Biochimica et Biophysica Acta, 1060 (1991) 75–81

ADONIS 000527289100223S

BBABIO 43476

Why is inorganic phosphate necessary for uncoupling of oxidative phosphorylation by Cd^{2+} in rat liver mitochondria?

Haruhiko Koike, Yasuo Shinohara and Hiroshi Terada

Faculty of Pharmaceutical Sciences, University of Tokushima, Tokushima (Japan)

(Received 15 May 1991)

Key words: Cadmium ion; Phosphate transport; Uncoupling; Phosphate/proton symporter; Oxidative phosphorylation; (Rat liver mitochondria)

The phosphate (P_i)-dependent uncoupling action of Cd^{2+} in oxidative phosphorylation in rat liver mitochondria was studied mainly in terms of P_i transport. Cd^{2+} at 2 μM caused full uncoupling in the presence of 10 mM P_i, but no uncoupling in the absence of P_i. Cd^{2+} released state 4 respiration after a certain lag-time, and then the respiration increased progressively with time. After its addition, Cd^{2+} was taken up by mitochondria in a similar period to the lag time before respiratory release. KIH-201, a potent and specific inhibitor of P_i transport via the P_i/H^+ symporter, abolished the uncoupling completely. Cd^{2+} caused dissipation of the electric transmembrane potential ($\Delta\Psi$) and swelling of mitochondria in a P_i-dependent manner. Uncoupling by Cd^{2+} was found to take place in parallel with the uptake of P_i into mitochondria via the P_i/H^+ symporter, suggesting that the uncoupling was due to acceleration of H^+ influx through the P_i/H^+ symporter activated by Cd^{2+}.

Introduction

The mechanism of uncoupling by weakly acidic uncouplers by their protonophoric action has been well documented [1–4]. However, uncoupling mechanisms other than protonophoric actions are not well understood. The hydrophobic peptide antibiotics alamethicin and hypelcin [5], a lipophilic SH-reactive maleimide [6], and amphipathic cations, such as cyanine dyes [7,8], crystal violet [9] and local anesthetics [10–13] induce uncoupling in mitochondria. P_i at high concentrations is also reported to cause uncoupling [14]. These compounds are not protonophores, and their uncoupling features are more or less different from those of weakly acidic uncouplers. Most of these uncoupling actions require P_i, and are accompanied by swelling of the mitochondria. Furthermore, the tertiary amine local anesthetic bupivacaine causes uncoupling by formation of a leakage-type pathway specific for H^+ in combination with amphipathic anions such as ANS^- and TPB^- [13]. Information on the uncoupling mechanisms of these compounds is important for full understanding of the mechanism of oxidative phosphorylation.

The uncoupling action of Cd^{2+} has received considerable interest [15–19], probably because Cd^{2+} is toxic in various biological systems. The mode of action of Cd^{2+} is similar in some respects to the well studied uncoupling action of Ca^{2+} [20,21]. Uncoupling by Cd^{2+} occurs only in the presence of P_i, and is abolished by the P_i-transport inhibitor NEM [22–24], like the action of some non-protonophoric uncouplers including Ca^{2+}. However, the role of P_i in the uncoupling has not been studied precisely, and the action of Cd^{2+} has been discussed mainly in terms of its inhibitory action on coupling factor B (F_B) after its electrogenic transport into the matrix space of mitochondria [25]. F_B, a com-

Abbreviations: KIH-201, 2-(4′-hydroxy-3′-methoxybenzylidene)-4-cyclopentene-1,3-dione; NEM, *N*-ethylmaleimide; P_i, inorganic phosphate; tri-S-C_4 (5), 2,2′-[3-[2-(3-butyl-4-methyl-2-thiazolin-2-ylidene)ethylidene]propenylene]-bis(3-butyl-4-methylthiazolinium iodide); tri-S-C_7 (5), 2,2′-[3-[2-(3-heptyl-4-methyl-2-thiazolin-2-ylidene)ethylidene]propenylene]-bis(3-heptyl-4-methylthiazolinium iodine); TPP^+, tetraphenyl phosphonium; TPB^-, tetraphenyl borate; SF 6847, 3,5-di-tert-butyl-4-hydroxybenzylidenemalononitrile; ANS^-, 1-anilino-8-naphthalenesulfonate; $\Delta\Psi$, electric transmembrane potential; ΔpH, transmembrane pH gradient; $\Delta\mu_{H^+}$, proton electrochemical potential; SDS, sodium dodecyl sulfate.

