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Influence of Dimethyl Sulfoxide on the Radiation Sensitivity of Catalase

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Recently, several investigators have demonstrated the radioprotective effect of dimethyl sulfoxide (DMSO) in animals (1,2), spores (3), and in isolated cell systems (4). It is the purpose of this communication to show that DMSO exhibits a protective effect also on a molecular level. Parts of the results have been reported previously (5).

MATERIALS AND METHOD

Two ml of lyophilized beef liver catalase solution (8.8×10^{-8}M, 0.05M phosphate buffer, pH 7.0; Worthington Biochemical Corp., Freehold, N.J.) were irradiated in a Lucite container with doses varying from 0 to 2.25×10^6 R. The dose rate of the beryllium-window x-ray tube (100 kV, 12 mA, HVL 0.065 mm Al, Philips Electronics Inc., Mt. Vernon, New York) was about 9×10^4 R/min. The x-ray tube was calibrated with an air wall ionization chamber. DMSO (Matheson Company, Inc., East Rutherford, N.J.) was added to the catalase solution prior to irradiation in concentrations varying from 0 to 3.48×10^{-3}M. The catalase activity was determined spectrophotometrically (at 240 mμ) using the method of Beers and Sizer (6). Details of the procedure were described previously (7). All measurements and irradiations were done at room temperature.

The electron spin resonance (ESR) spectra of the chlorides of Fe^{3+}, Mn^{2+}, and Cu^{2+} in water and DMSO, respectively, were obtained with a Varian V4500 100 kc ESR spectrometer at a frequency of 9500 Mc/sec using a liquid sample accessory. All solvents and hydrated salts used were of reagent grade quality. A Cary 14 spectrophotometer was used for the optical absorption studies of Fe^{3+}, Cu^{2+}, and Mn^{2+} in water and DMSO, respectively.

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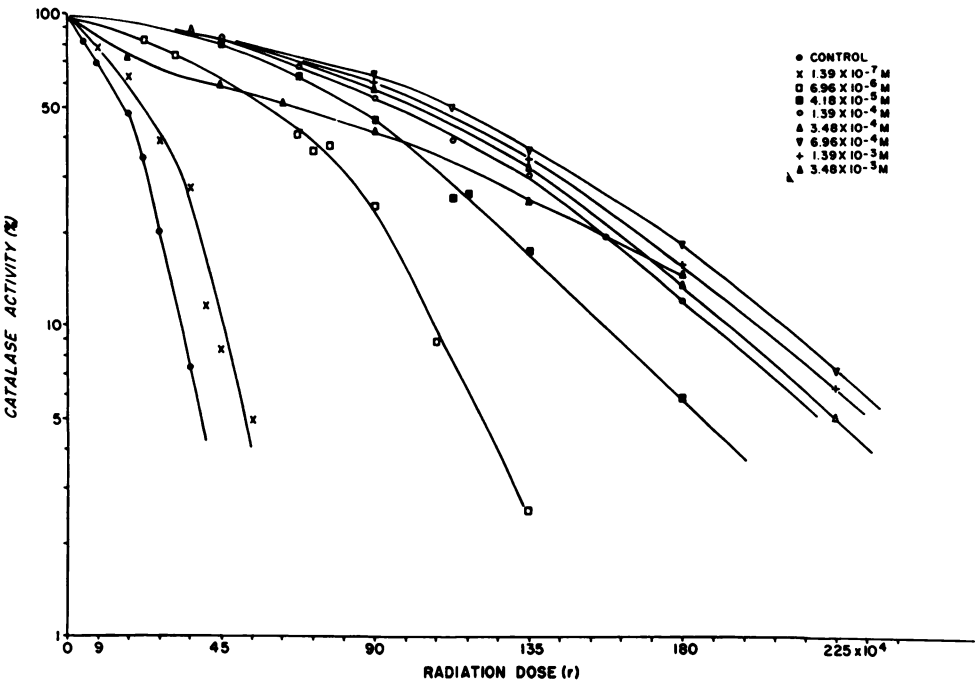
CATALASE ACTIVITY (%)
RADIATION DOSE (r)
• CONTROL
× 1.39 X 10⁻⁷ M
□ 6.96 X 10⁻⁶ M
■ 4.18 X 10⁻⁵ M
○ 1.39 X 10⁻⁴ M
▲ 3.48 X 10⁻⁴ M
▼ 6.96 X 10⁻⁴ M
+ 1.39 X 10⁻³ M
Δ 3.48 X 10⁻³ M

