EFFECT OF RIFAMPICIN DERIVATIVES ON THE ION COMPARTMENTATION OF BIOLOGICAL MEMBRANES

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Based on the experimental results that 3-formyl rifamycin SV acts as an uncoupler *in vitro* on rat liver mitochondria, the effect of some rifampicin derivatives on the K^+ and H^+ compartmentation of biological membranes was examined to obtain a chemiosmotic hypothesis for oxidative phosphorylation. The K^+ release from mitochondria was remarkably stimulated by 3-formyl rifamycin SV in accordance with uncoupling of the oxidative phosphorylation. 3-Formyl rifamycin SV also stimulated the K^+ release from red blood cells, though its action was not as effective as in mitochondria. It can be suggested that 3-formyl rifamycin SV interacts with biological membranes, causing a change in permeability to ions, especially of K^+ and H^+ through the mitochondrial membrane, resulting in uncoupling of the oxidative phosphorylation.

As a result of studies to elucidate the biological actions of rifampicin, it was found that 3-formyl rifamycin SV (3F·RFM SV), one of the rifampicin derivatives, acts as a rather potent uncoupler *in vitro* on rat liver mitochondria.[1] Most uncouplers are lipid-soluble in both forms of hydrated or unhydrated, therefore dissolve into the lipid layer of mitochondrial membrane. And then the uncouplers enhance dielectric constant of the membrane, causing a decrease in electrical resistance and an increase in electric conductivity, that result in increase of membrane permeability to H^+ and other ions.[2~4] Namely, it is considered that uncouplers act to diminish the H^+ gradient and cause a decrease in the membrane potential accompanied with change in permeability to K^+ of the biological membrane.[5~6]

From this point of view, this report describes the effect of rifampicin derivatives on the K^+ compartmentation of biological membranes which has a close correlation to the membrane potential not only in mitochondria but also in red blood cells.

Materials and Methods

Animals

Male Donryu-strain rats (200~250 g) and male albino rabbits (3 kg) were used.

Mitochondrial Preparation

Rats were fasted overnight, sacrificed by decapitation and liver mitochondria were isolated according to the method of SCHNEIDER.[7]

Red Blood Cell Preparation

Fresh red blood cells were prepared from venous blood of rabbit earlobe and washed twice with 150 mM choline chloride containing Tris-HCl (pH 7.4) at 0~4°C.

Measurements of K^+ and H^+ Movement

Mitochondria were incubated in an open vessel containing 5.0 ml of a reaction medium as shown

in the legends of figures at 25°C with continuous stirring. The measurements were carried out by using a glass K^+-selective electrode (Beckman No. 39137) and a glass H^+-electrode connected to a pH meter. The signal of K^+ or H^+ concentration change was amplified with a D.C. amplifier and recorded with an autorecorder.

Red blood cells were incubated at 37°C and K^+ movement was measured in the same manner as in the case of mitochondria. For addition of rifampicin derivatives, dimethylsulfoxide (DMSO) was used as solvent, the final concentration of DMSO added being less than 1%. Only DMSO of the same concentration was present in the controls.

Measurement of Oxygen Consumption

Oxygen consumption of mitochondria was measured by using a Galvani-type oxygen electrode at 25°C. Mitochondria were incubated in a closed vessel containing 5.0 ml of a reaction medium for oxidative phosphorylation. The vessel can be equipped with both an oxygen electrode and a K^+-electrode, or both an oxygen electrode and a H^+-electrode as occasion demands. Therefore simultaneous measurements of oxygen uptake and K^+ movement, or oxygen uptake and H^+ movement were carried out.

Determination of Mitochondrial Protein

Protein concentration was determined by the Biuret reaction using bovine serum albumin as a standard.

Results and Discussion

During the incubation of mitochondria in the reaction medium, a moderate K^+ release from mitochondria was observed. Remarkable stimulation of K^+ release from mitochondria was induced by the addition of 3F·RFM SV added (Fig. 1). The effects of 3F·RFM SV on the K^+ compartmentation and the respiratory activity of mitochondria are summarized in Table 1. As shown in this table, there is a close correlation between the acceleration of K^+ release from mitochondria and increase in the state 4 oxygen uptake, *i.e.* uncoupling by 3F·RFM SV. The same concentration of rifampicin

Fig. 1. Potassium release from mitochondria by treatment with 3-formyl rifamycin SV.
Mitochondria (3.9 mg protein) were incubated in the medium containing 0.3 M mannitol, 5 mM Tris-HCl buffer (pH 7.4) at 25°C. Total incubation mixture was 5.0 ml. Intramitochondrial content of potassium was estimated by measuring the K^+ depleted by adding a surfactant Triton X-100. For further details, refer to the text.