Correspondence: H. Terada, Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi-1, Tokushima 770, Japan.

ponent of F_0 existing in the matrix space, is proposed to be essential for the coupling of electron transport and ATP synthesis [26–28].

We previously reported that the uncoupling actions of the cyanine dyes tri-S-C_4(5) and tri-S-C_7(5), and the (*o*-phenanthroline)-Cu^{2+} complex are dependent on P_i, and that the P_i transporter (P_i/H^+ symporter) or adenine nucleotide translocator may participate in the uncoupling [7,8,29]. The fact that all these compounds are divalent cations and require P_i for induction of uncoupling prompted us to study the uncoupling action of Cd^{2+} from the viewpoint of the role of P_i. We found clear dependence of its uncoupling effect on the amount of P_i transported into mitochondria.

Materials and Methods

KIH-201 was synthesized as reported previously [30]. SF 6847 was purchased from Wako Pure Chemical Industries, Osaka, Japan. Other reagents used were of the highest grade commercially available.

Mitochondria were isolated from adult male Wistar rats as reported by Myers and Slater [31]. The amount of mitochondrial protein was determined by the Biuret method [32] with bovine serum albumin as a standard.

The respiration of mitochondria was monitored polarographically with a Clark-type oxygen electrode (Yellow Spring, YSI 5331). The incubation medium consisted of 200 mM sucrose and 2 mM $MgCl_2$ in 10 mM potassium phosphate buffer (pH 7.4). In experiments on the effect of P_i, 10 mM Tris-HCl buffer (pH 7.4) was used instead of potassium phosphate buffer. Mitochondria were added at 0.7 mg protein/ml in a total volume of 2.53 ml. Succinate (final concentration 10 mM) plus rotenone (1 μg/mg protein) was used as a respiratory substrate.

Uptake of P_i by mitochondria during respiration was determined by use of [^{32}P]P_i (specific radioactivity, 925 kBq/mmol) at 20°C. Mitochondria (2 mg protein/ml) were incubated in medium consisting of 200 mM sucrose, 2 mM $MgCl_2$ and 10 mM potassium phosphate buffer (pH 7.4), with 10 mM succinate (plus rotenone at 1 μg/mg protein) and a known amount of Cd^{2+} was added to induce uncoupling. After 4 min, 1 ml of mitochondrial suspension was transferred to an Eppendorf tube. Then, [^{32}P]P_i (final concentration, 10 mM) was added, and 5 s later, the uptake of P_i was terminated by addition of the phosphate transport inhibitor KIH-201 (50 nmol/mg protein). The mitochondria were promptly precipitated by centrifugation at 12000 rpm for 1 min in a Kubota centrifuge, model KM 15000, and the pellet was washed twice with incubation medium. The mitochondrial pellet was solubilized in 200 μl of 4% sodium dodecyl sulfate (SDS) and its radioactivity was determined in an Aloka liquid scintillation counter LSC-700.

Uptake of ADP by mitochondria was measured as described previously at 20°C [33].

Uptake of Cd^{2+} was determined by atomic absorption spectrophotometry. To the suspension of mitochondria (0.7 mg protein/ml) in a total volume of 2.53 ml, Cd^{2+} (final concentration 2 μM) was added, and after an appropriate period, 1 ml of the suspension was removed and promptly centrifuged at 12000 rpm for 1 min in a Kubota centrifuge, model KM 15000. The supernatant was diluted 50-fold with 1 M HNO_3, and its Cd^{2+} concentration was determined in an Atomic absorption flame emission spectrophotometer, model Jarrell Ash AA-8500 connected with a Flame less Atomizer, Jarrell Ash FLA-10.

The volume change of mitochondria was monitored as the absorbance change at 540 nm in a Shimadzu recording spectrophotometer, model UV-3000.

The membrane potential of mitochondria was determined with a TPP^+ electrode by the method of Kamo et al. [34] in the incubation medium containing 10 μM TPP^+. The value of ΔpH was determined with [^{14}C]-CH_3COONa (specific radioactivity, 1.85 GBq/mmol) as described previously [35].