Fig. 1

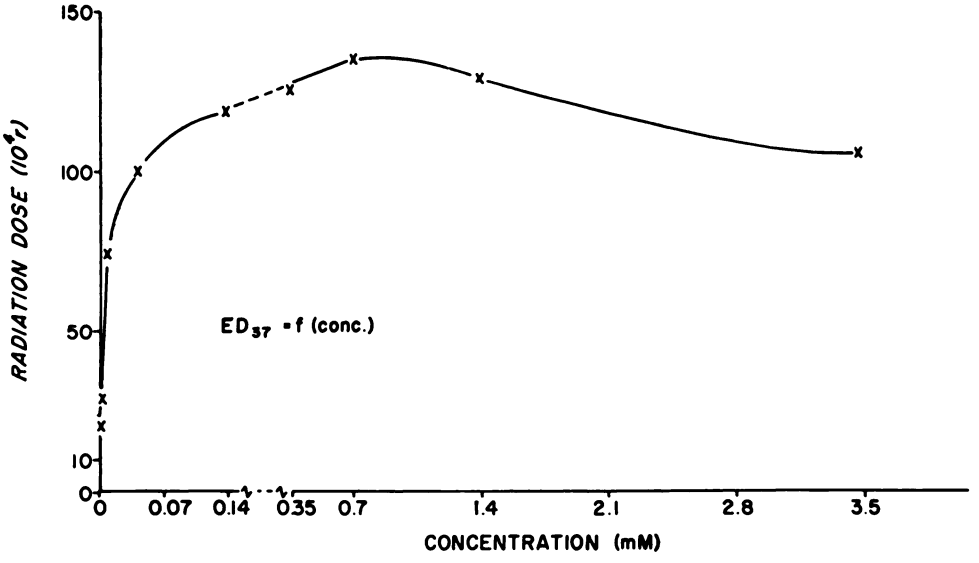
RADIATION DOSE (10⁴r)
ED37 = f (conc.)
CONCENTRATION (mM)

Fig. 2

RESULTS

The effect of DMSO on the radiation induced inactivation of catalase is shown in Fig. 1. Unirradiated catalase-DMSO mixtures were used as controls and their activity was set 100 per cent. As can be seen, DMSO exhibits a good radioprotective effect. It should be noted from the experimental results using high DMSO concentrations that at lower radiation doses the protective effect is less than that observed for smaller concentrations. At higher radiation doses the extent of protection seems to be proportional to the DMSO concentration.

The radiation dose which was required to reduce the catalase activity to 37 per cent is plotted in Fig. 2 as a function of the DMSO concentration. Results from Fig. 1 were used in obtaining these values. The protective effect exhibited by DMSO was found to be several times greater than that observed using diglycyl glycine (8). Experimental results showed that with increasing DMSO concentration the protective effect increased to a maximum and was then observed to decline slowly.

From the results obtained with mice, van der Meer *et al* (2) have recently concluded that a hypoxia mechanism might be responsible for the protective effect of DMSO. Experimental results, however, seem to indicate that another mechanism might be involved, since DMSO also protects cells in tissue culture

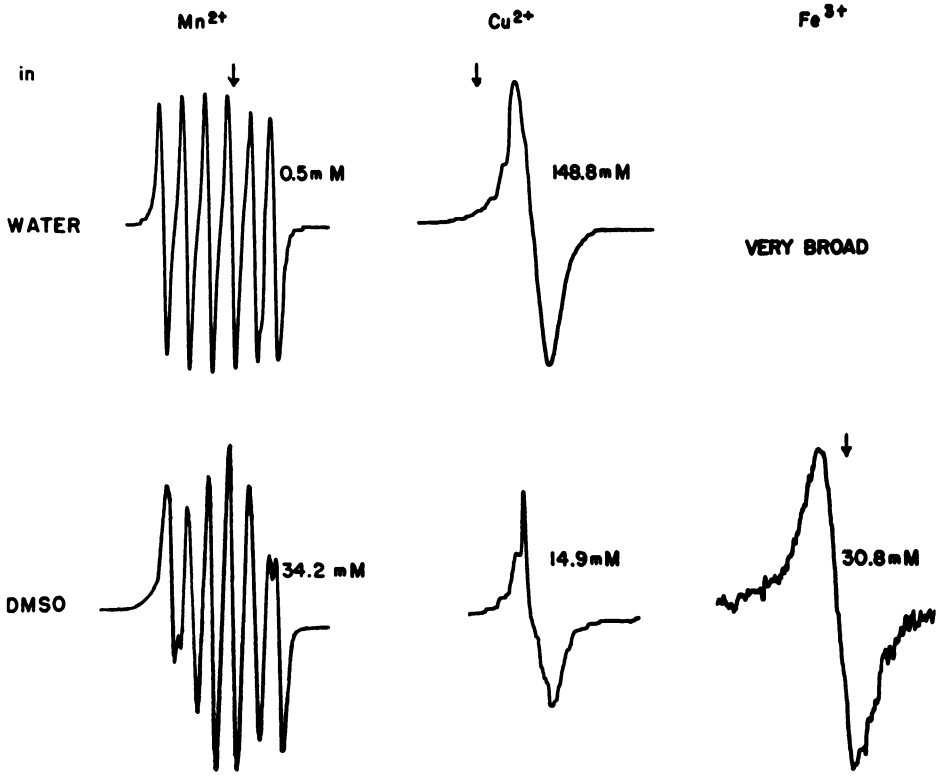

Fig. 3

(4) while other compounds acting by the hypoxia mechanism show no effect in *in vitro* systems. Thus, it seems that some other mechanism is responsible for its radioprotective ability. As was the case with glycerol, a complex formation between DMSO and the iron centers in the catalase molecule seems to protect the active site against radiation damage. Further evidence for this concept was obtained by ESR and optical absorption studies.

