PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 156, 88-91 (1977)

The Effects of Dimethylsulfoxide on the Enzymes of Isolated Brush Border of Rat Kidney (39881)

BETTY YEE-LI HSU,[1] PAMELA D. McNAMARA, AND STANTON SEGAL

Division of Biochemical Development and Molecular Diseases, Children's Hospital of Philadelphia, and the Departments of Pediatrics and Medicine, Medical School of the University of Pennsylvania, Philadelphia, Pennsylvania

The use of dimethylsulfoxide (DMSO) as a cryoprotective agent has been studied in depth (1). With regard to the drug's interaction with cell membranes, it has been clearly shown that it crosses animal cell membranes rapidly (1, 2) and, indeed, is able to enhance the movement of other drugs through cell membranes (3). In an evaluation of DMSO on a specific physiologic function of renal tubule cells, i.e., concentrative uptake of amino acids *in vitro*, Holtzapple *et al.* (4) found no deleterious effect. They also reported that DMSO enables rat renal cortex slices to be frozen at −30° and thawed without impairment of amino acid uptake. Although Abbot's studies (5) have shown no deleterious effects of DMSO on rabbit renal cortical slices as judged by enzymatic criteria, Pillion *et al.* (6) have reported an effect of DMSO on enzymes in perfused isolated rabbit kidney. After perfusing for 60 min at 37° with solutions containing DMSO, they found a marked increase in urinary γ-glutamyl transpeptidase, an enzyme normally associated with the brush border membrane.

We have examined the effects of *in vitro* incubation with DMSO on isolated rat renal brush border membranes with emphasis on changes in the localization of protein and enzyme activity as well as morphologic appearance determined by electron microscopy. The results of thus utilizing DMSO as a membrane probe form the basis of this report.

Materials and methods. Experiments were performed using 250- to 300-g male Sprague-Dawley rats (Charles River, Mass.) fed ad libitum on Purina rat chow. Rats were sacrificed by decapitation and the kidneys were removed, decapsulated, and placed in ice-cold saline. The microvillar fraction from the kidney cortex was isolated in ST buffer (0.25 *M* sucrose, 0.01 *M* triethanolamine hydrochloride, pH 7.6) by the modified method of Kinne and Kinne-Saffran (7, 8). The increase of specific activity of alkaline phosphatase (9) in the isolated brush border fraction over the homogenate was four- to fivefold in water suspensions. Electron-microscopic control of the brush border fraction indicated reproducibly good quality of microvillar preparations.

The membrane fraction to be treated was suspended in ST buffer. Protein concentration of the suspension, as determined by the method of Lowry *et al.* (10), was adjusted to the range of 3 to 3.5 mg/ml. For treatment with DMSO, constant ionic concentration was maintained by replacing solvent water with DMSO. No other substance could be found which would act as a control for changes in osmotic pressure of the incubation medium caused by the presence of DMSO. Replacement of 10, 15, and 20% of solvent water with DMSO resulted in final DMSO concentrations of 1.4, 2.1, and 2.8 *M*, respectively. All experiments were conducted at 37° for the incubation time designated. Control samples containing the same amount of membrane protein without DMSO were incubated at 37° along with treated samples. After incubation, the isolated brush border membrane fractions were centrifuged for 20 min at 35,000*g* at 4°. The protein and enzyme activities which did not sediment after centrifugation at 35,000*g* were called the solubilized

[1] Send reprint requests to Dr. Betty Yee-Li Hsu, Division of Biochemical Development and Molecular Diseases, Children's Hospital, Philadelphia, Pa. 19146.

ISSN 0037-9727

portion of the brush border fraction. The solubilized fractions and the sediments were dialyzed separately against Tris-NaCl buffer (0.15 *M* NaCl, 0.01 *M* Tris-HCl, pH 7.6) for 18 hr at 4°. Protein concentration and enzyme activities of each fraction were determined and expressed as percentage of total activity recovered.

Alkaline phosphatase (EC 3.1.3.1) determinations were performed according to a standard procedure (9). Aminopeptidase (EC 3.4.1.2.) was assayed according to the method of Goldmann *et al.* (11). Analyses of γ-glutamyl transpeptidase and γ-glutamyl hydrolase (EC 2.3.2.2) activities were performed according to the procedure of Glossmann and Neville (12). Maltase (EC 3.2.1.20) activity was determined according to the procedure of Dahlqvist (13).