Results

There are reports that Cd^{2+} uncouples oxidative phosphorylation only in the presence of P_i, and that its action is inhibited by the P_i transport inhibitor NEM [22–24]. Thus, we first examined the effect of P_i on the Cd^{2+}-induced release of state 4 respiration of rat liver mitochondria with succinate (plus rotenone) as a respiratory substrate at 25°C. Fig. 1 shows the induction of P_i-dependent acceleration of state 4 respiration by 2 μM Cd^{2+} (2.86 nmol/mg protein). This concentration of Cd^{2+} did not have any effect on mitochondrial

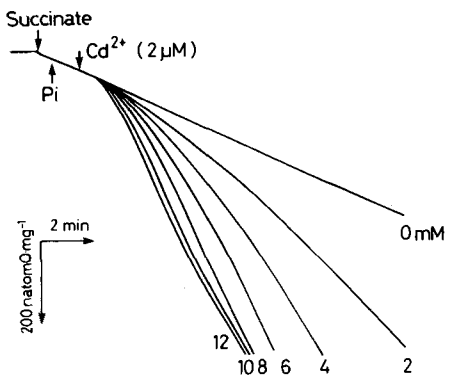

Fig. 1. Effect of P_i on stimulation of state 4 respiration by 2 μM Cd^{2+} at 25°C. Mitochondria were suspended in medium consisting of 200 mM sucrose, 2 mM $MgCl_2$ and 10 mM Tris-HCl buffer (pH 7.4). Cd^{2+} was added 1 min after addition of P_i. Numbers beside traces are P_i concentrations in mM. Mitochondria were added at 0.7 mg protein/ml in a total volume of 2.53 ml. Succinate (10 mM) plus rotenone (1 μg/mg protein) was used as a respiratory substrate.

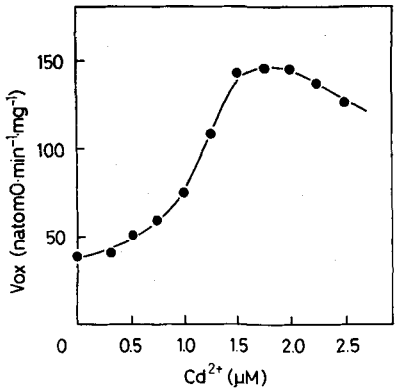

Fig. 2. Acceleration of respiratory rate (V_{ox}) of state 4 mitochondria by various concentrations of Cd^{2+} in the presence of 10 mM P_i at 25°C. Experimental conditions were as for Fig. 1, except that 10 mM phosphate buffer was used instead of 10 mM Tris-HCl buffer. V_{ox} was determined 4 min after addition of Cd^{2+}.

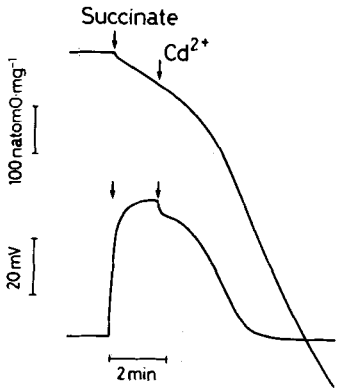

Fig. 3. Time-dependent change in $\Delta\Psi$ (lower trace) caused by acceleration of state 4 respiration by 2 μM Cd^{2+} (upper trace) in the presence of 10 mM P_i at 25°C. Experimental conditions were as for Fig. 2. The membrane potential was monitored with a TPP^+-electrode in the presence of 10 μM TPP^+.

respiration in the absence of P_i in the incubation medium, because the weakly acidic uncoupler SF 6847 fully released state 4 respiration of mitochondria incubated with Cd^{2+} in the absence of P_i (data not shown). However, in the presence of P_i, Cd^{2+} released state 4 respiration, its effect depending on the P_i concentration. The maximum effect was observed with about 10 mM P_i. There was always a lag phase before induction of respiratory release, and the rate of respiratory release after the lag-phase increased progressively with time, attaining a maximum after a certain periods. This- period was dependent on the concentration of P_i. The time required for induction of maximal respiratory release by 2 μM Cd^{2+} decreased with increase in the concentration of P_i, e.g., 6 min at 2 mM P_i and 2 min at 10 mM P_i after addition of Cd^{2+}.

Because 10 mM P_i had the maximum effect on the release of state 4 respiration in the uncoupling of Cd^{2+}, we next determined the concentration dependence of uncoupling by Cd^{2+} in the presence of 10 mM P_i. The time required for induction of maximal release of the respiratory rate decreased with increase in the concentration of Cd^{2+}, as observed with the effect of higher concentrations of P_i at a fixed concentration of Cd^{2+} (cf. Fig. 1). Fig. 2 shows the respiratory rate (V_{ox}) as a function of Cd^{2+} concentration at 25°C. The V_{ox} values shown were measured 4 min after addition of Cd^{2+} when the uncoupled respiration by 2 μM Cd^{2+} had attained a maximum. Under these conditions, 2 μM Cd^{2+} (2.86 nmol/mg protein) caused maximal release of V_{ox}, and higher concentrations inhibited respiration.

