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Biochemical Interactions of Dimethyl Sulfoxide I: Respiratory Inhibition

ROBERT L. BEAMER▲, JAMES E. WYNN, and RAFAEL E. LEDESMA*

Abstract □ The effect of dimethyl sulfoxide upon rabbit liver homogenates was determined with the Warburg respirometer. Lineweaver-Burk treatment of the data indicated that the respiration was competitively inhibited by dimethyl sulfoxide.

Keyphrases □ Dimethyl sulfoxide—effect on rabbit liver homogenates, Warburg respirometer □ Respiratory inhibition—dimethyl sulfoxide, effect on rabbit liver homogenates, Warburg respirometer □ Warburg respirometer—effect of dimethyl sulfoxide on rabbit liver homogenates

During recent years there has been widespread interest in the clinical effects and applications of dimethyl sulfoxide. Toxicological studies have demonstrated a low acute toxicity, as indicated by higher LD_{50} values from intravenous, oral, and subcutaneous administration (1, 2).

Long-term administration of dimethyl sulfoxide produced changes in tissue morphology, such as reduced relucency of the lens cortex in dogs (3) and changes in hepatic cell morphology in rats (4). Teratogenic effects of dimethyl sulfoxide have been observed in various experimental animals (*e.g.*, chicks and golden hamsters) (5-7). However, few investigations have been reported concerning the effects of dimethyl sulfoxide on biochemical systems (8). This study was designed to provide preliminary data concerning the effects of dimethyl sulfoxide at the enzyme level.

EXPERIMENTAL

Materials—*Dimethyl Sulfoxide*—Experimental drug grade dimethyl sulfoxide[1] was redistilled under reduced pressure (100° at 2 mm.).

Serum Substrate—The substrate was prepared by diluting lyophilized bovine serum[2] to a specific gravity of 1.0210 with glass-redistilled water.

Apparatus—*The Warburg Instrument*—A Warburg respirometer[3], equipped with one-arm, 15-ml. reaction vessels with center wells, was used. The center wells contained 0.2 ml. of 15% KOH and a Whatman No. 1 filter paper wick. The amplitude of flask travel was 4 cm. at a rate of 60 c.p.m.; the water bath temperature was 37°. Warburg manometers were filled with Kreb's manometric fluid (9, 10), density 1.0334.

C-H-N Analyzer—A carbon-hydrogen-nitrogen analyzer[4] was utilized to determine the nitrogen content of animal tissue.

Preparation of Animal Tissues—Four albino rabbits, weighing approximately 1.4 kg. (3 lb.) each and in apparent good health, were quarantined for 2 weeks; they were fed rabbit pellets[5] and given water *ad libitum*. Each day they were examined for symptoms of disease.

At the end of the quarantine period, the animals were sacrificed by placing them in a closed container with dry ice. The livers immediately were removed, rinsed in cold Ringer's solution, weighed, inspected for gross abnormalities, and placed in cold (0–4°) 0.06 M sodium phosphate buffer at pH 7.4 (1 ml. buffer/g. wet tissue). The wet liver appeared normal and weighed 54.80–56.51 g. Small segments of each liver were dried at 100°, and their dry-to-wet ratios and nitrogen contents were determined. The mean value of the dry-to-wet weight ratio was 0.7735 with a standard deviation of 0.256; the mean nitrogen content was 10.62% with a standard deviation of 0.21.

The livers were homogenized individually in a cold Virtis homogenizer, pooled, and further homogenized in a test tube homogenizer[6]. The homogenates were placed in test tubes, sealed with sulfide-free rubber stoppers, and rinsed with glass double-distilled water. The homogenates were stored in a freezer.

Inhibition Studies—Inhibition studies were performed using the Warburg technique (11). The homogenates were thawed for approximately 60 min. at 6°. A constant liver homogenate volume containing 3.84×10^{-2} g. of nitrogen was used throughout. The flasks and their contents were incubated for 10 min. at 37° prior to initiating the reaction. The lyophilized serum substrate concentration for apparent maximal liver respiration (apparent V_{max}) (12) was found to be 1.7×10^{-2} g./ml.

Studies were then performed to determine the minimal concentration of dimethyl sulfoxide that would produce significant respiratory inhibition. The results were analyzed statistically and the kinetics of inhibition were evaluated by Lineweaver–Burk plots con-

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[1] Crown Zellerbach Corp.

[2] Nutritional Biochemicals Corp.

[3] Model 18U, Precision Scientific.

[4] Hewlett-Packard model 185.

