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REVERSIBLE EFFECT OF SODIUM DODECYL SULFATE ON HUMAN ERYTHROCYTE MEMBRANE ADENOSINE TRIPHOSPHATASE

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SUMMARY

A study was made on the effect of sodium dodecyl sulfate on human erythrocyte membrane ATPase. The ATPase was activated by low concentrations of sodium dodecyl sulfate and was inhibited by the detergent above 0.4 mM. At optimum concentration of sodium dodecyl sulfate the membrane ATPase was stimulated about two-fold by either Na^+ or K^+ alone. This stimulation was not affected by ouabain. The effect of sodium dodecyl sulfate was abolished by a preincubation at 50° for 5 min.

Tracer studies indicated that within the range tested less than 1% of sodium dodecyl sulfate in the medium was bound to the membrane fragments. Heating at 50° did not dissociate the bound sodium dodecyl sulfate. The evidence indicated that the reversible alteration of membrane ATPase characteristics by sodium dodecyl sulfate was not simply due to the binding and unbinding of the detergent molecules to the enzyme system.

INTRODUCTION

One of the essential features of human erythrocyte membrane ATPase is the effect of alkali metal ions on the rate of hydrolysis. The ATPase is stimulated by the presence of both Na^+ and K^+, and this stimulation can be abolished by low concentration of ouabain[1-3]. Neither of these cations alone has detectable effect on the ATPase under normal conditions. However ASKARI AND FRATANTONI[4] observed a 20–30% stimulation by either Na^+ or K^+ alone when the membrane preparation was treated with sonic oscillation. They suggested that the mechanical rupturing of the membrane enzyme complex had in some way uncoupled the sites of activation of the $(Na^+ + K^+)$-activated ATPase (ref. 5).

Similar alteration of characteristics in membrane ATPase by low concentrations of sodium dodecyl sulfate was observed in this study. In the presence of this detergent the ATPase could be stimulated about two-fold by either Na^+ or K^+. However, the effect of sodium dodecyl sulfate was abolished by preincubation at 50°.

Preliminary results have been reported elsewhere[6].

METHODS AND MATERIALS

Human erythrocyte membrane fragments were prepared according to the procedure of DODGE, MITCHELL AND HANAHAN[7] with some modifications described earlier[8].

Protein concentration of the membrane preparation was determined according to the method of LOWRY *et al.*[9].

The conditions for ATPase assay are described in Table I. ATP in the reaction mixture was always in excess and was not a rate-limiting factor.

Sodium dodecyl sulfate labeled with ^{35}S was purchased from the Radiochemical Center, Amersham, England. It was recrystallized from absolute ethanol without loss of specific activity.

Alkali metal chlorides, reagent grade, were obtained from Baker Chemical Co. Potassium chloride (99.9% pure) contained 0.005% sodium, and sodium chloride (99.6% pure) contained 0.0004% potassium.

RESULTS AND DISCUSSION

The alkali metal ion effect on erythrocyte membrane ATPase in the absence of sodium dodecyl sulfate shown in Table I was similar to earlier reports by POST *et al.*[1], DUNHAM AND GLYNN[2], and others. The ATPase was not stimulated by either Na^+ or K^+ alone; but in the presence of both of these cations the activity in this preparation was stimulated over two-fold, and this stimulation was abolished by the addition of

TABLE I

EFFECT OF SODIUM DODECYL SULFATE

The incubation mixture in a final volume of 2.0 ml contained 2.5 mM $MgCl_2$, 1.25 mM ATP (as Tris salt), 5 mM cysteine, 50 mM imidazole–HCl (pH 7.5), additions as indicated, and membrane fragments with a final concentration equivalent of 0.25 mg of protein per ml. Unless specified otherwise all components except ATP were preincubated at 37° for 5 min and then 0.05 ml of 0.05 M ATP was added to initiate the reaction. The incubation was for 30 min at 37°, and the P_i released was assayed as described earlier[8].

