Vol. 86, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 14, 1979 Pages 704-709

ACTIVATION OF CALCIUM-ATPase OF LIVER PLASMA MEMBRANE BY DEXTRAN SULFATES

Kyoichi Kagawa and Setsuo Tomizawa

Department of Pharmacology, School of Pharmaceutical Sciences,
Kitasato University, Tokyo 108, Japan

Received December 15, 1978

SUMMARY. Dextran sulfate (DS) with average molecular weight (AMW) of 20,000 and sulfur content of 18%, which has a high lipemia clearing activity, enhanced Ca^{2+} binding to the plasma membrane of rat liver, and the DS itself bound the membrane, whereas there was little binding of DS and Ca^{2+}. Various DSs slightly activated Na^{+}-K^{+}-ATPase, but not Mg^{2+}-ATPase activity of the membrane. These results suggest that DSs, especially with high AMW of 20,000, bind the plasma membrane, resulting in enhancements of the Ca^{2+} binding to there and Ca^{2+}-ATPase activity.

INTRODUCTION

Acid polysaccharides, heparin (1) and DSs (2), cause osteoporosis and a fall in blood Ca^{2+} level in many species (3, 4). This may be due to the activation of lysosomal protease which may result in the degradation of bone matrix (5) and the release of lipoprotein lipase (6) which may result in the binding of blood Ca^{2+} and free fatty acids increased (3). Acid polysaccharides, however, may affect Ca^{2+} transport itself.

Our previous report (7) indicated that DS transport to the lysosomal fraction of intestinal mucosa was inhibited by EDTA. Further, DSs increased calcium contents in bile (8), kidney and liver cells, and their lysosomal fractions (4). From these findings, we believe that acid polysaccharides, at least, DSs, are pinocytosed, which may probably be affected by Ca^{2+}.

In recent year, Folger et al. (9) showed that the inhibition of phagocytosis by tertiary amine local anesthetics was reversed by addition of Ca^{2+}. Their results may support our speculation that Ca^{2+} is necessary for endocytosis.

Endocytosis has been considered to be energy dependent process (10, 11). We already showed that DS with high AMW enhanced Ca^{2+}-ATPase in the lysosomal frac-

Abbreviations used in this paper: DSs, dextran sulfates; IV, intrinsic viscosity; AMW, average molecular weight; SC, sulfur content; Ka, association constant; N, the number of binding sites.

0006-291X/79/030704-06$01.00/0

Copyright © 1979 by Academic Press, Inc.
All rights of reproduction in any form reserved.

tion of intestinal mucosa (7) and speculated that the ATPase may involved in an endocytic transport.

To confirm these two possibilities, we examined the effect of DSs on Ca^{2+}-ATPase activity of the plasma membrane of rat liver and their relation to Ca^{2+}.

MATERIALS AND METHODS

Composition of DSs. DS-L, IV 0.027, AMW 3000, SC 18.3%; DS-M1, IV 0.082, AMW 20,000, SC 18.1%; DS-M2, IV 0.083, AMW 20,000, SC 8.3%. DS-M1 labeled with [^{35}S] had a specific activity of 1.5 μCi/mg and used in some experiments. These DSs were generous gifts of Meito Sangyo Co., Nagoya.
Liver Plasma Membrane. The livers of female Donryu rats (about 200 g, Nihon Rat Co., Urawa) were used and their plasma membranes were collected according to the method of Fitzpatrick et al. (12), as previously described (13).
Ca^{2+} Binding to the Plasma Membrane. The stocked membrane solution was centrifuged and the pellet was washed once with 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA before use. The final pellet obtained was suspended in 100 mM Tris-HCl buffer (pH 7.5). Various concentrations of [^{45}Ca]Cl_2 (New England Nuclear Corp., Boston, Mass.) were added to the membrane solution containing about 0.2 mg of the membrane protein with or without 0.5 mM DS-M1. Ten min after incubation at 37°C, the solution was centrifuged at 105,000 g for 10 min to produce the membrane pellet bound to [^{45}Ca]. The pellet was suspended in 0.1% Triton X-100 and the sample was used to determine the amount of Ca^{2+} bound to the membrane.
DS Binding to the Plasma Membrane. To the membrane solution, various concentrations of [^{35}S]DS-M1 were added and 1 ml of the solution containing 1 mg of the membrane protein were incubated at 37°C for 10 min. After incubation, the solution was centrifuged to produce the membrane pellet bound to [^{35}S]DS-M1. The pellet was suspended in 0.1% Triton X-100. The sample was counted to determine the amount of DS-M1 bound to the membrane.
Ca^{2+} Binding to DS. Free Ca^{2+} was measured by the method of Urist et al. (14) using a Calcium Specific Electrode, Orion Res., Inc., Cambridge, Mass., before and after addition of DS. Incubation system consisted of 134 mM NaCl, 7 mM KCl, 5 mM $CaCl_2$ and 6.7 mM or 67.0 mM DS-M1 at 20±1°C. Final readings were taken 30 min after DS addition.
ATPase Activities. Na^+-K^+-ATPase and Mg^{2+}-ATPase activities were assayed by the method of Quigley and Gotterer (15). Ca^{2+}-ATPase activity was measured according to the method of Wins and Schoffeniels (16) with a slight modification, as previously descibed (7).
Determinations. Hofstee plots of the bound Ca^{2+} and DS-M1 were used to determine their Ka and N. Protein was measured by the method of Lowry et al. (18). The radioactivities were measured with a Liquid Scintillation Spectrophotometer, Aloka 651.

