JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
J. Am. Chem. Soc., 1996, 118(45), 11014-11025, DOI:10.1021/ja962032l

Supplementary Material

Experimental

Materials and solutions

All chemicals used were of highest commercially available purity. Dimethyl sulfide (DMS) and diethyl sulfide (DES) (both from Aldrich) were extracted with water, pH 14 (adjusted with KOH), prior to destillation in order to remove traces of mercaptan impurities. Aldrich further supplied dimethylsulfoxide (DMSO), 4-carboxybenzophenone (CBP), ethylenediaminetetraacetate (EDTA), 3-trimethylsilylpropionic acid (TMSPA), formaldehyde (37% v/v in water), NaOD, and D_2O (99.8 % atom D). Sigma supplied sodium acetate, catalase (bovine liver; EC 1.11.1.6), superoxide dismutase (SOD; bovine erythrocyte; EC 1.15.1.1), xanthine, xanthine oxidase, and ferricytochrome C (bovine heart). Sodium phosphate (dibasic) and hydrogen peroxide were from Fisher Scientific, and $H_2{}^{18}O$ (95-98% atom O pure) was from Cambridge Isotope Laboratories.

Stock solutions of sodium phosphate in destilled deionized water (Millipore; 18 MΩ) were treated overnight with Chelex resin (Bio-Rad, 100-200 mesh). Deuterated phosphate buffer was prepared by dissolving sodium phosphate in D_2O, followed by lyophilization to dryness. After redissolving in D_2O, this solution was passed over a column containing Chelex resin which was pre-equilibrated with D_2O. The adjustments of pD were made with NaOD and H_3PO_4 where small amounts of protons introduced through H_3PO_4 are negligible compared to the concentration of D_2O. All glassware was washed with HCl and distilled deionized water prior to use in order to minimize contamination with iron.

Demetallated SOD, des-Cu-SOD (apo-SOD), was prepared according to the method of McCord and Fridovich[18], involving partial denaturation of SOD at pH 3.8 in the presence of EDTA, followed by refolding at neutral pH. The homogeneity of the protein preparations was confirmed by HPLC, employing a DEAE (TSK-Gel 5 PW WAX) column, eluted with a mobile phase containing 0.125 M potassium phosphate, pH 5.8, and 10% (v/v) acetonitrile. The activities of

SOD and apo-SOD were verified by their efficiency at inhibiting the xanthine/xanthine oxidase coupled reduction of ferricytochrome c[18]. The activity of catalase was tested by the ability to protect DMS against oxidation by 5.0×10^{-2} M H_2O_2.

Isotopically labelled DMS^{18}O was synthesized by oxidation of DMS by iodine[43] in alkaline $H_2{}^{18}O$ where the content of ^{18}O was ca. 94 atom % due to the use of $Na^{16}OH$ for the adjustment of the alkaline conditions. The incorporation of ^{18}O into DMSO was confirmed by GC-MS.

Reactions

Reactions were generally carried out in 0.5-2.0 ml volumes of solution in quartz cuvettes which were saturated with N_2, O_2, or air. In order to avoid loss of the volatile sulfides, these were gently saturated separately, before aliquots were added through septa into the quartz cuvettes. Samples were placed in a Rayonet Merry-Go-Round Photoreactor (Southern New England Ultraviolet Company) and irradiated for various times with four RPR-3500Å lamps, emitting light between 305 and 420 nm (λ_{max}=350 nm). The photoreactor output was determined as 1.48×10^{-5} photons/sec by ferrioxalate dosimetry[44]. Because of the broad spectrum of the incident light we prefer to report our steady-state photolytic product yields in units of Ms^{-1} rather than quantum yields based on the photon flux. This should avoid confusion with quantum yields obtained from laser experiments employing a defined wavelength of incident light. The yield of triplet 4-carboxybenzophenone was quantified through the loss of CBP and the formation of 4,4'-dicarboxybenzpinacol. Immediately after photolysis (i.e. at maximum 180 seconds after start of the photolysis), catalase was added to the samples at a final concentration corresponding to 2,000 U/ml in order to minimize formation of sulfoxide through oxidation of the sulfides by photolytically generated hydrogen peroxide.

Product characterization and quantification

The formation of sulfoxide was confirmed and quantified by reversed-phase HPLC, ^{1}H-NMR, and gas chromatography-mass spectrometry (GC-MS). Authentic standards were either obtained

commercially (DMSO) or, for diethylsulfoxide (DESO), by oxidation of the sulfide with excess H_2O_2. For ^{1}H-NMR experiments, the reactions were carried out entirely in D_2O. When applicable, either integrated peak areas or peak heights (with consistent band broadening) were used for the quantification of the components. The HPLC separations were achieved on a SGE 4.0 x 250 mm polymer coated C18 column, and the eluates monitored by UV detection at 214 nm: DMS and DMSO were separated isocratically with a mobile phase consisting of 3% (v/v) acetonitrile in water, containing 0.1% trifluoroacetic acid. For DES and DESO, the acetonitrile content was raised to 10%. After the elution of the peaks of interest, the column was washed with a mobile phase of high acetonitrile content in order to elute CBP and 4,4'-dicarboxybenzpinacol. The separation and quantification of CBP and 4,4'-dicarboxybenzpinacol involved the SGE polymer coated C18 column, eluted isocratically with a mobile phase consisting of 40% (v/v) acetonitrile in water, containing 0.1% trifluoroacetic acid. The eluate was monitored at 254 nm.

Formaldehyde was quantified by HPLC after derivatization with 2,4-dinitrophenylhydrazine, essentially as described[45], employing the SGE polymer coated C18 column eluted isocratically with 40% acetonitrile (v/v) in water, containing 0.1% trifluoroacetic acid.

For GC-MS experiments, any proteins (catalase and, when applicable, SOD) were removed from the reaction mixtures by centrifugation of the solutions in Microcon microconcentrators (Amicon) of 3 kD cutoff. Electron ionization (EI, 70 eV) spectra were acquired on a Nermag (Paris, France) R10-10 quadrupole GC-MS system equipped with a SPECTRAL 30 data system and a Girdel GC, modified with a J&W Scientific (Folsom, CA) split/splitless capillary injector. The DMSO molecular ion was recorded by selected ion monitoring of m/z 77-82 with 20 msec dwell time for each ion. The source temperature was 250°C and the emission current 200 mA. Chromatographic separations were carried out on a 30m DB1 (bonded dimethylpolysiloxane; J&W Scientific) capillary column. Helium was the carrier gas at 25cm/sec linear velocity, and splitless injections were made with 45 sec reduced flow delay into an injector maintained at 250°C. The capillary column passed through a 220°C heated zone and the effluent was introduced directly into the source. The GC oven program was held for 3 minutes at an initial temperature of 60°C,

followed by an increase to 250°C at 10°C/ min. The retention time of DMSO was t_R=4:14 min.

References

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