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# UNCOUPLER HAS A WEAK IONOPHORIC ACTIVITY

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The potassium ion transport across liposomal membranes induced by uncoupler was studied. Both SF6847 and DNP induced $K^+$ leak from liposomes containing KSCN, but did not induce the leak from liposomes containing KCl. A powerful uncoupler, SF6847, was also a stronger $K^+$ ionophore than a weak uncoupler, DNP.

DNP (2,4-dinitrophenol) and SF6847 (3,5-di-ter-butyl-4-hydroxybenzylidenemalononitrile) are known to be typical uncouplers. The uncoupling effect of SF6847 is stronger than that of DNP by a factor of 1,000[1,2]. The mechanism of action of uncoupler is attributed to dissipation of the proton motive force in energy-transducing membranes according to the chemiosmotic theory, i.e. uncoupler is generally a protonophore[1]. Recently, the chemical behavior of SF6847[3,4] and the mechanism of its mediated proton-uptake in liposome[5] were studied. In this report we show that uncoupler also functions as an ionophore.

Multilayered liposomes were prepared as follows: The inner surface of 50 ml of round-bottomed flask was coated with 10 mg of egg lecithin containing 2% of phosphatidic acid by evaporation of solvent. Then 15 ml of 150 mM KSCN or KCl solution was poured into the lecithin coated flask and the flask was gently shaken until all lipid was removed from the flask. The liposome suspension was centrifuged for 20 minutes at 27,000 g at 4°C. The liposome preparations were washed with isotonic 150 mM $MgSO_4$ solution

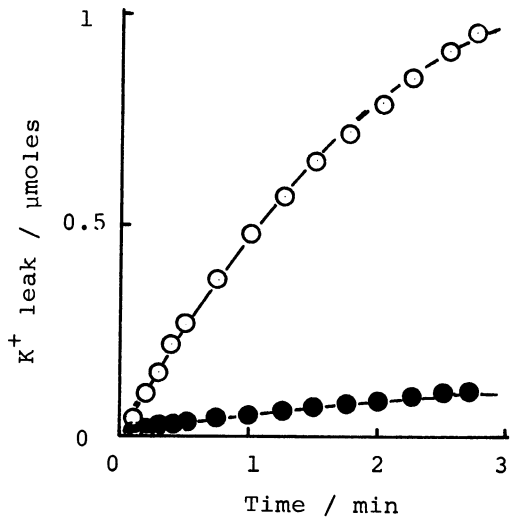


Fig. 1 $K^+$ leak from liposomes containing 150 mM KSCN (○) and 150 mM KCl (●) after addition of 100 nmoles SF6847.

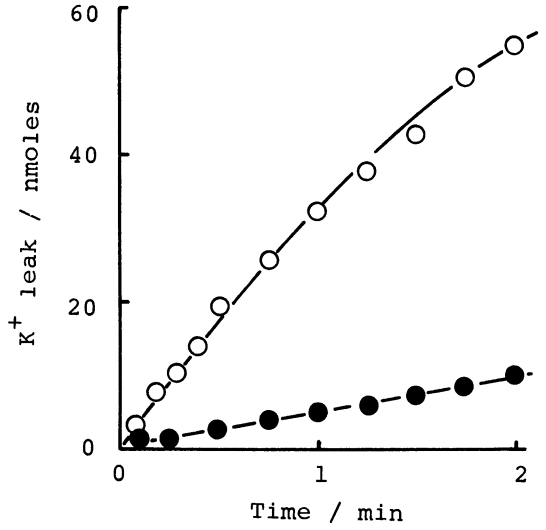


Fig. 2 $K^+$ leak from liposomes containing 150 mM KSCN (○) and 150 mM KCl (●) after addition of 100 nmoles DNP.

