CHEMISTRY LETTERS, pp. 303-306, 1982.

MECHANISM OF UNCOUPLER-MEDIATED CATION TRANSPORT AND CORRELATION BETWEEN THE IONOPHORIC ACTIVITY OF UNCOUPLER AND ITS UNCOUPLING ACTIVITY

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Uncoupler-mediated cation transport across liposomal membrane was studied. The pH dependencies of cation leaks were different between counter ions. The mechanism was proposed and ion selectivity was discussed. The correlation between the ionophoric activity of the uncoupler and its uncoupling activity was also shown.

Green et al. proposed that uncoupler acts as a carrier of both H^+ and cation,[1-3] and also proposed the mechanism for uncoupler-mediated cyclical transport of cation driven by electron transfer in mitochondrial electron transfer complexes.[4] In previous paper, we showed that uncoupler has an ionophoric activity on liposomal membrane and that a powerful uncoupler is also a stronger K^+ ionophore than a weak uncoupler.[5] In this paper, we show that the extent of cation transport mediated by uncoupler in its dissociated form or undissociated form at several pH was governed by counter ion, and propose the mechanism of uncoupler-mediated cation transport on liposomal membrane. We also show the correlation between the uncoupling activity of uncoupler and its ionophoric activity.

Multilayered liposomes were prepared as follows: The sample solution, 150 mM of Na, K, Rb, or CsSCN or KCl solution (pH 5-9), was poured into Egg lecithin (containing 2% of phosphatdic acid) coated flask and the flask was gently shaken until all lipid was removed from the flask. The liposomes were centrifuged and diluted in 10 ml of isotonic 150 mM $MgSO_4$, 10 mM Tris/H_2SO_4 solution at pH 5-9. Uncoupler, dissolved in methanol, was added with a microsyringe as 20 µl of methanol solution to the liposome dispersed solution. The ion leaks from the liposomes were measured by an ion-meter (Orion Research) with appropriate ion-selective electrodes. Details were shown in previous paper.[5]

In Fig. 1, SF6847-mediated K^+ leak from the liposomes containing 150 mM KSCN at several pH and SCN^- leak at pH 7 were shown. The more the solution was made acid, the more the K^+ leak from the liposomes increased. The leaks of K^+ and SCN^- at pH 7 were almost the same. From these observations and the result on the previous paper that the K^+ leak mediated by SF6847 was proportional to the concentration of SF6847,[5] It was suggested that K^+ was carried through liposomal membrane in the form of $SFHK^+SCN^-$ at neutral or acid in aqueous phase, where SFH was undissociated form of SF6847. In Fig. 2, K^+ leak from the liposomes containing KCl at several pH was shown. In contrast with the result for the liposomes containing KSCN, the more the solution was made alkaline, the more the K^+ leak from the liposomes increased. From the observation and the report by Green and Zande,[4] it was suggested that K^+

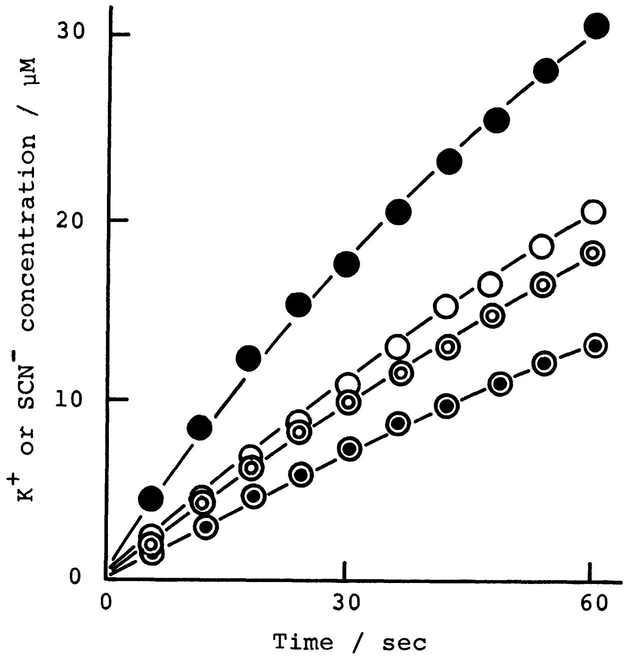

Fig. 1 K^+ or SCN^- concentration increase in the medium by K^+ or SCN^- leak from liposomes containing 150 mM KSCN after addition of 10 nmol/mg lipid of SF6847; ●: K^+ at pH 5.0, ○: K^+ at pH 7.0, ◎: SCN^- at pH 7.0, ◉: K^+ at pH 9.0.