RESULTS AND DISCUSSION

Fig. 1 shows that DS-M1 enhanced Ca^{2+} binding, including the uptake, to the plasma membrane vesicles of rat liver. In the absence of DS-M1, the apparent Ka and N of the Ca^{2+} binding are 6.4×10^2 M^{-1} and 73 nmoles/mg of membrane protein. These values are similar to those reported by Chambault et al. (19) and those for

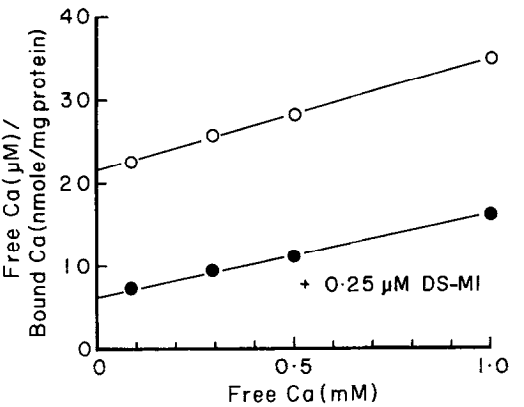


Fig. 1. Enhancement of Ca^{2+} Binding to the Plasma Membrane of Rat Liver by DS. See text for assay condition. Each point shows the mean of seven experiments.

low affinity, as previously reported by us (13). DS-MI in a concentration of 0.25 μM remarkably increased the Ka to 15.9 x 10^2 M^{-1} and the N to 102 nmoles/mg of the membrane protein. This DS action might depend on Ca^{2+} binding to DS-MI itself. The complex of acid polysaccharide and protein have been considered to a model of bone. Urist et al. (14) have shown that chondroitin sulfates alone could bind Ca^{2+}. Similarly, DS-MI also could bind Ca^{2+} in vitro, as shown in Table I. However, the binding was little. Thus, even in the presence of DS-MI, Ca^{2+} is probably bound mainly to the plasma membrane.

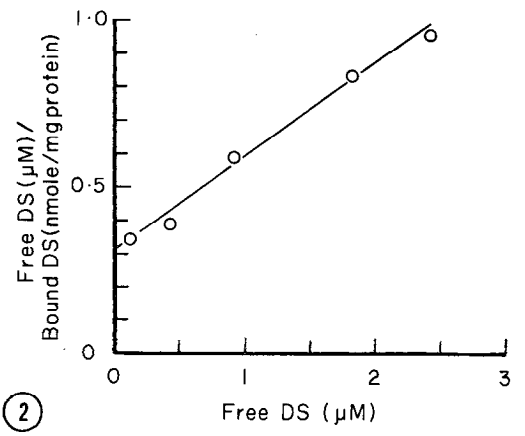
Equilibrium constant for DS-calcium complex varied with DS concentration added. Lages and Stivalas (20) have shown that, when heparin bound Ca^{2+} or Cu^{2+}, its random coil changed. Accordingly, the conformational change of DS-MI may probably occur with the binding of Ca^{2+}.

Waite and Sisson (21) have shown that heparin bound the plasma membrane of rat liver and caused the release of phospholipase A . Here, DS-MI also bound the plasma membrane, as shown in Fig. 2. The maximal amount of DS-MI that can be bound was 3.6 nmoles/mg of the membrane protein. From their result, the bound heparin can be estimated as about 1 nmole/mg of the membrane protein. Heparin causes the release of lipoprotein lipase in vivo (6). This action varys with its AMW and anionic group (22). Although we do not know the AMW, SC and number

Table I. Ca^{2+} Binding to DS

Initial concentration (mM)		Final concentration (mM)	
[DS]	[Ca]	[Ca]	[DS·Ca]
6.7	5.02±0.24	4.75±0.30	0.27±0.07
67.0	4.76±0.31	4.17±0.26*	0.58±0.12

See text for assay condition. Values show the mean and S.D. of four experiments. Statistically different from the initial concentration, * $P<0.05$.



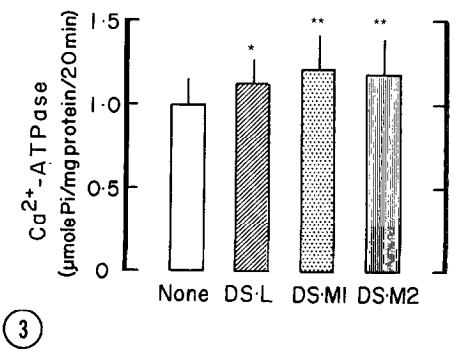


Fig. 2. Binding of DS to the Plasma Membrane of Rat Liver. See text for assay condition.

