requires some care. The titration, if carried too far, may not lead immediately to precipiation, but this may still occur after a period of several hours.

Radiation damage was observed in terms of the degradation and loss of crystalline diffraction patterns. In order to find suitable crystals, the specimen was scanned at a magnification of 5,000X and a current density of approximately 1×10^{-4} A/cm^2 on the specimen. When a crystal of appropriate thickness was found, it was then subsequently examined by the usual selected area diffraction technique. Heavy specimen irradiations were carried out under conditions suitable for normal electron microscopy at a magnification of 50,000X. The illumination spot had a diameter of 5 microns and the current density at the specimen was approximately 1×10^{-2} A/cm^2. In these conditions one could observe the sublimation of crystalline N_2 in a matter of seconds, but no sublimation of condensed H_2O was noticeable. No sublimation of crystalline N_2 could be noticed under the less intense conditions of irradiation that were used to scan over the

specimen and to record the electron diffraction patterns. The electron exposures involved in conducting these experiments were measured in an approximate way by use of the fluorescent screen as a flat plate "Faraday cage", in the same way previously employed by Grubb (1971) and by Grubb & Groves (1971). The current densities reported here have been corrected for the reemission effect, according to the factor given by Grubb (1971).

EXPERIMENTAL RESULTS

The first observation made was that the electron diffraction intensities for crystals at low temperature did not extend to as high a resolution as for crystals at room temperature. At "helium" temperature no diffraction patterns were observed to extend beyond the second order of the 68 Å period, while at room temperature—on the same specimen grid—diffraction patterns could routinely be obtained to the fourth order of the 68 Å period. Figure 1a shows a diffraction pattern obtained at room temperature, while Figure 1b shows a pattern obtained from another crystal at low temperature. The total electron exposure given to the crystals in obtaining patterns of this type varied between 2×10^{-3} and 1×10^{-2} Coulomb/cm^2.

The second effect observed is that the uranyl stained catalase structure became disordered to a much greater extent under conditions of intense irradiation at low temperature than is the case at room temperature. Figure 1c shows the room temperature diffraction pattern for the same crystal as shown in Figure 1a, but after the crystal

had been exposed to an electron flux of approximately 1.0 Coulomb/cm^2. Similarly, Figure 1d shows the diffraction pattern obtained at low temperature after irradiation by approximately 1.0 Coulomb/cm^2. As reported previously by Glaeser (1971), the electron diffraction pattern of uranyl stained catalase at room temperature fades down to a stable pattern at a resolution of approximately 25Å. This has not turned out to be the case, however, at helium temperature. Instead the diffraction pattern frequently fades down to a resolution of approximately 70Å, and in some of the cases it has been observed that all of the diffraction spots are lost.

In an attempt to further define the correct cause for the exceptional radiation sensitivity at low temperature of the uranyl stained catalase, experiments were done in which the diffraction pattern of a single crystal was observed continuously while the specimen was cooled from room temperature to helium temperature. In these experiments it was observed that the diffraction pattern remained essentially unchanged until the temperature reached approximately 35°K. At this point, a sudden loss of crystalline structure was observed, and the pattern was reduced to a resolution of approximately 25Å, giving a pattern such as the one shown in Figure 1b. This reduction in crystalline structure was irreversible in the sense that no subsequent improvement in the pattern was observed if the crystal was warmed again to room temperature. This reduction in crystallinity was not due, however, to the simple act of cooling the specimen to helium temperature. Other crystals, on the same grid, which were

not exposed to the electron beam during the period of specimen chilling could be observed to produce an excellent diffraction pattern if they were observed at room temperature. This remained the case even after the specimen had been cycled from room temperature to helium temperature and back to room temperature a number of times.

It seemed important to determine whether the damage that occurs at helium temperature includes some effect in addition to the damage which can occur at room temperature. To test this point a specimen which initially showed an excellent diffraction pattern at room temperature was irradiated by approximately 1.0 Coulomb/cm^2 in order to reduce the pattern to the typical 25Å resolution state. At this point the specimen was then cooled to helium temperature and the same crystal was given a further exposure of 1.0 Coulomb/cm^2. Virtually no further change appeared in the electron diffraction pattern. From this observation we conclude that the exceptional radiation sensitivity of the uranyl stained catalase at low temperature is in some sense a synergistic effect involving both low temperature and extensive electron irradiation.

DISCUSSION

The results obtained in this study at low temperature are just the opposite of what had been our original expectations, and these results remain difficult for us to understand and explain. Nevertheless, it is significant and important to note that the uranyl stained specimen clearly is not any more resistant to the damaging effects of electron

irradiation at helium temperature than at room temperature. The fact that very low specimen temperature is to some extent a disadvantage in the electron microscopy of these stained specimens is perhaps less significant from an operational point of view than the fact that low temperature does not provide any advantage.

One possible suggestion for the increased radiation sensitivity at low temperature may be related to the fact that chemical radicals and low molecular weight species will be trapped in the specimen until they reach very high concentration. At this point they will begin to come in contact with one another, and a relatively rapid reaction may take place. The interaction of these products of radiolysis, at high concentration, may have a more cataclysmic effect upon the structure than if the products could have been consumed or released at the same rate that they were produced. Presumably the latter is what occurs at room temperature.

A second hypothesis regarding the increased radiation sensitivity at low temperature is related to the relative thermal contraction which the specimen must undergo upon being cooled by nearly 300°C. It may be suggested that if local or microscopic strains exist in the specimen due to thermal contraction, then these may render the specimen more susceptible to the occurrence of extensive disorder than in comparison to the disorder occurring at room temperature.