[5] Purina.
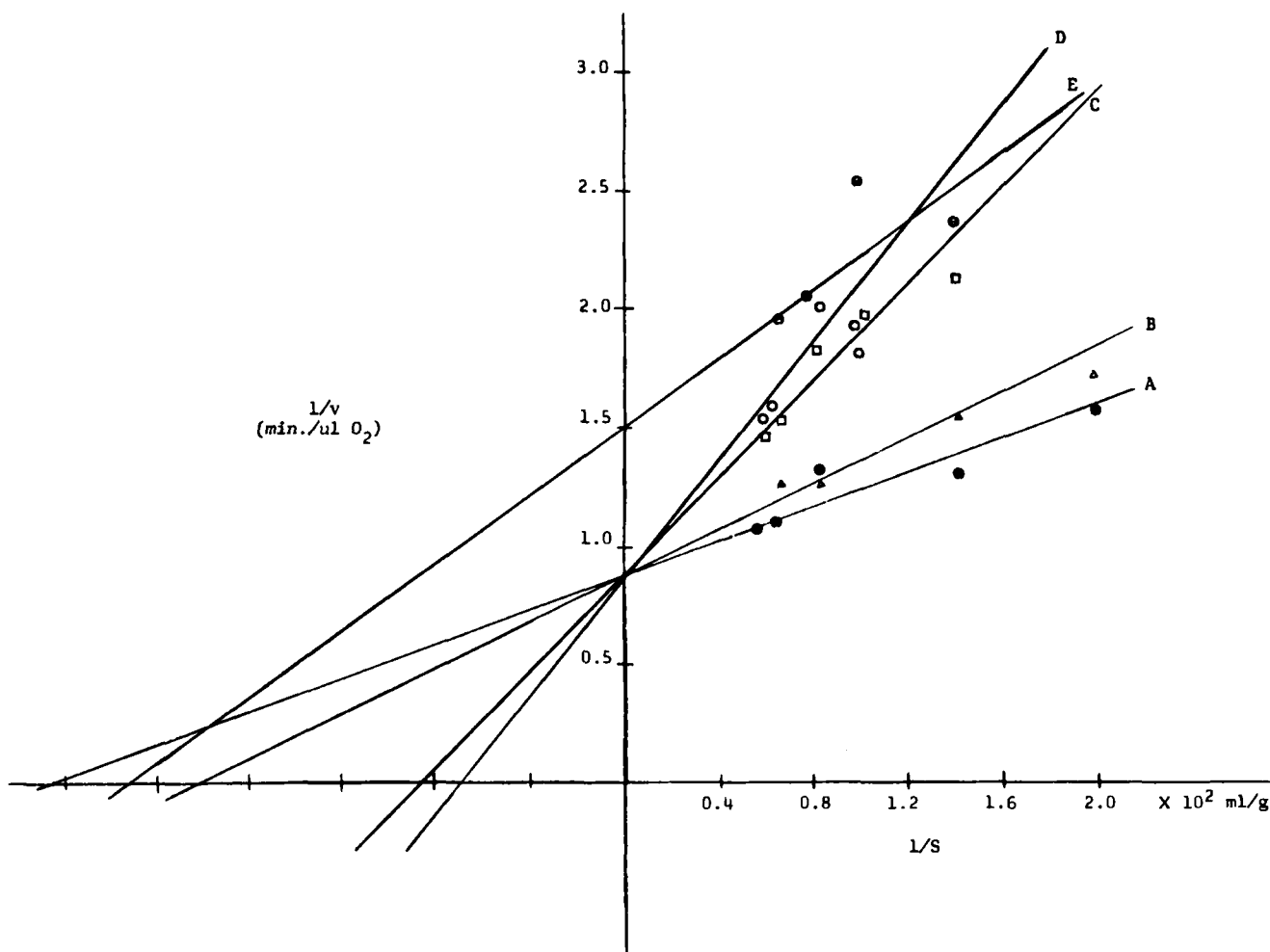
[6] Model 8335, Ace Glass Co.

Figure 1—*Standard Lineweaver-Burk plots of the inhibited versus noninhibited liver homogenate. Key: A, ●, control; B, △, 10^{-3} M dimethyl sulfoxide; C, □, 10^{-2} M dimethyl sulfoxide; D, ○, 10^{-1} M dimethyl sulfoxide; and E, ⊖, 10^{-3} M sodium cyanide.*

structed by the method of least squares (13). Values for the apparent Michaelis constant of the uninhibited reaction (apparent K_m)[7] and for the inhibited reactions (apparent K_m')[7] were obtained by extrapolation. Calculations of the apparent inhibitor constant (apparent K_i)[7] were made by utilizing the equation (14):

$$K_i = \frac{K_m\,(\mathrm{I})}{K_m' - K_m} \qquad \text{(Eq. 1)}$$

Comparative experiments were carried out using 1×10^{-3} M sodium cyanide as the inhibitor.

RESULTS

The maximal rate of the control reaction was obtained with a substrate concentration of 1.7×10^{-2} g./ml. of lyophilized serum. The rates of reaction were linear up to 40 min., at which time the rates decreased. This decrease in rate is typical of Warburg studies with animal tissues and can be attributed to either complete utilization of the substrate or product inhibition. This deviation from linearity was observed in all inhibited and control studies at approximately 40 min. Therefore, the kinetic studies are based on 40-min. oxygen uptake values.

A concentration of 10^{-3} M dimethyl sulfoxide was observed to be the minimal concentration producing a significant inhibition of the reaction. A concentration of 10^{-4} M dimethyl sulfoxide produced an insignificant decrease in the reaction rate.