Additions	*ATPase (μmoles P_i per mg protein per h)*	
	Without sodium dodecyl sulfate	*+0.2 mM sodium dodecyl sulfate*
None	0.27	0.53
0.15 mM ouabain	0.29	0.52
125 mM NaCl	0.26	0.85
125 mM NaCl + 0.15 mM ouabain	0.24	0.91
125 mM KCl	0.21	0.97
125 mM KCl + 0.15 mM ouabain	0.22	1.03
100 mM NaCl + 25 mM KCl	0.74	1.54
100 mM NaCl + 25 mM KCl + 0.15 mM ouabain	0.25	1.12

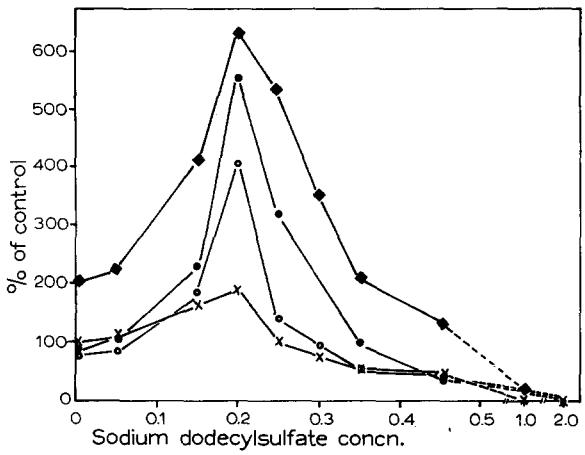

Fig. 1. Effect of sodium dodecyl sulfate on erythrocyte membrane ATPase. The conditions for ATPase assay are described in Table I. ×—×, no addition (the first point without sodium dodecyl sulfate is arbitrarily chosen as 100% of control); ○—○, *plus* 100 mM NaCl; ◆—◆, *plus* 100 mM NaCl and 25 mM KCl; ●—●, *plus* 100 mM NaCl, 25 mM KCl, and 0.15 mM ouabain. Sodium dodecyl sulfate concn. in mM.

0.15 mM ouabain. The extent of activation varied somewhat from preparation to preparation. Either in the absence of added Na^+ and K^+ or in the presence of both Na^+ and K^+, the range of stimulation by 0.2 mM sodium dodecyl sulfate was 180 to 250%. In the presence of either Na^+ or K^+ at optimum concentration the range of stimulation by 0.2 mM sodium dodecyl sulfate was 350–500%.

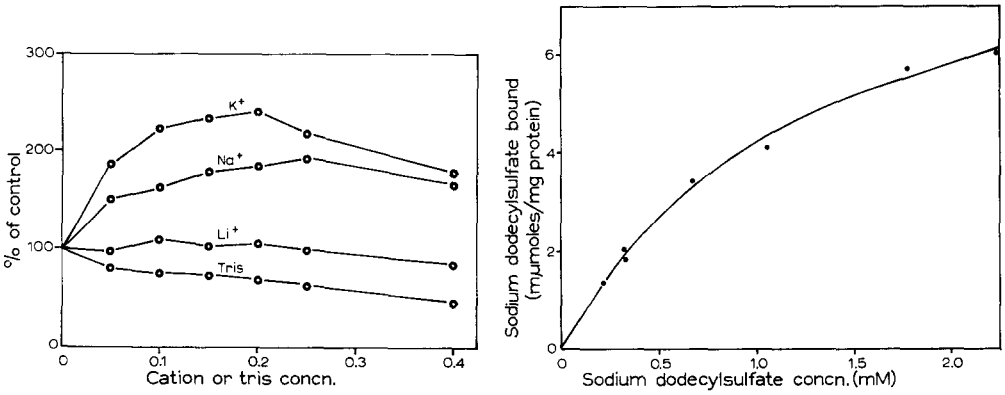

Fig. 2. Monovalent cation effect on membrane ATPase in the presence of 0.2 mM sodium dodecyl sulfate. The conditions for ATPase assay are described in Table I. K^+, Na^+ and Li^+ were added as chloride salts. Tris was neutralized with HCl to pH 7.5. Cation or Tris concn. in M.

Fig. 3. Relationship between the amount of sodium dodecyl sulfate bound and its concentration in the medium. A total volume of 6.0 ml containing membrane fragments equivalent to 5–6 mg of protein, 0.05 M imidazole–HCl (pH 7.5) and varying amount of ^{35}S-labelled sodium dodecyl sulfate, was incubated at 37° for 5 min and then put through a Sephadex G-100 column (120 ml) in the same buffer. The eluate was collected in 4.9-ml fractions at a rate of about 25 min per fraction. The absorbance at 280 mμ and the radioactivity of each fraction were measured. Over 80% of the 280-mμ absorbing materials came through in the first void column volume in 3 or 4 tubes (see typical runs in Fig. 4). The amount of sodium dodecyl sulfate bound per mg protein was calculated from the radioactivity and the protein concentration in the combined fraction of this peak.