Results and discussion. The effect of prolonged incubation and DMSO concentration on the solubilization of protein and enzyme activities from brush border membranes is shown in Table I. Incubation for 10 min in the presence of increasing concentrations of DMSO did not result in any significant increase in amount of membrane protein solubilized. Enzyme activities showed an increased solubility with increasing concentration of DMSO. Incubation of brush border membranes at 37° for 10–60 min resulted in a release of protein and enzyme activities into the 35000*g* supernatant (Table I). γ-Glutamyl transpeptidase was the only enzyme activity which was not solubilized to some extent after 10 min of incubation at 37°, but was after 30 min in the absence of DMSO. Other than this, no significant effect of prolonged incubation time on the solubilization of total protein or enzyme activity could be seen in the absence of DMSO. When added to the membrane incubations, DMSO caused a greater release of enzyme activities than of total protein for all time periods studied. After incubation

TABLE I. Effect of Prolonged Incubation on Percentage Recovery of Protein and Enzyme Activities from DMSO-Treated Membranes.[a]

DMSO concentration (*M*)	Protein or enzyme	Percentage of total in supernatant fraction		
		10 min	30 min	60 min
0	Protein	10.33 ± 0.51	10.27 ± 0.61	11.79 ± 0.69
	Alkaline phosphatase	3.19 ± 0.23	3.17 ± 0.10	3.24 ± 0.28
	Aminopeptidase	2.62 ± 0.25	2.77 ± 0.05	3.16 ± 0.27
	γ-Glutamyl transpeptidase	0.07 ± 0.04	2.99 ± 1.08	2.97 ± 1.29
	γ-Glutamyl hydrolase	1.93 ± 0.67	1.24 ± 0.66	1.38 ± 0.72
	Maltase	2.35 ± 0.12	2.37 ± 0.13	2.92 ± 0.23
1.4	Protein	10.72 ± 0.78	11.84 ± 0.79	14.31 ± 0.20
	Alkaline phosphatase	5.43 ± 0.51	6.53 ± 0.47	9.34 ± 0.85
	Aminopeptidase	5.66 ± 1.17	4.95 ± 1.06	7.80 ± 1.22
	γ-Glutamyl transpeptidase	2.25 ± 0.40	7.65 ± 0.72	8.95 ± 1.50
	γ-Glutamyl hydrolase	4.45 ± 0.56	1.86 ± 0.75	2.93 ± 1.30
	Maltase	4.31 ± 1.06	4.87 ± 1.05	5.77 ± 1.20
2.1	Protein	12.38 ± 1.34		
	Alkaline phosphatase	6.17 ± 0.51		
	Aminopeptidase	7.04 ± 0.95		
	γ-Glutamyl transpeptidase	5.14 ± 0.93		
	γ-Glutamyl hydrolase	7.10 ± 0.39		
	Maltase	5.06 ± 0.50		
2.8	Protein	13.59 ± 1.55		12.94 ± 0.02
	Alkaline phosphatase	7.63 ± 0.68		9.24 ± 0.16
	Aminopeptidase	8.43 ± 1.13		7.79 ± 0.07
	γ-Glutamyl transpeptidase	7.00 ± 0.83		7.89 ± 0.40
	γ-Glutamyl hydrolase	7.58 ± 1.01		7.26 ± 0.52
	Maltase	5.80 ± 0.60		7.10 ± 0.05

[a] The isolated brush border membranes were incubated at 37° for 10, 30, or 60 min, with or without 1.4, 2.1, or 2.8 *M* DMSO, after which the samples were centrifuged at 35,000*g* for 20 min at 4°. Protein concentration and enzyme activities were measured in pellets and supernatants after dialysis against 0.15 *M* NaCl in 0.01 *M* Tris, pH 7.6. Results are the means ± SE of four to six determinations and are expressed as the percentage of total appearing in the supernatant fraction.

of membranes with 1.4 *M* DMSO for 10, 30, or 60 min, γ-glutamyl transpeptidase activity in the soluble fraction increased dramatically (three- to fourfold) with time. Protein and alkaline phosphatase showed a slight but significantly increased solubility after 60 min of incubation with 1.4 *M* DMSO; γ-glutamyl hydrolase activity in the solubilized fraction was slightly decreased with time, although the decrease is not statistically significant after 60 min. After incubation with 2.8 *M* DMSO for 10 min, no further release of protein or enzymes from the brush border membranes with time was seen.

