THE INTERACTION OF HYDROXYL RADICALS WITH DIMETHYLSULFOXIDE PRODUCES FORMALDEHYDE

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1. Introduction

Dimethylsulfoxide is a potent hydroxyl radical scavenging agent [1,2]. This compound reacts with hydroxyl radicals to produce methyl radicals ($^{\cdot}CH_3$) [3–5], which can give rise to methane gas by hydrogen abstraction. The production of methane from dimethylsulfoxide has been used to detect the generation of hydroxyl radicals by several biological systems [6–9]. However, investigations with dimethylsulfoxide, 3-thiomethylpropanal (methional) and 2-keto-4-thiomethylbutyric acid as hydroxyl radical scavengers have demonstrated that the production of methane from dimethylsulfoxide is at least one order of magnitude less than the generation of ethylene gas from either methional or from 2-keto-4-thiomethylbutyric acid under the same experimental conditions [7,8]. Therefore, the detection of methane gas in these systems is limited by the small amounts which are generated.

Here we show that formaldehyde is also produced when dimethylsulfoxide interacts with hydroxyl radicals. The oxidation of xanthine by xanthine oxidase was used as a model hydroxyl radical generating system. Hydroxyl radicals, which are generated during the oxidation of xanthine by xanthine oxidase, have been shown to be directly responsible for the production of ethylene from methional [10] and for the oxidation of ethanol to acetaldehyde [11]. The production of formaldehyde from dimethylsulfoxide may also provide a useful assay for detecting the presence of hydroxyl radicals and for evaluating the role of these radicals in biological reactions.

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2. Experimental

The standard reaction mixture used for the production of formaldehyde from dimethylsulfoxide consisted of 50 mM potassium phosphate buffer (pH 7.4), 0.4 mM xanthine, 0.1 mM tetrasodium EDTA, 0.018 units xanthine oxidase (0.45 units/mg protein, Boehringer Mannheim) and either 33 mM or 3.3 mM dimethylsulfoxide in 3.0 ml final vol. All incubations were carried out in triplicate in a Dubnoff metabolic shaking incubator at 37°C. The reactions were initiated by the addition of xanthine oxidase and were terminated by the addition of 1.0 ml ice-cold 17.5% (w/v) trichloroacetic acid. A 1.5 ml aliquot was then assayed for the presence of formaldehyde according to a fluorimetric modification [12] of the method in [13]. The amount of formaldehyde which was formed was measured with a Perkin-Elmer fluorescence spectrophotometer (model 650-10S) at an excitation wavelength of 415 nm and an emission wavelength of 505 nm. It was found necessary to construct a standard curve for each of the various incubation conditions in order to compensate for the effect of the various constituents on the fluorescence emission intensity.

3. Results

Fig.1 illustrates the time course of the production of formaldehyde from dimethylsulfoxide during the oxidation of xanthine by xanthine oxidase. After an

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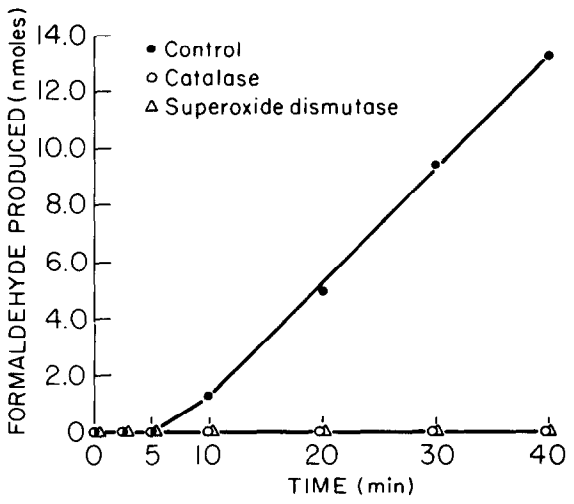

Fig.1. The production of formaldehyde from dimethylsulfoxide during the oxidation of xanthine by xanthine oxidase and the effects of superoxide dismutase and catalase. All incubations were done as in section 2. Superoxide dismutase and catalase were added at zero time to final concentrations of 58 μg/ml and 67 μg/ml, respectively. The results of a typical experiment are presented.

initial lag period of ~5 min, the rate of formaldehyde production was linear for at least an additional 35 min. The lag period is generally considered to represent the amount of time required for the accumulation of sufficient hydrogen peroxide to serve as a precursor of hydroxyl radicals [10]. In control experiments, formaldehyde was not produced when dimethylsulfoxide or xanthine or xanthine oxidase was omitted from the reaction mixture. Formaldehyde was not produced when hydrogen peroxide (0.4 mM) was added to the reaction mixture in the absence of either xanthine or xanthine oxidase. Therefore, formaldehyde production does not occur as a result of a direct reaction between dimethylsulfoxide and hydrogen peroxide.

