indirect control of the respiration rate by oxygen is also possible.

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BIOCHEMISTRY

Sub-unit Nature of Catalase Compound II

THE absorption spectrum of catalase compound II is well established and provides the principal basis for the characterization of this species. The nature of the reactions involved in the conversion of catalase to compound II is not well understood, but it is clear that compound II plays a very important part in the reversible deactivation of catalase by its substrates. Although there has been no suggestion in the literature that the formation of compound II involves a major disruption of the catalase molecule, recent kinetic studies in this laboratory[1] suggested that compound II may be a sub-unit species. This compound is sufficiently stable to permit a direct test of this idea by sedimentation velocity studies in the analytical ultracentrifuge.

The catalase used in our experiments was prepared from *M. lysodeikticus* and had an activity[2] of $6{\cdot}5 \times 10^7$ M^{-1} s^{-1} and an RZ value of 0·8; sedimentation velocity experiments showed no lower molecular weight impurities. Catalase was partially converted to compound II by the addition to catalase solutions ($\sim 10^{-5}$ M, phosphate buffer *p*H 7·0) of either several very small amounts of H_2O_2 (allowing the catalatic reaction to subside after each addition) or, in much milder conditions, by a single addition of dilute aqueous alkyl hydroperoxide solution. The results obtained were independent of the mode of formation of compound II. The absorption spectra of the solutions were time invariant for several hours and these measurements permitted calculation of the percentage of compound II using the data of Chance and Herbert[3].

Fig. 1 shows the sedimentation velocity pattern obtained with the Beckman Model E Ultracentrifuge, from a catalase solution containing 17·5 per cent compound II by spectrophotometric estimation; the faster peak ($S_{20,w} = 10{\cdot}7\ S$) corresponds to catalase (ref. 4; S_{20} bacterial catalase = 11·0 *S*). The fraction of slower moving material ($S_{20,w} = 2{\cdot}7\ S$) corresponded well with the percentage of compound II in the solution (for example, 15 per cent slower moving material for the experiment shown in Fig. 1, corrected for sectorial dilution). We therefore assign the slower peak to compound II and, if compound II resembles a globular protein, the reduced *S* value implies a much lower molecular weight than that of catalase. Using the method of Van Holde[5], we have calculated a diffusion coefficient for compound II of $5{\cdot}4 \times 10^{-7}$ cm^2 s^{-1}, which yields a molecular weight of about 5×10^4 (compare ref. 4, bacterial catalase $2{\cdot}3 \times 10^5$). A more detailed quantitative characterization of compound II is in progress and other catalase compounds are also being investigated.

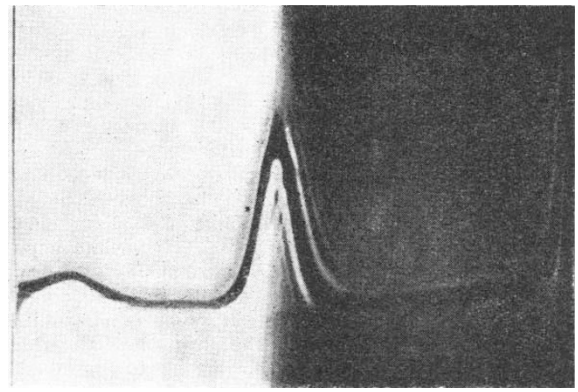

Fig. 1. Sedimentation velocity pattern of mixture of catalase and compound II. Speed 60,000 r.p.m.; $T = 22°$ C; photograph taken 31 min after reaching full speed; bar angle 60°. Sedimentation occurs to the right.

The occurrence of catalase sub-units has been reported in extreme conditions[6] and after lyophilization[7]. Caravaca *et al.*[8] have shown that a hepatocatalase sub-unit, which lacks significant catalatic activity, is a powerful peroxidase *in vitro*. They have also shown that, *in vivo*, this sub-unit may be of value in the prevention of induced atherosclerosis[9]. Our result that compound II is a sub-unit species opens a new chapter in fundamental studies of the catalase system. We believe that a variety of sub-unit species play important parts in catalase action and our evidence to support this more general proposition will be published in detail elsewhere.

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Evidence that there are Two Forms of Fluoride in Human Serum

IT has been assumed that there is only one form of fluoride in serum, the inorganic F ion. It would therefore seem that either the value for serum fluoride which I found (1 μM) (refs. 1 and 2) or that found by Singer and Armstrong (7·5 μM) (ref. 3) must be in error. While the diffusion method of Singer and Armstrong has been shown to produce erroneous values, the same cannot be