Under all the conditions tested the rate of ATP hydrolysis was activated by 0.2 mM sodium dodecyl sulfate. Moreover, in the presence of sodium dodecyl sulfate an alteration was found in the cation effect, the ATPase was stimulated by either Na^+ or K^+. The stimulation by either alkali metal ion was not diminished by ouabain addition. When both Na^+ and K^+ were added in the presence of sodium dodecyl sulfate there was a further increase of ATPase. Ouabain partially abolished the stimulation by the combination of both cations.

Fig. 1 shows that the optimum concentration of sodium dodecyl sulfate under the conditions tested was 0.2 mM. The enzyme activity was inhibited by sodium dodecyl sulfate above 0.35 mM and was essentially abolished in 1.0 mM of the detergent. The optimum concentration of sodium dodecyl sulfate varied somewhat with varying concentration of membrane fragments. It is of interest to note that ASKARI AND FRATANTONI[4] also tested sodium dodecyl sulfate in their sonicated erythrocyte membrane preparation and found only an inhibitory effect on the ATPase.

In the presence of 0.2 mM sodium dodecyl sulfate the activation by single alkali metal ions was not merely due to a non-specific ionic effect. As shown in Fig. 2, Li^+ did not have significant effect up to 0.25 M and slightly inhibited the enzyme activity at a higher concentration, whereas at optimum concentration either Na^+ or K^+ stimulated the ATPase about two-fold. The effect of Li^+ was in contrast with the observation of ASKARI AND FRATANTONI[4]. In the sonic oscillation-treated membrane preparation they found single cation stimulation of ATPase by all three alkali metal ions: 30% by K^+, 18% by Na^+, and 12% by Li^+. An organic cation, Tris, was also tested in the presence of 0.2 mM sodium dodecyl sulfate. ATPase was inhibited by addition of Tris–HCl (pH 7.5).

Attempts to reverse the effect of sodium dodecyl sulfate on the membrane preparation with the following methods were unsuccessful: Sephadex chromatography, dialysis, and treatment with albumin, mixture of phospholipids, or erythrocyte membrane lipid extract prior to chromatography. However, Table II shows that the effect of sodium dodecyl sulfate could be reversed if the reaction mixture (including sodium dodecyl sulfate) was preincubated at 50° for 5 min prior to addition of ATP. The activating effect of sodium dodecyl sulfate as well as the stimulation by single alkali metal ions in the presence of sodium dodecyl sulfate were abolished by the heat treatment.

Sodium dodecyl sulfate with ^{35}S was used to determine the extent of binding of the detergent molecules to the membrane fragments and also to study the effect of heating on the binding. Fig. 3 shows that only a small fraction of sodium dodecyl sulfate in the medium was bound to the membrane fragments. The amount of detergent bound per unit weight of membrane increased with increasing concentration of sodium dodecyl sulfate in the medium. The binding sites of the membrane were not saturated at 2 mM sodium dodecyl sulfate, ten times as concentrated as the amount of sodium dodecyl sulfate used in most of the ATPase assay.

Fig. 4 shows the Sephadex chromatography patterns of two sodium dodecyl sulfate-treated preparations, one of which was chromatographed without heat treatment (a), and the other was incubated for 5 min at 50° prior to chromatography (b). It was a surprise to find that the extent of sodium dodecyl sulfate binding was similar in both cases: about 0.6% of the added sodium dodecyl sulfate was bound to the protein peak which came through in the first void column volume. In five different

TABLE II

SODIUM DODECYL SULFATE EFFECT REVERSED BY HEATING

The conditions for the assay were as described in Table I. Preincubation with all reaction components except ATP was carried out at 50° for 5 min and then the tubes were transferred to a 37° water bath and equilibrated for 2 min prior to addition of ATP at zero time.

*Additions**	*Preincubation at 50°*	*ATPase (μmoles P_i per mg protein per h)*	
		Without sodium dodecyl sulfate	*+0.2 mM sodium dodecyl sulfate*
None	—	0.29	0.65
NaCl	—	0.26	0.96
KCl	—	0.25	0.93
NaCl + KCl	—	0.66	1.57
NaCl + KCl + ouabain	—	0.23	0.92
None	+	0.17	0.16
NaCl	+	0.17	0.13
KCl	+	0.17	0.17
NaCl + KCl	+	0.66	0.72
NaCl + KCl + ouabain	+	0.16	0.11

* When added NaCl was 100 mM, KCl was 25 mM, and ouabain was 0.15 mM.

TABLE III

ATPASE OF THE CHROMATOGRAPHY FRACTIONS

Tubes 7, 8, and 9 from Fig. 4 (a) and (b) were combined and assayed for ATPase under conditions described in Tables I and II.