Cd^{2+} also activated ATPase and inhibited the synthesis of ATP at similar concentrations to those inducing respiratory release, and these effects also were observed only in the presence of P_i (data not shown). Thus, Cd^{2+} induced uncoupling of oxidative phosphorylation in rat liver mitochondria in cooperation with P_i. Furthermore, Cd^{2+} caused dissipation of the membrane potential, $\Delta\Psi$, measured as uptake of TPP^+ monitored with a TPP^+-selective electrode, in accordance with the acceleration of respiration (Fig. 3). Cd^{2+} had no effect on $\Delta\Psi$ during the lag-phase, but gradually caused dissipation of $\Delta\Psi$ in parallel with the degree of respiratory release. In the absence of P_i, Cd^{2+} had no effect on $\Delta\Psi$ (data not shown).

As shown in Fig. 4, uptake of 2 μM Cd^{2+} by respiring mitochondria with succinate had been completed less than 30 s after its addition in the presence of 10 mM P_i, although induction of full respiratory release required about 2 min. The time required for complete uptake of Cd^{2+} seemed to correspond to the lag time before induction of respiratory release. In

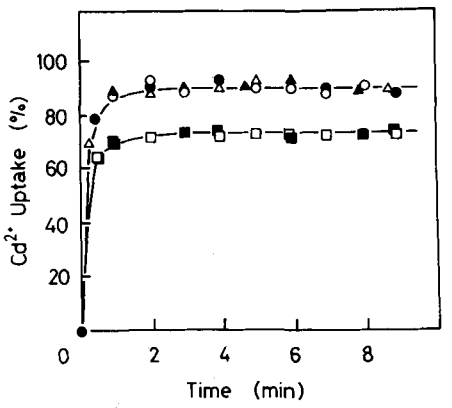

Fig. 4. Uptake of Cd^{2+} by mitochondria under various conditions. Change in Cd^{2+} concentration (initial concentration, 2 μM) was monitored by measuring the Cd^{2+} concentration remaining in the incubation medium at 25°C under various conditions (closed circles) energized with succinate with 10 mM P_i; open circles, energized with succinate without P_i; closed triangles, deenergized with antimycin A (2.5 μg/mg protein) with 10 mM P_i; open triangle, de-energized with antimycin A without P_i; open square, de-energized with 40 nM SF 6847; closed square, deenergized with valinomycin (45.7 ng/mg protein). Experimental conditions were as for Fig. 2.

contrast to Ca^{2+} [21], no discharge of the trapped Cd^{2+} was observed during uncoupling, and about 90% of the added Cd^{2+} was taken up by respiring mitochondria both in the presence and absence of 10 mM P_i. Furthermore, the same amount of Cd^{2+} uptake was observed with mitochondria deenergized by antimycin A either with or without P_i. The amounts of Cd^{2+} taken up by mitochondria deenergized by valinomycin plus K^+ and by the weakly acidic uncoupler SF 6847 were slightly smaller than those by de-energized mitochondria induced by antimycin A, by mitochondria that had not been treated with reagent in the absence of P_i, and by mitochondria under uncoupling by Cd^{2+} (i.e., without reagent in the presence of P_i), but they can be regarded as essentially the same. Thus, the affinity for Cd^{2+} can be regarded to be essentially independent of the energized state of the mitochondria, the presence of P_i, and the time of incubation. It is noteworthy that uptake of 2 μM Cd^{2+} by respiring mitochondria with P_i did not affect ΔpH at all, when the P_i/H^+ symporter was inhibited by KIH-201 to avoid the effect of H^+ influx via the P_i/H^+ symporter: ΔpH in state 4 was 8.0 mV, and that on addition of Cd^{2+} was 8.8 mV. In contrast, valinomycin in the presence of K^+ caused increase in ΔpH (= 62.2 mV) in the presence of KIH-201.

