

Uncoupling by Sodium Alkyl Sulfates of Energy-Linked Functions of Rat Liver Mitochondria (36706)

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Lees (1) and Lees and Loncharich (2) reported that sodium dodecyl sulfate may uncouple phosphorylation from mitochondrial respiration since the P:O ratio for succinate was depressed by the detergent. We have extended Lees' work by examining the effects of four sodium alkyl sulfates (containing 8, 10, 12 and 14 carbon atoms) upon respiratory control in rat liver mitochondria supplied with L-glutamate or succinate as substrate. In addition, the influences of the alkyl sulfates on high amplitude volume changes of the organelles were investigated. The relative effectiveness of this series of compounds was a function of their lipophilicity.

Materials and Methods. Liver mitochondria, obtained from 150 to 200 g male Sprague-Dawley rats (Holtzman Company) fed Purina rat chow and water *ad libitum*, were prepared following a modified method of Hogeboom, Schneider and Pallade (3). The 0.25 M sucrose solution contained 0.5 mM EDTA. The liver was homogenized in a Teflon and glass Duall tissue grinder and centrifuged for 10 min at 700g. The precipitate was washed once and the combined supernatates were centrifuged for 10 min at 8500g. After two washings the mitochondrial pellet was resuspended in the sucrose-EDTA solution and used immediately. All preparative operations were at 0 to 4°.

Sodium octyl, decyl, dodecyl, and tetradecyl sulfates were prepared as described previously (4). Other reagents, ATP, ADP, L-glu-

tamate, sodium succinate and bovine serum albumin were purchased from Sigma Chemical Co. Sodium deoxycholate, tris(hydroxymethyl)methylamine(Tris), ethylenediaminetetraacetic acid (EDTA), and sucrose were from Fisher Scientific Co. 2, 4-Dinitrophenol (DNP) and mannitol were from Eastman Organic Chemicals and Pfaltz Laboratories, respectively.

Mitochondrial respiratory control (control of respiration by phosphate acceptor) (5) was assayed polarographically as described previously (6) and high amplitude swelling and energized contraction of the organelles were followed as absorbance changes at 520 nm (7). Protein was estimated in the presence of 0.8% deoxycholate by a biuret method (8).

Results and Discussion. Evaluations of respiratory control and high amplitude volume changes of rat liver mitochondria in the presence of four sodium alkyl detergents (8, 10, 12 and 14 carbon atoms) were conducted both in the presence and absence of bovine serum albumin. Albumin has been found to enhance the contraction of swollen mitochondria by ATP (9) and to bind sodium dodecyl sulfate (10).

The influence of sodium dodecyl sulfate on the mitochondrial properties of respiratory control and chemo-mechanical functions are summarized in Figs. 1 and 2. Respiratory control was depressed by sodium dodecyl sulfate during both succinate and glutamate oxidation. This indicated that the detergent uncoupled oxidative phosphorylation in these preparations. The concentration of sodium dodecyl sulfate required for 40% depression of respiratory control (I_{40}) was the same for both substrates. Although omission of bovine serum albumin depressed considerably res-

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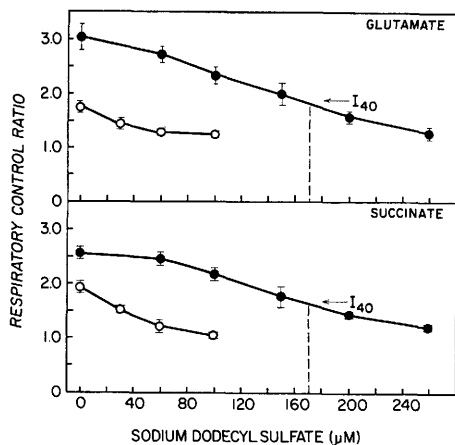


FIG. 1. Effects of sodium dodecyl sulfate on mitochondrial respiratory control. Oxygen consumption (n atoms of oxygen/min/mg of mitochondrial protein) was monitored by a Clark fixed voltage electrode. Glutamate and succinate concentrations were 1.4 mM. The absence and presence of bovine serum albumin (3 mg/ml) in the mitochondrial mixture is designated by (○) and (●), respectively. The average from six different mitochondrial preparations is presented with its standard error of the mean in brackets, as a single data point for a given concentration of sodium dodecyl sulfate. I_{40} refers to the concentration of detergent that diminished respiratory control by 40%. Note that I_{40} values were similar whether L-glutamate or succinate was substrate. Additional experimental details are given in the text.

piratory control ratios, increasing concentrations of sodium dodecyl sulfate further diminished these functional activities (Fig. 1).

Sodium dodecyl sulfate was not an inhibitor of phosphorylating oxidation since 33 μ M dinitrophenol (not shown) did not elevate the depressed ADP induced respiration (State 3) produced by 0.25 mM detergent alone. Only if the apparent State 3 respiratory rate were increased by 2,4-dinitrophenol could sodium dodecyl sulfate be considered an inhibitor of phosphorylating oxidation (11).