Fractions	*Additions**	*Preincubation at 50°*	*ATPase (μmoles P_i per mg protein per h)*
Fig. 4 (a) 7–9	None	—	0.41
	NaCl	—	0.60
	KCl	—	0.58
	NaCl + KCl	—	0.91
	NaCl + KCl + ouabain	—	0.58
Fig. 4 (a) 7–9	None	+	0.29
	NaCl	+	0.24
	KCl	+	0.25
	NaCl + KCl	+	0.57
	NaCl + KCl + ouabain	+	0.22
Fig. 4 (b) 7–9	None	—	0.15
	NaCl	—	0.15
	KCl	—	0.13
	NaCl + KCl	—	0.47
	NaCl + KCl + ouabain	—	0.11

* When added NaCl was 100 mM, KCl was 25 mM, and ouabain was 0.15 mM.

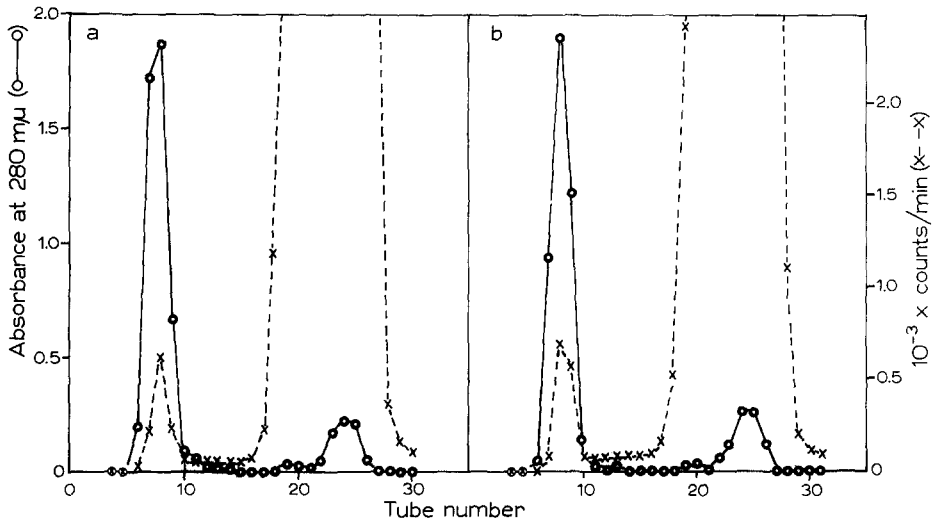

Fig. 4. Sephadex chromatography of two ^{35}S-labelled-treated sodium dodecyl sulfate membrane preparations: (a) control, (b) incubated at 50° for 5 min. Chromatography procedure was similar to that described in Fig. 3. In each preparation, membrane fragments equivalent to 5.8 mg protein were incubated with 0.3 mM ^{35}S-labelled sodium dodecyl sulfate ($1.4 \cdot 10^5$ counts/min per mole) and 0.05 M imidazole–HCl (pH 7.5) in a final volume of 4.0 ml at 37° for 5 min. Preparation (a) was put through the column directly and preparation (b) was further incubated at 50° for 5 min and then put through the column. The absorbance and the radioactivity patterns are similar in both preparations. 0.6% of the total radioactivity coincided with the protein peak (tubes 7, 8 and 9). The major radioactive peak representing over 99% of the total (tubes 17–29) is not completely presented in the figure.

runs with similar concentrations of sodium dodecyl sulfate, the per cent of detergent bound ranged from 0.53 to 0.63.

Each of these protein fractions was assayed for its ATPase and the results are shown in Table III. The ATPase in the fraction from Fig. 4(a) exhibited the characteristics similar to a typical sodium dodecyl sulfate-treated preparation without chromatography. This indicated that once the membrane preparation was altered by sodium dodecyl sulfate treatment, the unbound sodium dodecyl sulfate (over 99%) was not required for the single cation activation. Likewise, this activation was also abolished by a 5-min preincubation at 50° as shown in the second series of reaction tubes.

On the other hand, although the fraction from Fig. 4(b) was still bound to a similar amount of sodium dodecyl sulfate, there was no detectable single cation activation. Moreover, further addition of sodium dodecyl sulfate to this fraction could not regenerate the sodium dodecyl sulfate effect in a normal preparation (data not shown). The heat treatment appeared to have reversed the sodium dodecyl sulfate effect without changing the amount of sodium dodecyl sulfate bound to the membrane fragments. Consequently, it may be concluded that the change of membrane ATPase with respect to alkali metal ion activation was not simply due to the extent of sodium dodecyl sulfate binding to the membrane preparation.

