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DMSO IS A SUBSTRATE FOR CHLOROPEROXIDASE

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Dimethyl sulfoxide has been used as a nonaqueous organic solvent in haloperoxidase reactions. However, it has been found that this solvent is not inert under chloroperoxidase reaction conditions, forming the halosulfoxide, the sulfone, and the halosulfone. The biological significance of this finding is briefly discussed.

Nonaqueous solvents, like dimethyl sulfoxide (DMSO), can affect enzyme catalysis by 1) altering the chemical activity of the substrate, 2) altering protein conformation of the enzyme, or 3) altering the local environment at the active site. Cooney et al., (1) have shown that chloroperoxidase chlorinates monochlorodimedon at 47 percent of its activity in DMSO concentrations at 10 percent (v/v). Adams et al., (2) studied the properties of horseradish peroxidase in mixtures of DMSO and water and they have shown that the enzyme retains its ability to oxidize guaiacol up to 74 percent (v/v) of DMSO. Duran et al., (3) observed an increase in oxidase activity toward indole-3-acetic acid for horseradish peroxidase in the range of 1-5 percent (v/v) DMSO. However, DMSO should not necessarily be considered an inert solvent. Enzymes are known that specifically oxidize (to a sulfone) or reduce (to a sulfide) DMSO (4,5). This paper reports that DMSO is not an inert solvent for a specific haloperoxidase, chloroperoxidase (EC1.11.1.10, chloride/hydrogen peroxide oxidoreductase), but is in fact a substrate.

MATERIALS AND METHODS

Chloroperoxidase (CPO), from Caldariomyces fumago) was purchased from Sigma Chemical. Specific activity was measured as approximately 20 units/mg based upon the chlorination of monochlorodimedon at pH 3.0. Lactoperoxidase (LPO, from bovine milk), and horseradish peroxidase (HRPO,

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from horseradish) were also purchased from Sigma Chemical. Both specific activities were measured as approximately 10 units/mg based upon the peroxidation of pyrogallol at pH 6.0. DMSO (99.9%, A.C.S. reagent grade) was from Aldrich Chemical Co.

The reactions were run in 50 ml glass beakers, constantly stirred by magnetic stir bars and containing the following mixture: 20 ml of 0.1 M potassium phosphate buffer, 0.2 ml DMSO, and 1 mg of enzyme. The buffer pH was the pH optimum for each enzyme - 3.0 for CPO, 6.0 for LPO and HRPO. One vial contained 200 µmoles KCl, another vial contained 200 µmoles KBr, and a third vial had no added halide salt. Appropriate controls (i.e., no enzyme added) were also run. Three (3) additions of 1 ml of 3 percent H_2O_2 were made into each vial, each addition ten minutes apart.

Analysis of the reaction mixtures was made by injecting 10 µl into a Finnegan 4021 GCMS equipped with a 1.8 m x 4 mm coiled, glass column packed with Tenax-GC (80/100 mesh). The carrier gas was helium set at 25 ml/minute. The column temperature was programmed from 100° to 230°C at a rate of 10°C/minute. The injector and jet separator temperatures were set at 240°C. The mass spectrometer was operated in the electron impact (EI) mode at 70eV. The mass range from m/z 41 to 400 was scanned every two seconds.

RESULTS AND DISCUSSION

As shown in Figure 1a, chloroperoxidase oxidized DMSO in the absence of added halide salt. Dimethyl sulfone was formed. This product eluted from the column at 11 minutes and had the following characteristic mass spectrum: molecular ion at mass 94; base ion at mass 79 (loss of CH_3). Approximately 20 percent of the added DMSO was oxidized to the sulfone. With lactoperoxidase or horseradish peroxidase, or in the absence of added chloroperoxidase, no reaction occurred. The peroxidative uniqueness of chloroperoxidase among the other haloperoxidases has previously been observed in the ability of CPO, and CPO alone, to form nitroso derivatives

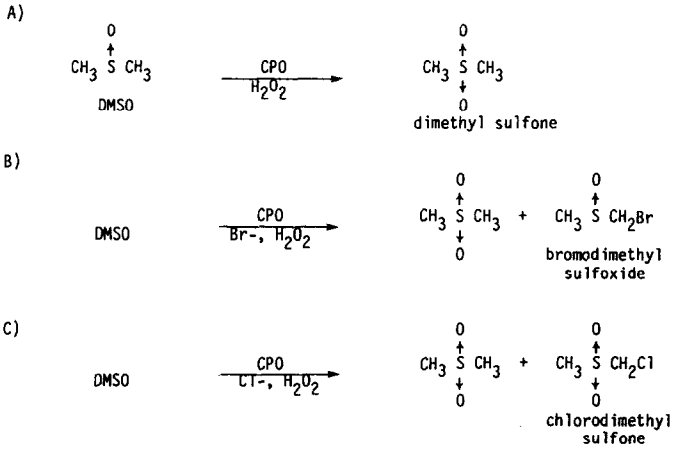

Figure 1. Chloroperoxidase (CPO) reaction on DMSO. A) in the absence of halide ion; B) in the presence of bromide ion; and C) in the presence of chloride ion.

from amino groups (6), to dismutate chlorine dioxide (7), to oxidize iodine to iodate (8), and to oxidize alcohols to aldehydes (9).

As shown in Figures 1b and 1c, chloroperoxidase can also halogenate DMSO and its oxidized product. In the presence of added bromide, dimethyl sulfone was still the major product formed, but minor amounts (approximately 1 percent of added DMSO) of bromodimethyl sulfoxide was also formed. Bromodimethyl sulfoxide eluted from the column at 14 minutes and had the following characteristic mass spectrum: molecular ion at masses 156 and 158 (1:1 in intensity); major fragment ions at masses 141 and 143 (1:1 in intensity; loss of CH_3), and at masses 93 and 95 (1:1 in intensity; the CH_2Br^+ ion). In the presence of added chloride, dimethyl sulfone was again the major product formed, but minor amounts (approximately one percent of added DMSO) of chlorodimethyl sulfone were formed. Chlorodimethyl sulfone eluted from the column at 13 minutes and had the following characteristic mass spectrum: molecular ion at masses 128 and 130 (3:1 in intensity); major fragment ions at masses 113 and 115 (3:1 in intensity; loss of CH_3), and at masses 49 and 51 (3:1 in intensity; the CH_2Cl^+ ion). No products were detected in the absence of added chloroperoxidase. Since all haloperoxidases produce hypohalous acids when halide is present, both lactoperoxidase and horseradish peroxidase should halogenate DMSO also.

Therefore, although the use of nonaqueous solvents like DMSO can offer advantages for the haloperoxidase reaction, one has to ascertain that the solvent is truly inert. We can speculate that the decrease in measured activity in DMSO solutions previously reported in the chloroperoxidase reaction (1) might, in part, be due to competition between the desired substrate and the solvent-substrate. Another suggestion from these data is that caution should be exercised when DMSO is used in biological experimentation. For example, in the human there are three known haloperoxidases that could halogenate DMSO: myeloperoxidase in leukocytes, lactoperoxidase in milk and saliva, and thyroid peroxidase in the thyroid. Halogenated derivatives of DMSO may be toxic.

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