As shown in Fig. 5A, the uncoupling action of Cd^{2+} (2 μM) in the presence of 10 mM P_i was inhibited by addition of the P_i-transport inhibitor KIH-201, which is a more specific and more effective inhibitor of P_i-transport via the P_i/H^+ symporter than NEM [36]. The inhibition of respiratory release by KIH-201 was reversed completely by addition of the typical protonophoric uncoupler SF 6847, indicating that the inhibition of the respiratory release was not due to inhibition of the respiratory chain. The commonly used P_i-transport inhibitor NEM also abolished the uncoupling by Cd^{2+}, but NEM was found to cause partial inhibition of the respiratory chain. Fig. 5B summarizes the dependence of the uncoupled respiratory rate of mitochondria on the concentration of KIH-201. KIH-201 inhibited uncoupling dose-dependently, and its dose-dependent inhibitory effect was similar to that of its inhibition of P_i uptake into mitochondria [36]. Thus, the transport of P_i into mitochondria was suggested to be closely related to the uncoupling by Cd^{2+}.

We next determined the effects of Cd^{2+} on the uptake of ADP and P_i by mitochondria, because some non-protonophoric uncouplers have effects on the transports of these anions [7,8]. Uptake of ADP and P_i into mitochondria are very rapid at 25°C [37,38], so we determined their rates at 20°C. Cd^{2+} at concentrations causing uncoupling did not have any effect on the transport of ADP (data not shown), but it enhanced P_i uptake. The amount of P_i transferred into mitochondria was measured as follows: mitochondria were incubated with 10 mM non-radioactive P_i in medium containing succinate to induce uncoupling, which was monitored with an oxygen electrode, and 4 min after addition of Cd^{2+} an aliquot of the suspension was transferred to an Eppendorf tube, and [^{32}P]P_i was added. After 5 s, uptake of P_i was terminated by addition of KIH-201, and the amount of [^{32}P]P_i incorporated into mitochondria was determined. As shown in Fig. 6, Cd^{2+} activated P_i transport into mitochondria. However, Cd^{2+} failed to accelerate P_i uptake, when the weakly acidic uncoupler SF 6847 was present. It also did not accelerate the transport of P_i in the presence of inhibitors of P_i transport via the P_i/H^+ symporter, KIH-201 and NEM, and the respiratory chain inhibitor antimycin A (data not shown).

There was a clear linear relationship between the uncoupled respiratory rate V_{ox} induced by Cd^{2+} and the rate of P_i transport into the uncoupled mitochondria determined at 20°C, as shown in Fig. 7. This linear relationship is expressed by Eqn. 1, where J_{P_i}

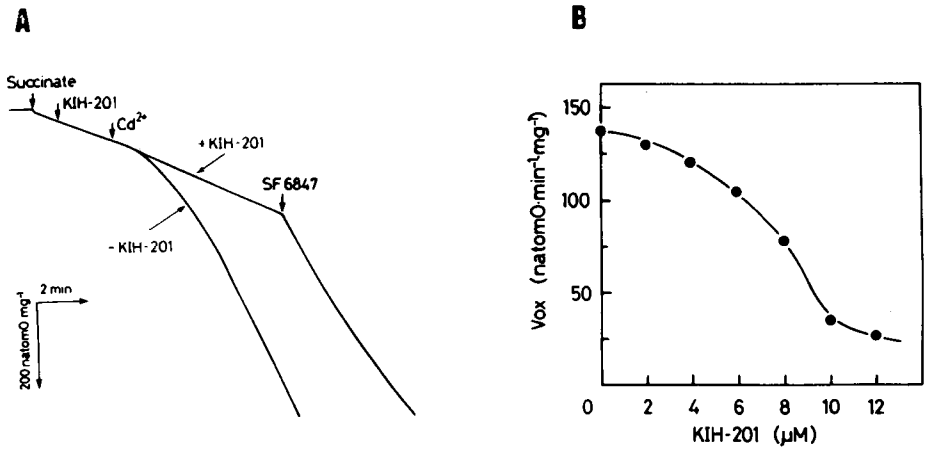

Fig. 5. Effect of the P_i transport inhibitor KIH-201 on uncoupling by 2 μM Cd^{2+} in cooperation with 10 mM P_i (A) and dose-response curve of the inhibition of uncoupling by KIH-201(B). Experimental conditions were as for Fig. 2. KIH-201 (final concentration, 10 μM) was added at the times indicated on the traces (A). The concentration of SF 6847 was 40 nM.