A third suggestion for the increased sensitivity at helium temperature is that residual gas molecules which are condensed at the surface could participate in a radiolytic attack upon the specimen. This

third suggestion would seem to be excluded, however, by the observation that specimens which are heavily irradiated at room temperature undergo no further, apparent damage when they are subsequently irradiated at helium temperature, and in fact, remain in a much more crystalline state than those specimens which are irradiated only at helium temperature.

It should be pointed out that the disappearance of the pattern at low temperature is not due to a simple masking of the pattern by diffuse scatter from the residual gas molecules that tend to condense on the specimen. Such an effect can indeed be seen after prolonged operation at low temperature. This type of effect must be excluded from consideration in the damage studies, however, by the fact that the patterns for irradiated crystals did not improve when the condensed gases were removed by bringing the specimen up to room temperature.

Finally it is important to emphasize that further experiments of this type are needed before concluding that helium specimen temperature is of little use in electron microscopy of stained biological specimens. While the results obtained with uranyl stained catalase may be quite discouraging, one cannot tell whether similar results will be obtained with different kinds of biological specimens or with different kinds of heavy metal stains. These are points that can only be determined by further experiments. In particular, it may be worthwhile to suggest that experiments should be done with heavy metal stains that are highly resistant to electron irradiation damage. These would have to be stains other than ones that have an organic molecular component,

which by itself is quite sensitive to radiolytic destruction.

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FIGURE LEGEND

Figure 1. Electron diffraction patterns recorded from uranyl acetate stained, crystalline catalase. Figure 1A is a pattern obtained at room temperature after an electron exposure of 1×10^{-2} Coulomb/cm^2, while Figure 1B is a pattern obtained from another crystal at helium temperature, again after an electron exposure of 1×10^{-2} Coulomb/cm^2. Figures 1C and 1D show the patterns for the same crystals as Figures 1A and 1B (at room temperature and at helium temperature, respectively) after a further exposure of 1.0 Coulomb/cm^2.

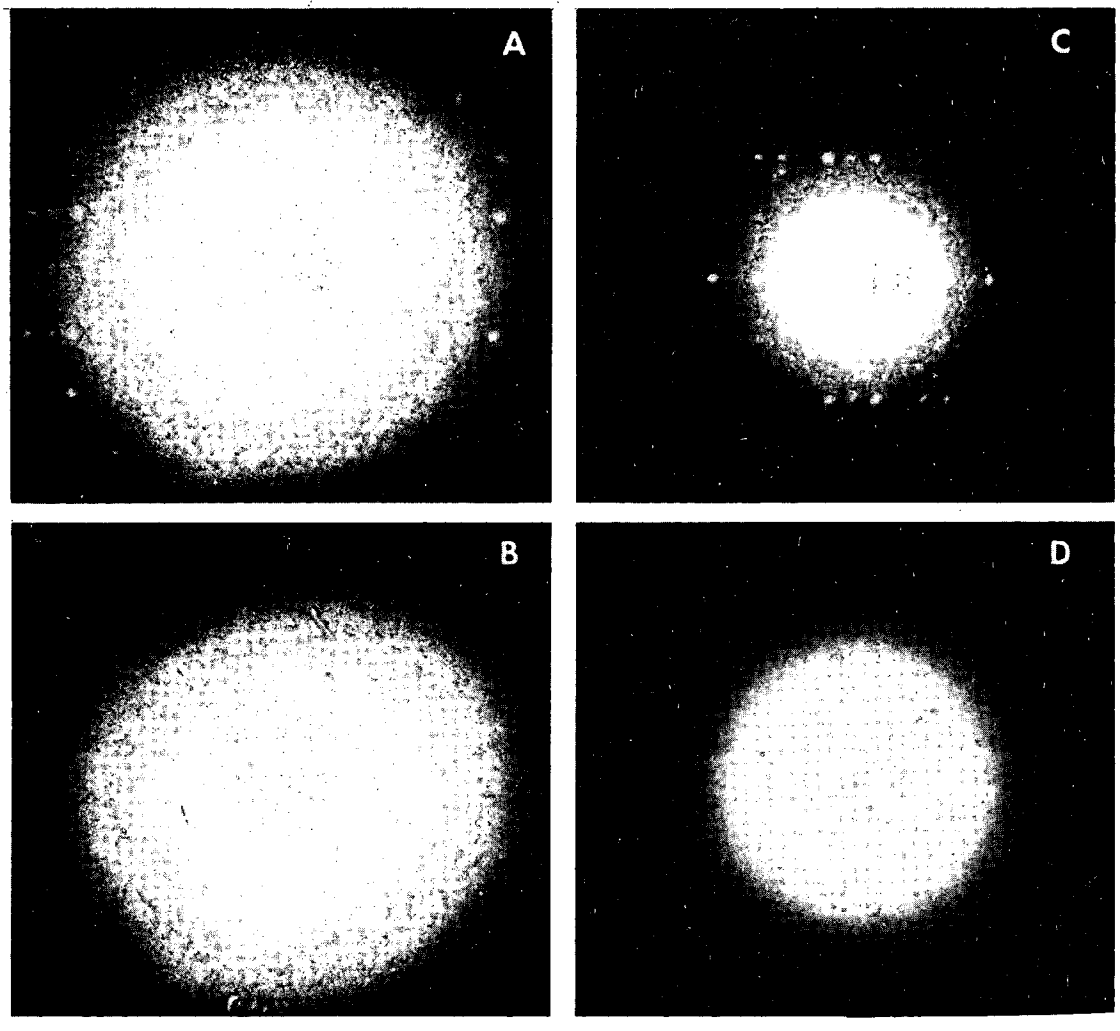

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Fig. 1

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