It was further demonstrated that most of the bound sodium dodecyl sulfate was not released from the membrane fragments by heat treatment. In a similar preparation as in Fig. 4(a), tubes 7, 8 and 9 were combined and heated at 50° for 5 min and

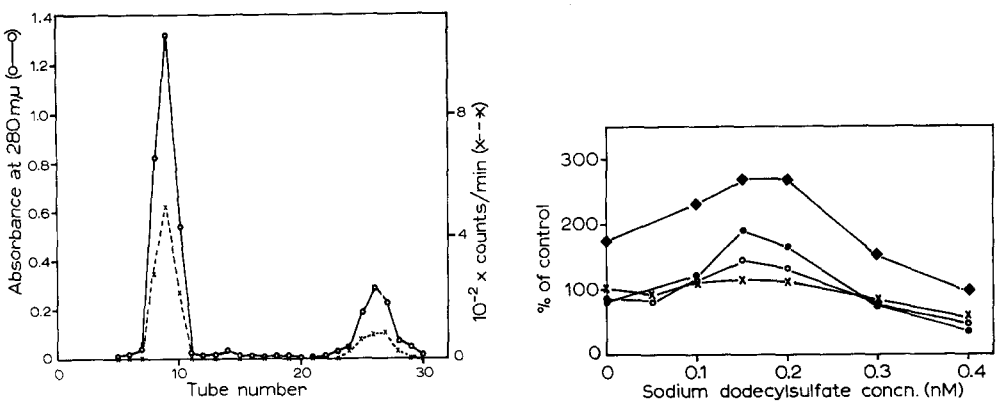

Fig. 5. Rechromatography of heat-treated membrane fragments. A sodium dodecyl sulfate-treated membrane preparation was passed through a Sephadex G-100 column as in Fig. 4 (a). Tubes 7, 8 and 9 were combined and heated at 50° for 5 min and then put through the column again. Sodium dodecyl sulfate concn. in mM.

Fig. 6. Effect of sodium dodecyl sulfate on the ATPase of a heat-treated membrane preparation. The conditions for ATPase assay are described in Table I. The membrane preparation in 0.05 M imidazole–HCl (pH 7.5) was incubated for 5 min at 50° prior to addition to the ATPase reaction mixture. ×—×, no addition (first point without sodium dodecyl sulfate is arbitrarily chosen as 100% of control); ○—○, *plus* 100 mM NaCl; ◆—◆, *plus* 100 mM NaCl and 25 mM KCl; ●—●, *plus* 100 mM NaCl, 25 mM KCl, and 0.15 mM ouabain.

then put through the Sephadex column again. Fig. 5 shows that 80% of the radioactivity still remained with the main protein peak.

Since preincubation at 50° abolished the effect of the detergent in the sodium dodecyl sulfate-treated membrane fragments, it was next attempted to find out if sodium dodecyl sulfate had any effect on heat-treated membrane fragments. Fig. 6 shows that activating effect of sodium dodecyl sulfate on the heat-treated preparation was greatly diminished as compared with the untreated preparation shown in Fig. 1. Moreover, the single ion activation was much less pronounced. At optimum concentration (0.15 mM sodium dodecyl sulfate) in this experiment the activation by 100 mM NaCl was 25%, while in a normal preparation as shown in Fig. 1, 100 mM NaCl activated 105% at optimum concentration of sodium dodecyl sulfate. It indicated some irreversible alteration on the membrane ATPase system as a result of heating at 50°.

It has been shown that certain structural integrity of the lipoprotein complex is essential for the ($Na^+ + K^+$)-activated ATPase in the membrane[3]. When the enzyme system is treated with either sonic oscillation or a hydrophobic bond-rupturing agent such as sodium dodecyl sulfate, some conformational changes may be expected. Extensive treatment by either of these two methods can abolish the ATPase activity (see ref. 4 and Fig. 1). Under properly controlled conditions, however, either of these treatments may bring about only alterations in the characteristics of the cation effect without destroying the activity. (In fact an increase of activity was observed, probably due to the dispersion of the aggregates of membrane fragments by the treatment and, consequently, the active sites were made more readily available.) The ATPase in a modified preparation can be partially activated by either Na^+ or K^+ alone, but the maximum activity still requires the combination of both cations. At present one can-

not be certain that the single alkali metal ion activated ATPase is a derivative of the ($Na^+ + K^+$)-activated ATPase. However, the cation specificity shown in sodium dodecyl sulfate-treated preparations (see Fig. 2) appears to lend support to this hypothesis.

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