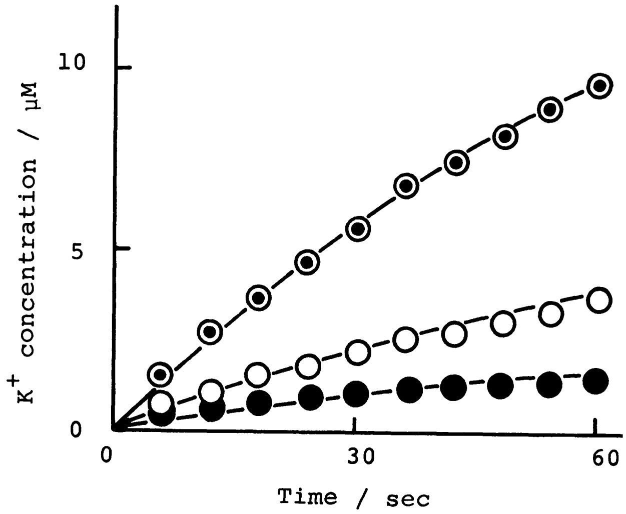

Fig. 2 K^+ concentration increase in the medium by K^+ leak from liposomes containing 150 mM KCl after addition of 10 nmol/mg lipid of SF6847; ●: at pH 5.0, ○: at pH 7.0, ◉: at pH 9.0.

leak by SF6847 from the liposomes containing KCl was governed by the formation of SF^-K^+, where SF^- was dissociated form of SF6847. On the basis of above results, we proposed the model for the mechanism of uncoupler-mediated cation transport through the membrane as shown in Fig. 3. In acid solution, $SFHM^+X^-$ is a carrier of cation transport through the membrane and it is necessary for X to be lipophilic anion such as SCN^-. In the case, net current of ions both of M^+ and X^- occurs through the membrane from inside to outside according to the concentration gradient of M^+ and X^-. The K^+ leak from the liposomes containing KSCN is dominated by such a mechanism, that is, the formation of $SFHK^+SCN^-$. In alkaline solution, SF^-M^+ is a carrier of cation

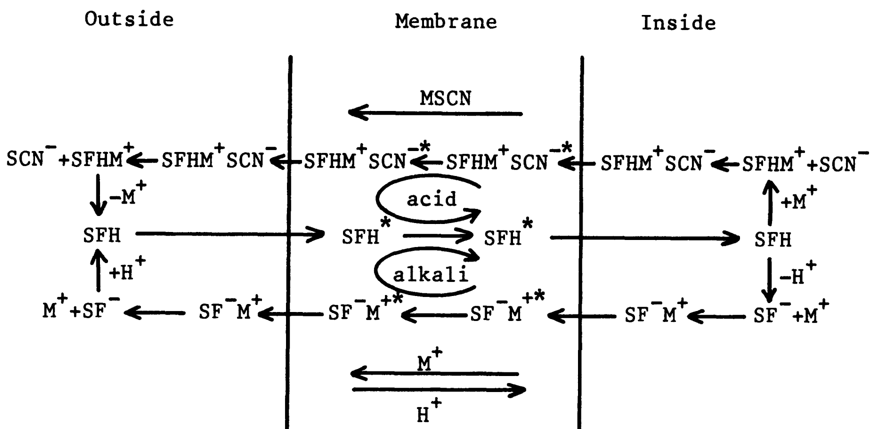

Fig. 3 Model for the mechanism of uncoupler-mediated cation transport through membrane

transport and it is not necessary for X^- to be lipophilic cation. In this case, net current of M^+ occurs through the membrane from inside to outside, and that of H^+ from outside to inside. The K^+ leak from the liposome containing KCl is dominated by such a mechanism, that is the formation of SF^-K^+.

Initial increases of several cation leaks from the liposomal membrane were plotted against the ionic radius as shown in Fig. 4. A large cation, such as, Cs^+, whose hydration energy is small, was carried by SF6847 through the membrane more than a small cation, such as, Na^+, whose hydration energy is large. Then ion selectivity of SF6847 was dominated by the effect of dehydration energy of the cation, on the contrast to a macrolide ionophore, such as, tetranactin, whose shape in its cation-complexed form was sphere in solution[6] and then whose ion selectivity was governed both by the conformational energy in its complexed form and by the desolvation energy of ion.[7,8] The relation between a parameter, (b'-a')/(b-a), which showed the effect of cations on the release of mitochondrial respiration by uncoupler and whose values had been given by Green and Zande,[4] and the initial increase of several cation leaks on Fig. 4 was shown in Fig. 5. Uncoupling effect of uncoupler and cation leak induced by uncoupler was showed good correlation. In Fig. 6, K^+ leaks by SF6847, FCCP and DNP from liposomes containing 150 mM KSCN at pH 7 were shown. The leaks induced by powerful uncouplers, SF6847 and FCCP showed similar behavior with each other and the extent was far larger than that by weak uncoupler, DNP. Karlish et al.