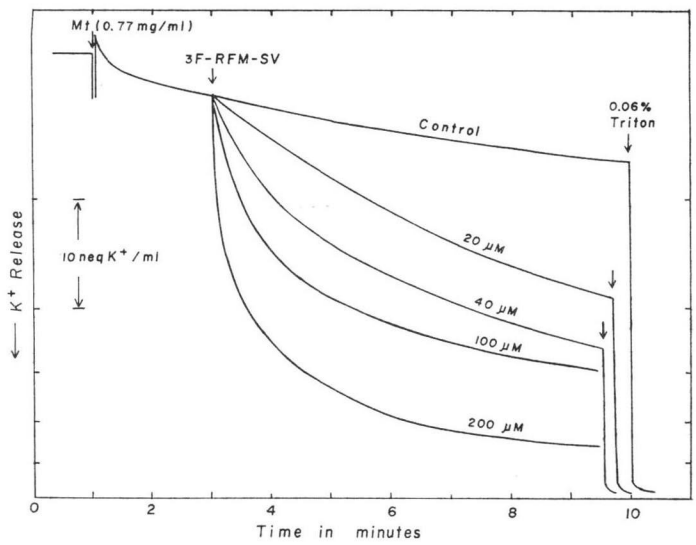


Fig. 2. Potassium release from mitochondria by treatment with rifampicin derivatives. Experimental conditions were the same as in Fig. 1.

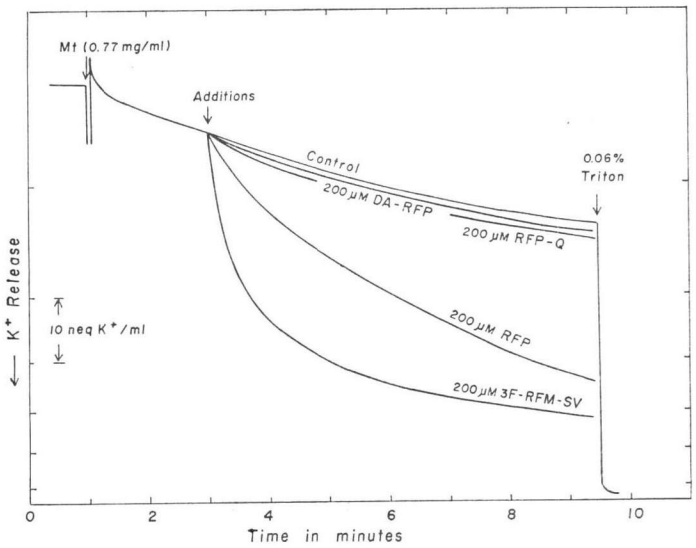
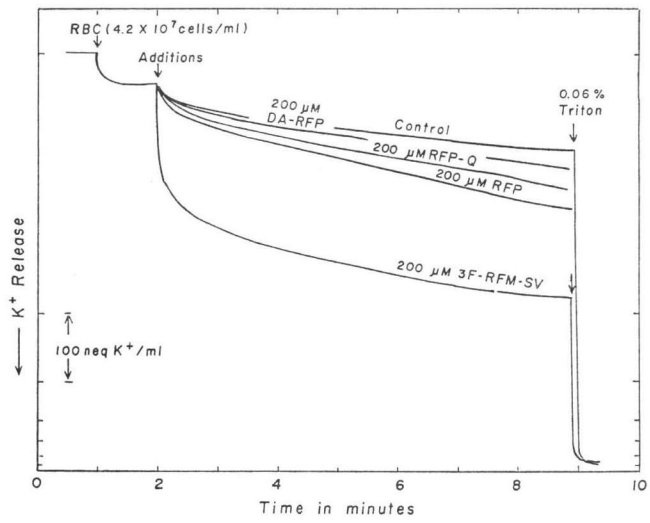


Fig. 3. Potassium release from red blood cells by treatment with rifampicin derivatives. Red blood cells (4.2×10^7 cells/ml) were incubated in the medium described in Fig. 1 at 37°C. Other conditions were as before.



induced K^+ release as well, though not as effective as 3F·RFM SV, and desacetylrifampicin (DA·RFP). Rifampicin quinone (RFP·Q) gave little effect on the K^+ compartmentation of mitochondrial membrane (Fig. 2).