Fig.1 also illustrates the effect of the addition of either 67 μg/ml catalase (65 000 units/mg protein, Boehringer Mannheim) or 58 μg/ml superoxide dismutase (1600 units/mg, Worthington) to the reaction mixture at zero time. The production of formaldehyde was completely inhibited by either enzyme. The addition of either enzyme 10 min after the initiation of the reaction, instead of at zero time, prevented further accumulation of formaldehyde after that time (data not shown). In contrast, bovine serum albumin (67 μg/ml) had no effect on the generation of formaldehyde.

Mannitol was used as a competing hydroxyl radical scavenging agent in the experiments described in table 1. Formaldehyde production was studied with either 3.3 mM or 33 mM dimethylsulfoxide. The control rates of formaldehyde production were similar at both concentrations of dimethylsulfoxide (table 1). Mannitol suppressed the production of formaldehyde from 3.3 mM dimethylsulfoxide in a dose-dependent manner (table 1). Urea, which is a weak scavenger of hydroxyl radicals (urea, $k = 7 \times 10^5$ M^{-1} s^{-1}; dimethylsulfoxide and mannitol, $k > 10^9$ M^{-1} s^{-1}) [1,2] had no effect on formaldehyde production

Table 1
The effect of mannitol on the production of formaldehyde from dimethylsulfoxide

Mannitol (mM)	3.3 mM Dimethylsulfoxide		33 mM Dimethylsulfoxide	
	Formaldehyde produced (nmol/30 min)	Effect of mannitol (%)	Formaldehyde produced (nmol/30 min)	Effect of mannitol (%)
0	10.88 ± 4.25	–	12.12 ± 3.64	–
10	8.12 ± 3.04	25	11.35 ± 3.97	6
25	5.98 ± 2.34	45[a]	11.03 ± 3.54	9
50	4.50 ± 2.74	59[b]	10.12 ± 2.75	17
100	2.45 ± 2.26	78[a]	8.38 ± 2.75	31

[a] $P < 0.05$; [b] $P < 0.005$

Incubations were done for 30 min as in section 2 with either 3.3 mM or 33 mM dimethylsulfoxide as indicated. Results are from 3 or 4 expt. Data represents the mean ± SD. Statistical analysis were performed by the paired Student's *t*-test

from 3.3 mM dimethylsulfoxide when added at 50 or 100 mM (not shown). Mannitol was a more effective inhibitor of formaldehyde production from 3.3 mM dimethylsulfoxide than it was from 33 mM dimethylsulfoxide (table 1). These results may be expected if both mannitol and dimethylsulfoxide are competing for hydroxyl radicals.

4. Discussion

It has been demonstrated that the production of ethylene from methional [10] and of acetaldehyde from ethanol [11] is dependent upon the interaction of these precursors with hydroxyl radicals that are produced secondarily during the oxidation of xanthine by xanthine oxidase. It is assumed that the generation of hydroxyl radicals by this system may occur as a result of an 'iron-catalyzed' Haber-Weiss type reaction between superoxide anions and hydrogen peroxide [14–16]. These data demonstrate that formaldehyde is produced from dimethylsulfoxide during the oxidation of xanthine by xanthine oxidase. Formaldehyde production appears to represent the interaction of dimethylsulfoxide with hydroxyl radicals generated by this system since the characteristics of formaldehyde production are similar in many respects of those of the production of ethylene from methional and of acetaldehyde from ethanol. These similarities include an initial time lag in the generation of product, inhibition by superoxide dismutase and by hydroxyl radical scavenging agents, sensitivity to catalase (for ethylene and formaldehyde production) and ineffectiveness of hydrogen peroxide in the absence of either xanthine or xanthine oxidase.

Preliminary evidence indicates that other systems which generate hydroxyl radicals also catalyze the production of formaldehyde from dimethylsulfoxide. For example, formaldehyde is produced from dimethylsulfoxide during NADPH-dependent electron transfer by rat liver microsomes and during iron-EDTA catalyzed oxidation of ascorbic acid (unpublished). Indeed, considerably greater amounts of formaldehyde than of methane are generated from dimethylsulfoxide by these two systems.

These data demonstrate that the production of formaldehyde from dimethylsulfoxide may represent a new, sensitive and convenient tool to detect the presence of hydroxyl radicals in some biological systems. There is much interest in the therapeutic properties of dimethylsulfoxide. The possibility that formaldehyde is a metabolite of the in vivo metabolism of dimethylsulfoxide remains to be evaluated. In view of the production of formaldehyde, dimethylsulfoxide should not be considered to be an inert and innocuous solvent in biological systems.

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