followed by centrifugation at 40,000g for 10 minutes at 4°C. The $K^+$ or $SCN^-$ leak was measured in 10 ml of 150 mM $MgSO_4$, 10 mM Tris/$H_2SO_4$ solution (pH7.0) by an ion-meter (Orion Research) with appropriate ion-selective electrodes. As shown in Fig. 1, when SF6847 was added to liposomes containing KSCN, the increase of the $K^+$ leak was maximal immediately following addition of the uncoupler, but when the uncoupler was added to liposomes containing KCl, the leak of $K^+$ was much less observed. Similar behavior, as shown in Fig. 2, was observed for DNP, although the leak of $K^+$ induced by DNP was far smaller than that by SF6847. The leak of $SCN^-$ after addition of SF6847 is shown in Fig. 3. The leaks of $K^+$ and $SCN^-$ were almost the same. This observation shows that the $K^+$ leak mediated by SF6847 is accompanied by $SCN^-$ leakage. Fig. 4 shows that the $K^+$ leak mediated by SF6847 was proportional to the concentration of SF6847. These observations suggest that $K^+$ leak by SF6847 was not induced by exchange between proton and potassium ion, but by stoichiometric complex formation between SF6847 and $K^+$[6], and the complex was accompanied by lipophilic anion or $SCN^-$, but not by $Cl^-$. In Fig. 5, IR spectra of SF6847 in methanol are shown. In the absence of KSCN, the signal asigned to C≡N stretching was observed at 2,230 $cm^{-1}$. On addition of KSCN, the signal at 2,230 $cm^{-1}$ was reduced but a new signal appeared at 2,205 $cm^{-1}$. The peak height of the new signal increased with time. These observations suggest that the interaction of KSCN with SF6847 in methanol may slowly induce a conformational difference near the C≡N group or dimer formation[7] of SF6847, although whether the OH group or the C≡N group is involved in the site of interaction was not established.

In this report, we showed that permeation of $K^+$ from liposomes is induced by uncoupler, and that the permeation necessitates the presence of lipophilic anion, such as $SCN^-$. It was also shown that a powerful uncoupler, SF6847, induces stronger $K^+$ leak than a weak uncoupler, DNP.

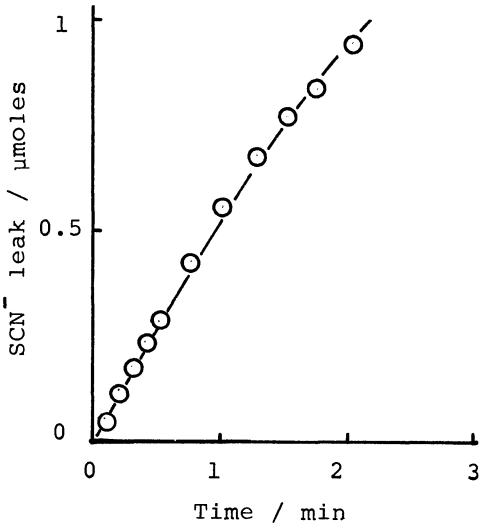


Fig. 3 $SCN^-$ leak from liposomes containing 150 mM KSCN after addition of 100 nmoles SF6847.

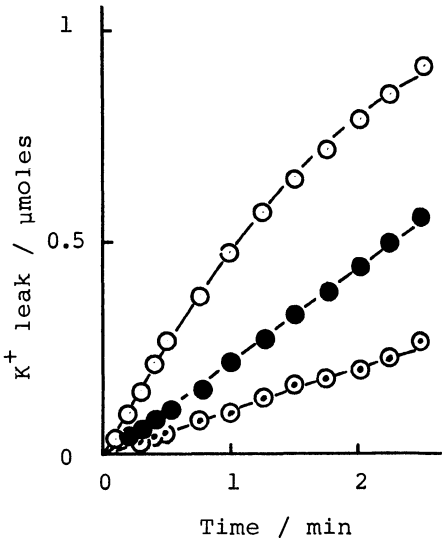


Fig. 4 $K^+$ leak from liposomes containing 150 mM KSCN after addition of 100 nmoles ◯, 50 nmoles ● or 25 nmoles ◎ SF6847.

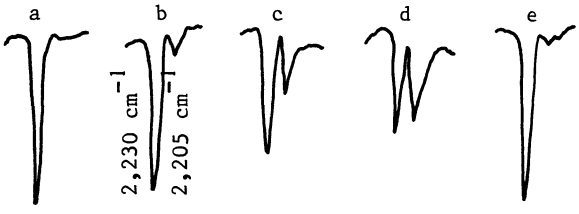


Fig. 5 IR spectra of SF6847 near 2,230 $cm^{-1}$ (0.02 M in methanol).
a) no KSCN. b) 10 min, c) 6 hr and d) 24 hr after addition of KSCN (0.1 M).
e) no KSCN 24 hr after solution.

References
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