Fig. 3. Activation of Ca^{2+}-ATPase of the Plasma Membrane of Rat Liver by DSs. Incubation system consisted of 2.7 mM $CaCl_2$, 0.3 mM $MgCl_2$, 0.5 mM ouabain, 5 mM ATP-Tris, 30 mM Tris-HCl buffer (pH 7.4), and about 0.4 mg of the membrane protein. Concentration of DSs was 0.5 mM. Each point shows the mean of four experiments and the vertical lines show S.D. Statistically different from control, * $P<0.05$, ** $P<0.01$.

of amino group in heparin they used, the binding of DS-M1 to the plasma membrane appears to be more than that of heparin. Tomizawa (23) already confirmed that, in the same dose, DS-M1 was stronger in lipemia clearing activity than heparin. This result may possibly be due to the difference in their binding ability to the plasma membrane.

Fig. 3 shows that all the DSs used in this experiment enhanced Ca^{2+}-ATPase activity of the plasma membrane. Especially, the action of DSs with AMW of

20,000 was stronger than that of DS with AMW of 3000. Thus, we think that the activation of Ca^{2+}-ATPase by DSs differs with their AMW and SC.

DSs in a concentration of 0.5 mM caused a slight increase in Na^{+}-K^{+}-ATPase (from 5.7±0.4 to 6.7±0.6 μmoles Pi/mg of the membrane protein/20 min), but did not affect Mg^{2+}-ATPase (from 19.7±2.9 to 20.8±1.3 μmoles Pi/mg of the membrane protein/20 min). From these results, we believe that DSs specifically cause an increase in Ca^{2+}-ATPase of the plasma membrane of liver.

Ca^{2+}-ATPase is involved in Ca^{2+} extrusion process, which was confirmed in erythrocyte (24) and kidney (25), and the conformational change in plasma membrane of macrophage (26). The ATPase activity in the plasma membrane we detected here appears to be involved in the latter function because of the absence of Ca^{2+} pump consuming ATP energy in this membrane (27). In mast cell also, the plasma membrane contains Ca^{2+}-ATPase, which associates with exocytic secretion of the granules containing some amines (28). According to these findings, we believe that the increasing of Ca^{2+}-ATPase activity induced by DSs reveals the conformational changing of the membrane. This may cause endocytosis. In addition, the activation of the ATPase by DSs may probably be due to the release of this enzyme from the membrane and unmasking of its active sites.

REFERENCES

1. Tourtellotte, C. D. and Dziewiatokowski, D. D. (1965) J. Bone Joint Surg. 47, 1185.
2. Walton, K. W. (1954) Brit. J. Pharmacol. 9, 1.
3. Goldsmith, N. W. and Parry, D. J. (1968) Clin. Chem. Acta 19, 429.
4. Kagawa, K. and Tomizawa, S. Life Sci. submitted.
5. Goldhaber, P. (1965) Science 147, 407.
6. Robinson, D. S. and Harris, P. M. (1959) Q. J. exp. Physiol. 44, 80.
7. Kagawa, K., Nishiyama, M. and Tomizawa, S. (1978) Biochem. Pharmacol. 27, 1931.
8. Kagawa, K. and Tomizawa, S. Biochem. Pharmacol. submitted.
9. Fogler, W. E., Gersten, D. M. and Fidler, I. J. (1978) Biochem. Pharmacol. 27, 2447.
10. Duncan, C. J. (1966) Nature 210, 1229.
11. Allison, A. C. and Davies, P. (1974) Symp. Soc. exp. Biol. 28, 419.
12. Fitzpatrick, D. F., Davenport, G. R., Forte, L. and Landon, E. J. (1969) J. biol. Chem. 244, 3561.
13. Kagawa, K., Kurohata, M. and Tomizawa, S. (1978) Biochem. Biophys. Res. Commun. 83, 1229.
14. Urist, M. R., Speer, D. P., Ibsen, K. J. and Stiates, B. S. (1968) Calc. Tiss. Res. 2, 253.

15. Quigley, J. P. and Gotterer, G. S. (1969) Biochim. biophys. Acta 173, 456.
16. Wins, P. and Schoffeniels, E. (1966) Biochim. biophys. Acta 120, 341.
17. Hofstee, B. H. J. (1952) Science 116, 329.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. biol. Chem. 193, 265.
19. Chambault, A., Leray-Pecker, F., Feldman, G. and Hanoune, J. (1974) J. gen. Physiol. 64, 104.
20. Lages, B. and Stivalas, S. (1973) Biopolymers 12, 127.
21. Waite, M. and Sisson, P. (1973) J. biol. Chem. 248, 7201.
22. Braswell, E. (1968) Biochim. biophys. Acta 158, 103.
23. Tomizawa, S. Unpublished data.
24. Schatzmann, H. J. (1966) Experientia 22, 364.
25. Brinley, F. J. (1973) Fed. Proc. 32, 1735.
26. Werb, Z. and Cohn, Z. A. (1971) J. exp. Med. 134, 1570.
27. Van Rossum, G. D. V. (1970) J. gen. Physiol. 55, 18.
28. Cooper, P. H. and Stanworth, D. R. (1977) Biochem. J. 168, 583.