Table II shows the relative enzyme activities and solubilization factors in the solubilized fractions of brush border membranes incubated with and without DMSO. The relative enzyme activity for each enzyme is the ratio of the total enzyme activity in the DMSO-treated brush border fractions (sum of the 35,000*g* supernatant + pellet) to that of the same amount of untreated (incubated without DMSO) membrane. A value of 1.0 would indicate no inhibition or enhancement and 100% recovery of total enzyme activity. All values of relative enzyme activity are very close to 1.0, indicating that DMSO had no deleterious effect on the enzyme proteins themselves. The enzymic proteins of the microvilli appear to be extremely durable under conditions of incubation at 37° with or without DMSO.

The solubilization factor is the ratio of the percentage of enzyme activity solubilized to that of the protein solubilized. A ratio greater than 1.0 would indicate the preferential release of enzymic protein to total protein. The solubilization factors in Table II show that 21–62% of the total protein solubilized after 10 min at 37° with varying concentrations of DMSO shows

TABLE II. EFFECT OF INCUBATION CONDITIONS ON THE RELATIVE ENZYME ACTIVITY AND SOLUBILIZATION FACTOR OF MARKER ENZYMES FOR BRUSH BORDER MEMBRANES TREATED WITH DMSO.

Enzyme	DMSO concentration (*M*)	Time of incubation at 37°					
		10 min		30 min		60 min	
		R.E.A.[a]	S.F.[b]	R.E.A.	S.F.	R.E.A.	S.F.
Alkaline phosphatase							
	0	–	0.31	–	0.31	–	0.27
	1.4	1.22 ± 0.25	0.51	0.85 ± 0.06	0.55	0.94 ± 0.06	0.65
	2.1	1.23 ± 0.31	0.50				
	2.8	1.12 ± 0.28	0.56			1.09 ± 0.002	0.71
Aminopeptidase							
	0	–	0.25	–	0.27	–	0.27
	1.4	1.21 ± 0.11	0.53	0.96 ± 0.16	0.42	0.93 ± 0.17	0.55
	2.1	1.06 ± 0.02	0.57				
	2.8	1.00 ± 0.09	0.62			1.21 ± 0.001	0.60
γ-Glutamyl transpeptidase							
	0	–	0.01	–	0.29	–	0.25
	1.4	1.19 ± 0.06	0.21	1.05 ± 0.02	0.65	0.83 ± 0.07	0.63
	2.1	1.01 ± 0.07	0.42				
	2.8	0.99 ± 0.12	0.52			1.14 ± 0.01	0.61
γ-Glutamyl hydrolase							
	0	–	0.19	–	0.12	–	0.12
	1.4	1.10 ± 0.05	0.42	1.11 ± 0.01	0.16	0.91 ± 0.13	0.20
	2.1	1.10 ± 0.08	0.57				
	2.8	0.97 ± 0.09	0.56			1.33 ± 0.01	0.50
Maltase							
	0	–	0.23	–	0.23	–	0.25
	1.4	1.28 ± 0.21	0.40	0.94 ± 0.18	0.41	0.97 ± 0.15	0.40
	2.1	1.06 ± 0.08	0.41				
	2.8	1.04 ± 0.14	0.43			0.95 ± 0.001	0.55

[a] Relative enzyme activity (R.E.A.) is calculated as the ratio of enzyme activity in the DMSO-treated brush border fractions (sum of 35,000*g* supernatant + pellet) to that in the same amount of untreated preparation. The results given are the means from three experiments with duplicate assays in each experiment ± the standard error.