Sodium dodecyl sulfate, in addition to uncoupling oxidative phosphorylation, altered the structural properties of mitochondria. An increase in active swelling of mitochondria was observed with increasing concentrations of the detergent in the range 0.05 – 0.25 mM in either the presence or absence of

albumin (not shown). The ability of detergent-treated mitochondria to recover from swelling (via energized contraction) was expressed as a function of the concentration of sodium dodecyl sulfate used (Fig. 2). The concentration of sodium dodecyl sulfate at which mitochondrial swelling was 60% reversible (or 40% inhibited) was designated R_{40} . Omission of bovine serum albumin from the mitochondrial preparation enhanced the sensitivity of the preparation to swelling by detergent and decreased the ability to contract. This was shown by varying the concentration of sodium dodecyl sulfate and measuring the recovery of original chemico-mechanical properties of the mitochondria, Fig. 2.

Uncoupling of oxidative phosphorylation and inhibition of mitochondrial recovery from detergent swelling by lipophilic anionic detergents. The effects of sodium octyl, de-

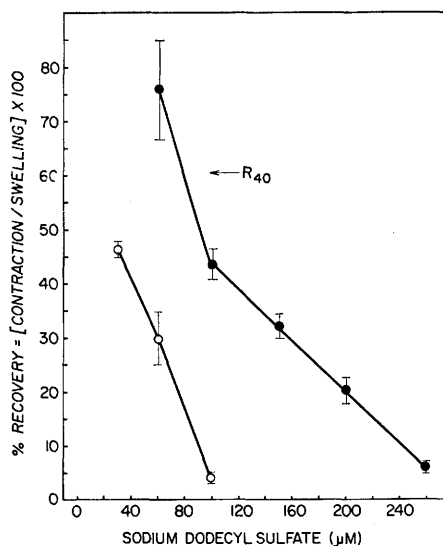


FIG. 2. Mitochondrial recovery as a function of sodium dodecyl sulfate concentration used for swelling. Experimental conditions are given in the text. Results are presented for mitochondria in the absence (○) and presence (●) of bovine serum albumin (1.8 mg/ml). Percentage recovery is expressed as the inverse ratio of absorbance changes for swelling to those for contraction. The concentration of detergent that diminished the reversibility of swelling by 40% was designated R_{40} . The average from four experiments is presented, with its standard error of the mean, as a single data point for a specific concentration of sodium dodecyl sulfate.

TABLE I. Depression of Mitochondrial Respiratory Control (pI_{40}) and Inhibition of Energized Mitochondrial Recovery from Detergent Swelling (pR_{40}) by Four Lipophilic Sodium Alkyl Sulfates.^a

	log P	pI_{40}		pI_{40} calc/ pI_{40} obs ^b
		Obs	Calc	
Sodium octyl sulfate	-0.40	2.46 (3.5)	2.46	1.000
Sodium decyl sulfate	0.60	3.30 (0.5)	3.30	1.000
Sodium dodecyl sulfate	1.60	3.77 (0.18)	3.76	0.997
Sodium tetradecyl sulfate	2.60	3.85 (0.14)	3.84	1.003
pR_{40}				
		Obs	Calc	pR_{40} calc/ pR_{40} obs ^b
Sodium octyl sulfate	-0.40	3.40 (0.40)	3.32	0.976
Sodium decyl sulfate	0.60	3.70 (0.20)	3.76	1.016
Sodium dodecyl sulfate	1.60	4.10 (0.08)	4.20	1.024
Sodium tetradecyl sulfate	2.60	4.72 (0.019)	4.64	0.983

^a The effects of four anionic detergents on two general mitochondrial properties are summarized. Negative logarithmic values of I_{40} and R_{40} , (pI_{40} and pR_{40} , respectively) were obtained from data plots similar to those of Figs. 1 and 2. The mitochondrial preparations examined contained added albumin. Experimental details are given in the text. Log P values [an index of lipophilicity (12)] were obtained from Ref. (13). The partition coefficient, an "equilibrium" constant, was the ratio of concentration of detergent in the nonpolar phase (*n*-octanol) to that in the polar phase (water). Calculated values of pI_{40} and pR_{40} were obtained from Eqs. 2 and 3, respectively. Values in parentheses represent mM concentrations.