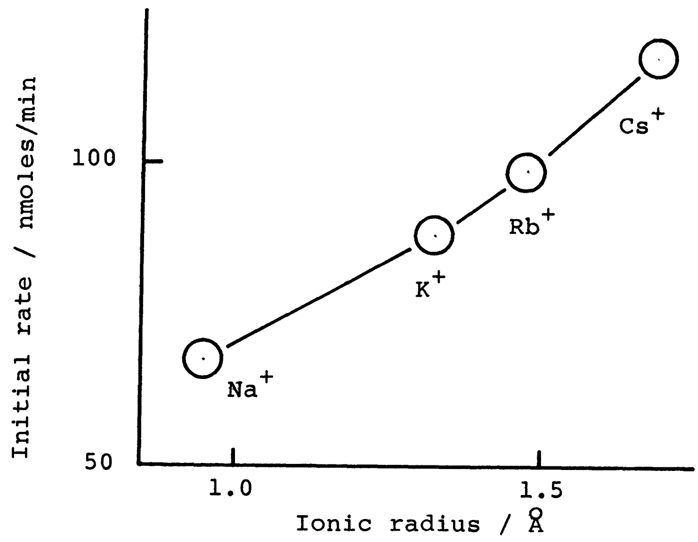

Fig. 4 Initial rate of cation leak from liposomes containig 150 mM MSCN after addition of 25 nmol / mg lipid of SF6847 vs. ionic radius.

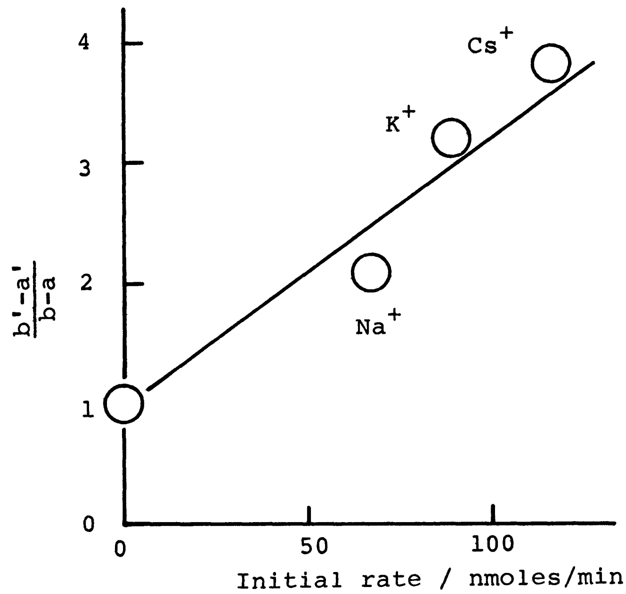

Fig. 5 Relationship between a parameter, (b'-a')/(b-a) and initial rate of cation leak (see context regarding to Fig. 5 and reference 3).

reported that DNP does not show uncoupling activity in the presence of K^+ alone without valinomycin, that is, both K^+ and valinomycin is necessary for exibition of its uncoupling activity in chloroplasts.[9,10] This may be ascribed to the very weak ionophoric activity of DNP.

In this report, we proposed the mechanism of uncoupler-mediated cation transport through liposomal membrane and showed the correlation between the uncoupling activity of uncoupler and its ionophoric activity.

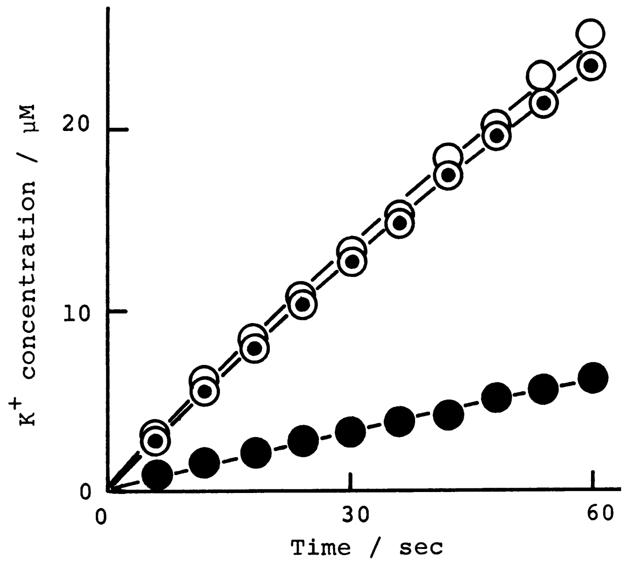

Fig. 6 K^+ concentration increase in the medium by K^+ leak from liposomes containing 150 mM KSCN after addition of 2.5 nmol/mg lipid of SF6847 (◉), FCCP (○) and DNP (●).

Acknowledgements

The authors are indebted to Dr H. Terada of Tokushima University for helpful discussion.

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(Received December 4, 1981)