[b] Solubilization factor (S.F.) is the precentage in Table I of enzyme activity solubilized divided by that of the protein solubilized. A ratio above 1.0 indicates preferential release of enzyme relative to protein and vice versa.

enzyme activities. This suggests the preferential extraction of nonenzymatic protein from the brush border membranes by treatment at 37° with or without DMSO. The data suggest that DMSO itself causes a greater release of enzyme activities than total protein, since most of the protein solubilization occurred during incubation in the absence of DMSO. For γ-glutamyl transpeptidase, treatment with 1.4 *M* DMSO for 10 min increases the percentage of protein with enzyme activity solubilized from 0 to 21%. The increasing solubility of γ-glutamyl transpeptidase and decreasing solubility of γ-glutamyl hydrolase in 1.4 *M* DMSO with time are emphasized by their respectively increasing and decreasing solubilization factors and the constancy of their relative enzyme activities. The decreasing solubilization factor of γ-glutamyl hydrolase with prolonged incubation time is not a result of enzyme inhibition or denaturation since the R.E.A. is not decreased. No explanation for this phenomenon has been found. In addition, the ratio of total enzyme activity in the 30- or 60-min samples to the total activity in the 10-min samples is very close to 1.0 in all cases.

Monitoring of preparations and treatments of brush border membranes with DMSO by negative-contrast electron microscopy showed no obvious morphological changes resulting from DMSO treatment.

Our findings indicate that DMSO when incubated at 37° with isolated rat renal brush border membrane causes little solubilization of the nonenzymatic protein components (0.4–3.3%) and a correspondingly slight release (2–9%) of intrinsic enzyme activities. The facts that amino acid uptake by rat cortical slices incubated with DMSO is unaltered (4) and that DMSO administered chronically to rats does not impair renal cortical transport mechanisms (14) provide evidence of functional integrity in the presence of DMSO and lend support to our observations. Pillion *et al.* (6), using isolated rabbit kidney perfused with and without DMSO, have shown an obvious increase in urinary γ-glutamyl transpeptidase levels throughout the course of a 60-min perfusion period in the absence of DMSO and a further increase in urinary enzyme upon perfusion with DMSO. Our data also show an effect of the drug superimposed upon that of the prolonged incubation of isolated brush border membranes.

Summary. Data from biochemical analyses indicate that isolated rat kidney brush border microvilli tolerate treatment with DMSO. Total membrane protein as well as the marker enzymes alkaline phosphatase, aminopeptidase, γ-glutamyl transpeptidase, γ-glutamyl hydrolase, and maltase are solubilized only slightly by DMSO treatment.

The authors wish to thank Dr. Harvey Schlesinger for electron-microscopic examination and Mrs. Louise Pepe for her technical assistance. Supported by Grant No. AM 10894 and Training Grant No. 00215 from the National Institutes of Health, Bethesda, Maryland.

1. Jacobs, S. W., Rosenbaum, E. E., and Wood, D. C. (eds.), "Dimethyl Sulfoxide," Vol. 1. Marcel Dekker, New York (1971).
2. Kolb, K. H., Jaenicke, G., Kramer, M., and Schulze, P. E., Ann. N.Y. Acad. Sci. **141,** 85 (1967).
3. Brobyn, R. D., Ann. N.Y. Acad. Sci. **243,** 497 (1975).
4. Holtzapple, P. G., Buchanan, W., and Segal, S., Biochem. Pharmacol. **21,** 833 (1972).
5. Abbot, W. M., Cryobiology **5,** 300 (1969).
6. Pillion, D. J., Jeske, A. H., and Leibach, F. H., Biochem. Pharmacol. **25,** 913 (1976).
7. Kinne, R., and Kinne-Saffran, E., Pflügers Arch. **308,** 1 (1969).
8. Pockrand-Hemstedt, H., Schmitz, J. E., Kinne-Saffran, E., and Kinne, R., Pflügers Arch. **333,** 297 (1972).
9. Sigma Technical Bulletin No. 104.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
11. Goldmann, D. R., Schlesinger, H., and Segal, S., Biochim. Biophys. Acta **419,** 251 (1976).
12. Glossmann, H., and Neville, D. M., Jr., FEBS Lett. **19,** 340 (1972).
13. Dahlqvist, A. *in* "Methods in Enzymology" (E. F. Neufeld, V. Ginsburg, S. P. Colowick, and N. O. Kaplan, eds.), Vol. 8, p. 584. Academic Press, New York (1966).
14. Small, A., and Ide, R. S., Cryobiology **13,** 328 (1976).

Received March 21, 1977. P.S.E.B.M. 1977, Vol. 156.