A change in the ESR spectra of various metal ions (Fe^{3+}, Cu^{2+}, and Mn^{2+}) was observed when DMSO was used as the solvent instead of water (see Fig 3). Considerable changes in the hf pattern of the manganous and cupric ions reflect

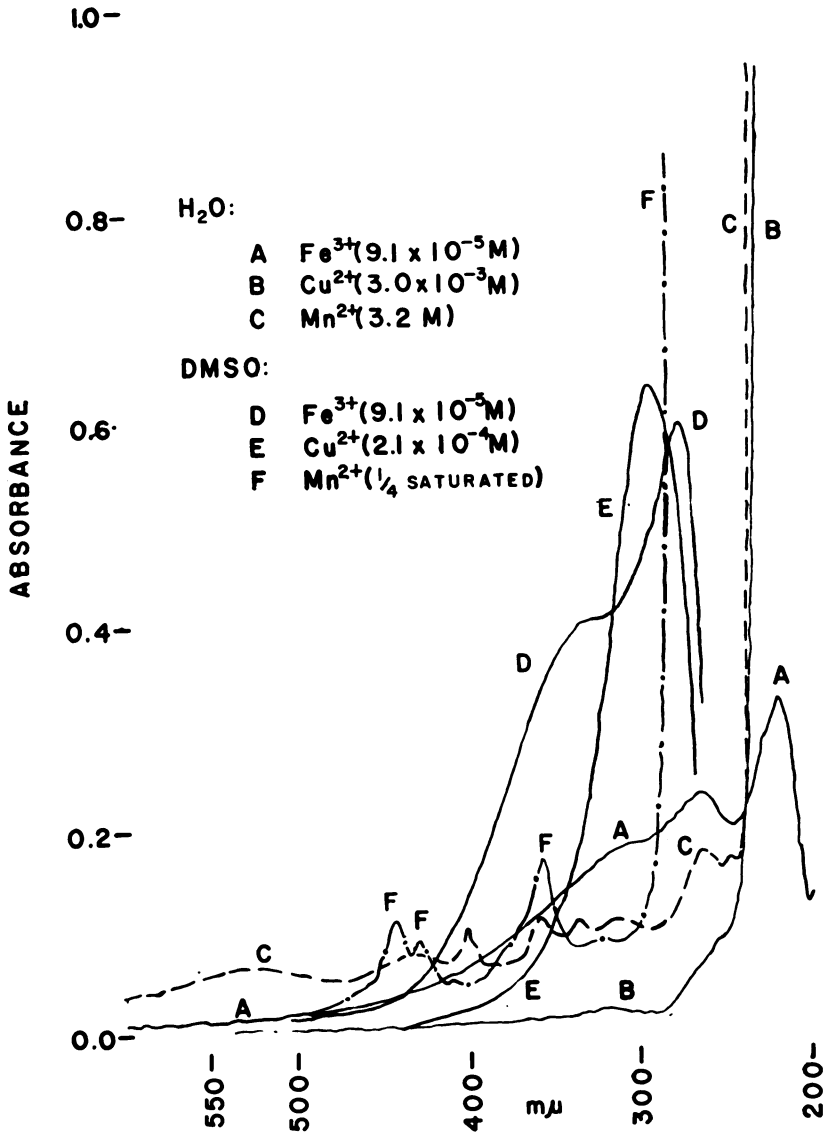

Fig. 4

the close solvent action of DMSO. With the ferric ion in DMSO, a narrow signal was observed near g = 2, while in water the signal was quite broad. The arrows indicate the position of the standard DPPH reference signal for which g = 2.0036. Optical absorption studies also revealed significant changes in the spectra of the various metal ions investigated, using both water and DMSO as solvents. "Red shifts" were observed when DMSO was used instead of water (see Fig. 4).

The changes in ESR line width and pattern as well as in the optical absorption maxima for various ions demonstrate the existence of a DMSO-metal ion complex interaction, differing from the hydrated species. The formation of a complex between DMSO and the active iron centers, present in the catalase macromolecule, appears to provide a basis for the protective effect.

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SUMMARY

The change in radiosensitivity of catalase by dimethyl sulfoxide was investigated spectrophotometrically. The enzyme-DMSO mixtures were irradiated with different doses of x-rays (2.25×10^6 R) at room temperature. The results obtained show that DMSO provides a good radioprotective effect. This protective effect might be explained by a complex formation between Fe^{3+}, the active site in catalase, and the protective compound. The existence of such a complex has been shown by electron spin resonance and optical absorption studies. Both the variation in line width (ESR spectra) and the optical "red shift" obtained suggest a change in crystal-field energy levels by spin-orbit mechanisms.

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