^b Ratio expresses the correlation between calculated and observed values; 1.000 is a perfect correlation.

cyl, dodecyl, and tetradecyl sulfates on the respiratory and physico-chemical properties of rat liver mitochondria are presented together with partition coefficient logarithms of the detergents in Table I. Differences in the I_{40} and R_{40} values only reflect different quantities of mitochondria that were used in the different experiments and thus these should not be taken as an indication that swelling of mitochondria occurred before loss in respiratory control. The extent of inhibitor effectiveness was correlated in part with lipophilicity or hydrophobic bonding (12) preference of the detergent by multiple linear least-squares regression equations. The equations were

$$pI_{40} = 2.83 + 0.46 \log P$$

$$(r = 0.939; SE = 0.27), \quad (1)$$

$$pI_{40} = 2.84 + 0.88 \log P - 0.19 (\log P)^2$$

$$(r = 0.999; SE = 0.003), \quad (2)$$

$$pR_{40} = 3.5 + 0.44 \log P$$

$$(r = .986; SE = 0.11), \quad (3)$$

where quantities in parentheses represented statistics for the equation: r , the correlation coefficient for the fit of experimental values, pI_{40} or pR_{40} to the equation; and SE, the standard error of the estimate (pI_{40} or pR_{40}) provided by the equation. A value of 1.00 for r would indicate perfect correlation. Comparison of the statistical values indicated that Eq. (2) described the correlation of log P with pI_{40} better than did Eq. (1). On the other hand pR_{40} was satisfactorily correlated with a linear function of log P . In either case, the lipophilicity of a detergent was sufficient to account for the biological properties determined, *i.e.*, depression of respiratory control, pI_{40} , and irreversible swelling of the mitochondria, pR_{40} . A linear correlation between log P and pR_{40} indicated (12) that hydrophobic bonding was important in the interaction of the detergent for swelling and subsequent contraction and that this interaction probably occurred at the first (12) lipid-like binding site encountered by the deter-

gent (perhaps the mitochondrial outer membrane). The observation that the properties of the detergents as inhibitors of respiratory control [Eq. (2)] required a parabolic expression (13) with $\log P$ indicated that the detergent must penetrate more than one membrane to exert its action (perhaps passage through the outer membrane of the organelle to combine with the inner membrane). Mechanistically, the outer membrane undergoes distortion and even rupture as an extreme consequence during high amplitude swelling, whereas agents affecting oxidative phosphorylation and respiratory control must penetrate to inner membrane sites in order to institute their action.

A hypothetical optimum value of $\log P$ for detergent inhibition of respiratory control was calculated (13) by taking the first derivative of Eq. (2) [$d(pI_{40})/d \log P$ was zero]. A value of 2.3 was obtained for $\log P^0$. This value was similar to that obtained in other studies involving cellular membranes, e.g., dove erythrocyte 2.4 (13) and reinforced the conclusion for lipophilic interaction of these detergents in their uncoupling activity with rat liver mitochondria.

Summary. Sodium octyl, decyl, dodecyl, and tetradecyl sulfates were tested for uncoupling activity against energy-linked functions of rat liver mitochondria. All four detergents diminished respiratory control with either succinate or L-glutamate as substrate. The alkyl sulfates augmented mitochondrial swelling and, at high concentrations, the detergent depressed energized (ATP) contraction. The latter incompetency may be partially due to lysis of the organelle by the detergent.

The potency of the detergents for both

depression of respiratory control and inhibition of energized recovery from swelling by 40% [$\log(1/I_{40})$ and $\log(1/R_{40})$, respectively] was a function of the number of methylene groups in the alkyl sulfate. This potency was equated with the lipophilicity of the molecules as measured by their partitioning across the interfacial barrier of *n*-octanol and water. Uncoupling and inhibition of recovery from swelling required lesser amounts of a molecule that was lipophilic than one that was hydrophilic.

1. Lees, H., *Biochim. Biophys. Acta* **105**, 187 (1965).
2. Lees, H., and Loncharich, K., *Biochim. Biophys. Acta* **113**, 181 (1966).
3. Hogeboom, G. H., Schneider, W. C., and Palade, G. E., *J. Biol. Chem.* **172**, 619 (1948).
4. Rogers, K. S., and Yusko, S. C., *J. Biol. Chem.* **247**, 3671 (1972).
5. Chance, B., and Williams, G. R., *Advan. Enzymol.* **17**, 65 (1956).
6. Banks, W. L., Jr., Kline, E. S., and Higgins, E. S., *J. Nutr.* **100**, 581 (1970).
7. Higgins, E. S., and Banks, W. L., Jr., *Biochem. Pharmacol.* **20**, 1513 (1971).
8. Gornall, A. G., Bardawill, C. J., and David, M. M., *J. Biol. Chem.* **117**, 751 (1949).
9. Lehninger, A. L., "The Mitochondrion", pp. 135, 188. Benjamin, New York (1964).
10. Steinhardt, J., and Reynolds, J. A., "Multiple Equilibria in Proteins", p. 244. Academic Press, New York (1969).
11. Huijung, F., and Slater, E. C., *J. Biochem. (Tokyo)* **49**, 493 (1961).
12. Hansch, C., *Accounts Chem. Res.* **2**, 232 (1969).
13. Hansch, C., and Glave, W. R., *Mol. Pharmacol.* **7**, 337 (1971).

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