The K^+ release from red blood cells was also induced in the presence of these antibiotics in such a manner as shown in Fig. 2 (Fig. 3). However the effects were not as remarkable as observed in mitochondria. This is probably due to the difference in the characteristics of these two different kinds of biological membranes, for example, the difference in lipid composition of these membranes.

Table 1. Acceleration of oxygen uptake and potassium release of mitochondria treated with 3-formyl rifamycin SV.

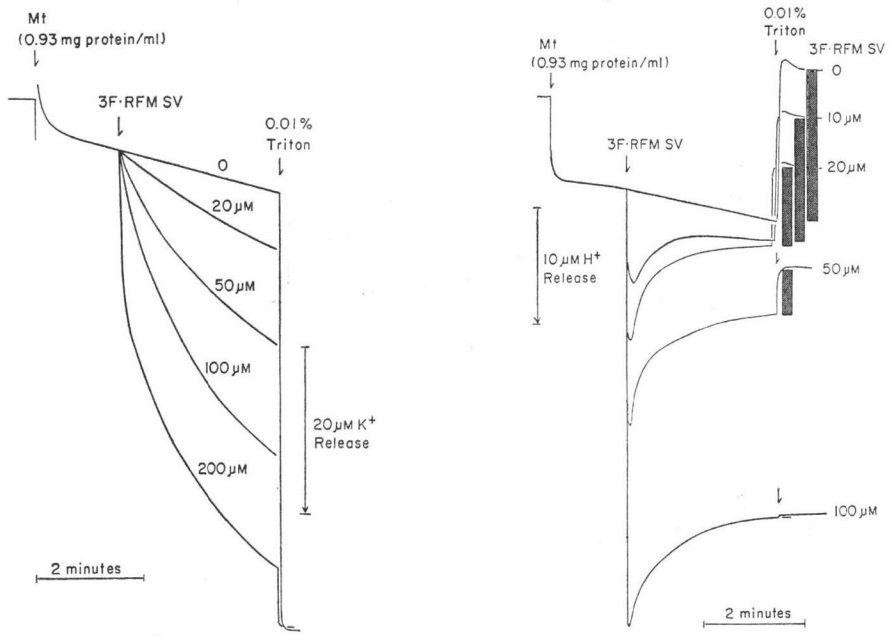
Mitochondria (4.5 mg protein) were incubated in 3.0 ml of a medium containing 0.3 M mannitol, 3 mM $MgCl_2$, 10 mM KCl and 3 mM phosphate buffer (pH 7.4) at 25°C. 3-Formyl rifamycin SV in varied concentration was introduced after the addition of 5 mM sodium succinate as a respiratory substrate. Then 450 μM sodium ADP was added. The velocity of oxygen uptake was calculated from the traces recorded. For the measurement of K^+ content, experimental conditions were the same as in Fig. 1. The figure in parenthesis means the endogenous K^+ content in control mitochondria.

	Oxygen uptake				Respiratory control index	K^+ release	
	State 4 natom/min/mg protein	%	State 3 natom/min/mg protein	%	State 3 / State 4	neq/mg protein	%
Control	19.2	100	97.6	100	5.09	0 (70.1)	0
3F·RFM SV							
20 μM	56.0	291.7	99.2	101.6	1.77	18.2	25.9
40	81.6	425.0	96.0	98.4	1.18	24.7	35.2
100	128.0*	666.7	128.0*	131.1	1.00	31.2	44.4
200	108.0*	566.7	108.8*	111.5	1.00	50.6	72.2

* Maximum values immediately after the addition of 3-formyl rifamycin SV.

Fig. 4. Relationship between K^+ (A) and H^+ movement (B) of mitochondria induced by 3-formyl rifamycin SV.

Mitochondria (4.66 mg protein) were incubated in a medium containing 0.15 M choline chloride, 1 mM Tris-HCl (pH 7.3) at 25°C. Total incubation mixture was 5.0 ml. The H^+ gradients across the mitochondrial membrane are shown by black vertical columns.