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[7] Since the investigations were carried out using a whole homogenate, the Michaelis constants (K_m and K_m'), the inhibitor constants (K_i and K_i'), and the maximal velocity (V_{max}) should be considered apparent.

Lineweaver-Burk plots for the data in Table I (dimethyl sulfoxide- and sodium cyanide-inhibited reactions) are presented in Fig. 1. The plots of the dimethyl sulfoxide-inhibited reaction are of the competitive type, and the plot of the sodium cyanide reaction is of the noncompetitive type. Sodium cyanide proved to be a stronger inhibitor than dimethyl sulfoxide. The V_{max} (0.925 μl. O_2/min.) of the plots remained constant, while both the Michaelis constant (K_m) and the inhibitor constant (K_i) increased with increased inhibitor concentration.

DISCUSSION

The choice of lyophilized bovine serum as the substrate for the studies was made because of the presence of a large number of possible reactions for liver respiration. A single substrate approach would have been too limited for a preliminary investigation.

In considering the inhibition of respiration in a liver homogenate, one must remember that he or she is dealing with a multienzyme system. At this time the points of inhibition or the mechanism by which inhibition is accomplished cannot be identified.

The overall resultant inhibition of the system was competitive and could result from any one or a combination of mechanisms, i.e., competitive, partially competitive, and substrate trapping.

Although V_{max} was constant in the dimethyl sulfoxide-inhibited reactions, K_m' and K_i increased with increasing inhibitor concentration. Variations in K_m' are expected for competitively inhibited reactions, and these values should deviate from one another by a factor of $1 + [(\mathrm{I})/K_i]$ in purified enzyme systems (15).

In complex multiple-enzyme systems, this constant deviation in K_m' usually is not observed. The inability to determine an adequate value for the inhibitor constant in multienzyme systems is a primary

Table I—Kinetics of Dimethyl Sulfoxide Inhibition of the Respiration of Rabbit Liver Homogenates

Inhibitor Concentration, M	Substrate Concentration, g./ml.	Oxygen Consumption, μl. O_2/40 min.	Percent Inhibition[a]	Standard Deviation	Apparent V_{max}, μl. O_2/min.	Apparent K_m, g./ml.	Apparent K_i, moles
0.00 Dimethyl sulfoxide	0.5×10^{-1}	24.88	—	5.17	1.162	4.25×10^{-3}	—
	0.7×10^{-1}	30.33	—	4.60			
	1.5×10^{-1}	35.28	—	4.91			
	1.7×10^{-1}	36.18	—	6.01			
10^{-3} Dimethyl sulfoxide	0.5×10^{-1}	23.07	7.28	0.95	1.162	5.81×10^{-3}	2.72×10^{-3}
	0.7×10^{-1}	25.89	14.64	1.40			
	1.0×10^{-1}	25.17	—	0.88			
	1.2×10^{-1}	30.99	—	2.66			
	1.5×10^{-1}	30.85	12.56	1.73			
10^{-2} Dimethyl sulfoxide	0.5×10^{-1}	18.73	24.72	2.86	1.162	1.01×10^{-2}	7.39×10^{-2}
	0.7×10^{-1}	18.70	38.35	3.32			
	1.0×10^{-1}	22.42	—	1.21			
	1.2×10^{-1}	21.73	—	3.36			
	1.5×10^{-1}	24.99	29.17	1.88			
	1.7×10^{-1}	27.21	24.80	2.13			
10^{-1} Dimethyl sulfoxide	0.5×10^{-1}	17.75	28.66	3.50	1.162	1.39×10^{-2}	4.39×10^{-1}
	0.7×10^{-1}	18.83	37.92	3.00			
	1.0×10^{-1}	21.65	—	1.56			
	1.2×10^{-1}	19.83	—	4.22			
	1.5×10^{-1}	24.97	29.23	4.64			
	1.7×10^{-1}	32.89	9.10	4.25			
10^{-3} Sodium cyanide	0.5×10^{-1}	15.92	36.02	2.97	0.675	4.94×10^{-3}	—
	0.7×10^{-1}	16.81	44.58	1.50			
	1.0×10^{-1}	15.63	—	3.36			
	1.2×10^{-1}	19.13	—	2.41			
	1.5×10^{-1}	20.26	42.58	1.70			

[a] Percent inhibition is given for those substrate concentrations in the inhibited reactions for which there were corresponding substrate concentrations in the controls. Additional points were obtained in the inhibited reactions to provide more points for the Lineweaver-Burk plots.

reason for the lack of a constant deviation in K_m' (15). The fact that K_i is observed to increase with increasing inhibitor concentration is evidence that more than one enzyme is being inhibited and that the inhibitor has a greater affinity for certain enzymes than for others (15).

The inhibition by sodium cyanide was used as a control to test the reliability of this system to detect competitive *versus* noncompetitive-type inhibitions. That sodium cyanide is a noncompetitor of respiration is well known, and the fact that it is a stronger inhibitor of respiration than dimethyl sulfoxide is not surprising.

This study was preliminary, and future investigations are planned to identify both the points of inhibition (*i.e.*, the enzymes being inhibited) and the mechanism of inhibition.

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