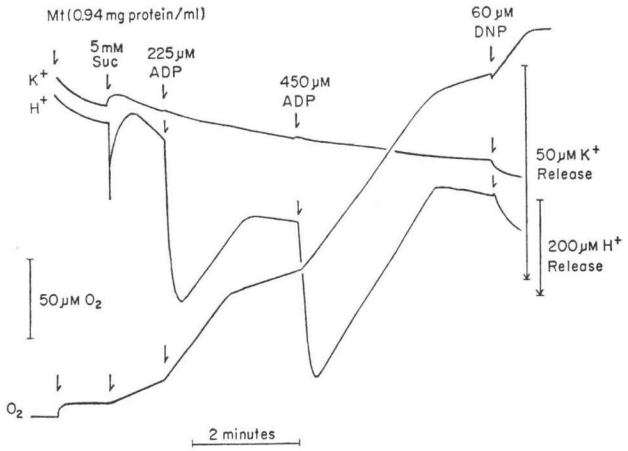


These data suggest that 3F·RFM SV interacts with biological membranes, causing a change in the permeability to ions, especially to K^+ in case of mitochondrial membrane. And this K^+ release from mitochondria probably influences on the H^+ gradient across the membrane, resulting in uncoup-

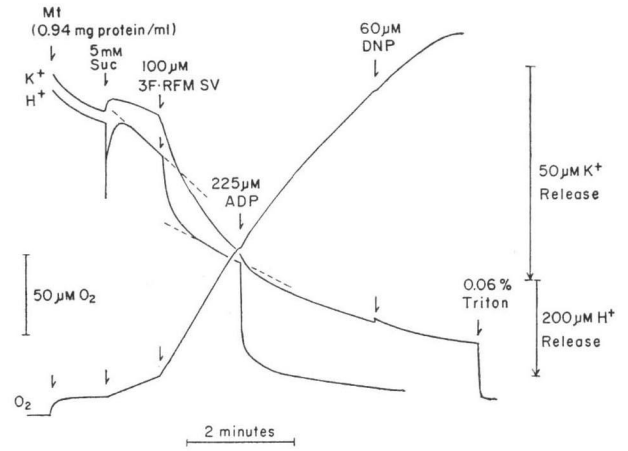
Fig. 5. Effect of 3-formyl rifamycin SV on the respiratory activity and ion compartmentation of rat liver mitochondria.

Mitochondria (4.7 mg protein) were incubated in 5.0 ml of the medium containing 0.3 M mannitol, 3 mM $MgCl_2$, 3 mM phosphate buffer (pH 7.4) at 25°C. 5 mM Tris-succinate, 225 or 450 μM Tris-ADP and 60 μM DNP were introduced at the points indicated. Simultaneous measurements of oxygen uptake with K^+ content or those of oxygen uptake with H^+ content were carried out.

A: Untreated mitochondria as control.



B: 3-Formyl rifamycin SV treated mitochondria.



ling of the oxidative phosphorylation by 3F·RFM SV.

Actually, as shown in Fig. 4B, the H^+ uptake of mitochondria was observed by the addition of 3F·RFM SV depending on its concentration. However, a strict stoichiometry between K^+ efflux and H^+ uptake could not be detected. This is probably due to the buffer capacity of the intramitochondrial space, or the transport of other cations or permeant anions.

Fig. 5 demonstrates the traces of simultaneous measurements of the respiratory activity and the ion compartmentation of mitochondria. In untreated control mitochondria, an appreciable K^+ movement was not seen during the oxidative phosphorylation (Fig. 5A). Abrupt changes of the H^+ concentration just after the additions of 3F·RFM SV and ADP were due to the slight acidity of their solutions. As shown in Fig. 5B, however, there occurred an increased K^+ release from mitochondria in

the presence of 3F·RFM SV, showing a close correlation between these phenomena. On the other hand, the H^+ gradient formed in the presence of succinate was decreased by 3F·RFM SV and H^+ uptake for phosphorylation was not seen. In this instance the stoichiometry between the K^+ and H^+ movement was less distinct, suggesting the transport of other ions across the mitochondrial membrane as described above.

The fact that 3F·RFM SV works not only on mitochondrial membrane but also on erythrocytes and stimulates the K^+ release from vesicles, indicates the possibility that this substance acts as the one causing instabilization of membranes. Therefore, it is concluded that such a property of 3F·RFM SV is a factor that induces uncoupling of oxidative phosphorylation in mitochondria.[1)]

However, the presence of 3F·RFM SV in the serum in measurable quantities has never been reported in subjects treated with therapeutic doses of rifampicin; on the other hand, very small amounts of 3F·RFM SV have been found in the urine of such subjects.[8)] Therefore further investigations are necessary as to whether it does work *in vivo* on biological membranes or not.

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