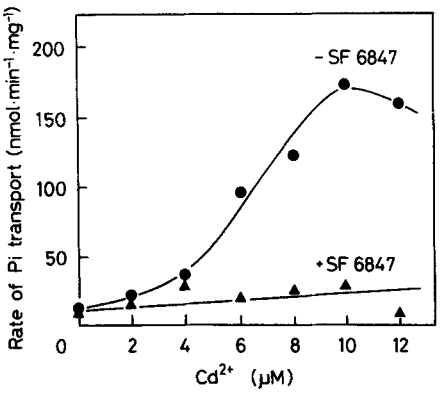

Fig. 6. Effect of Cd^{2+} on the transport of P_i into mitochondria in the presence of 10 mM P_i at 20°C. Uptake of [^{32}P] P_i by mitochondria (2.0 mg protein/ml) suspended in incubation medium in the presence of 10 mM P_i (total volume, 2.53 ml) was determined 4 min after addition of Cd^{2+} in the absence (closed circles) or presence (closed triangles) of the protonophoric uncoupler SF 6847 at 100 nM. Oxygen consumption by uncoupled mitochondria was monitored concomitantly. For details see Materials and Methods.

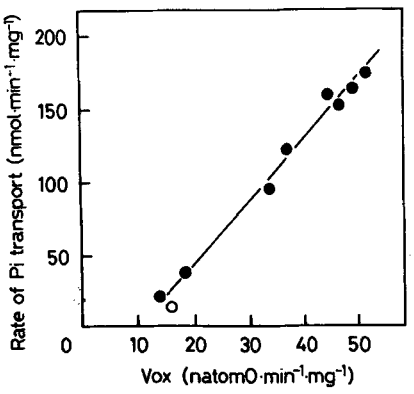

Fig. 7. Stimulation of P_i transport by uncoupling of Cd^{2+} at 20°C. The respiratory rates, V_{ox}, at 20°C are plotted as a function of rates of P_i transport via the P_i/H^+ symporter from the results of Fig. 6. The open circle shows the value in the absence of Cd^{2+}, i.e., state 4 mitochondria.

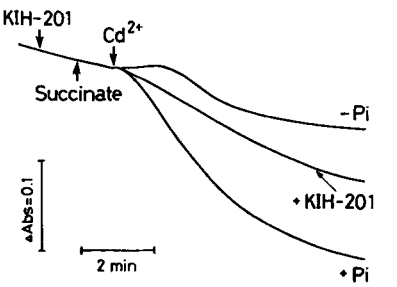

Fig. 8. Volume change of mitochondria caused by 2 μM Cd^{2+} in the presence or absence of 10 mM P_i in the incubation medium. Change in the optical absorbance at 540 nm was monitored at 25°C. Downward deflection indicates swelling of mitochondria [38]. Mitochondria (0.7 mg protein/ml in a total volume of 2. 50 ml) energized with succinate were suspended in the incubation medium as described in the legend of Fig. 2. For the trace without P_i, 10 mM Tris-HCl buffer was used instead of phosphate buffer. The effect of 10 μM KIH-201 was also examined (+KIH-201).

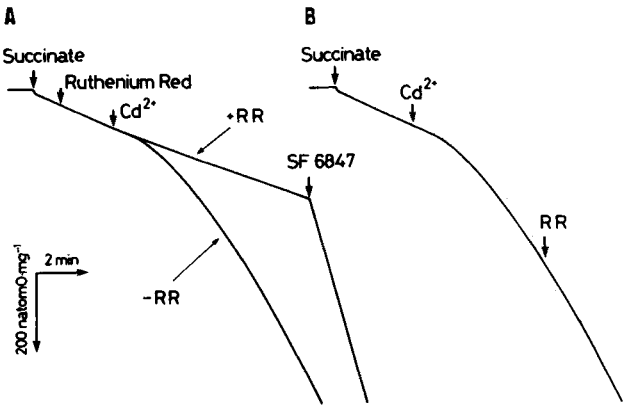

Fig. 9. Effect of ruthenium red (RR) on the uncoupling of Cd^{2+}. Ruthenium red at 100 nM was added either before (A) or after (B) addition of 2 μM Cd^{2+} to mitochondrial suspension at 25°C. SF 6847 was added at a concentration of 40 nM. Experimental conditions were as for Fig. 2.

and J_0 are the rates of P_i transport and oxidation, respectively.

$$J_{P_i} = 4.38J_0 - 46.14 \quad (1)$$

The significant correlation of Eqn. 1 with a correlation coefficient of 0.991 suggests that uncoupling of Cd^{2+} is directly related to P_i transport via the P_i/H^+ symporter.

Fig. 8 shows the volume change of mitochondria monitored as change in the absorbance at 540 nm of a mitochondrial suspension in the presence of 2 μM Cd^{2+} (2.86 nmol/mg protein), which induced full uncoupling in the presence of P_i. A decrease in absorbance density is interpreted as indicating swelling of the mitochondria [39]. In the presence of 10 mM P_i in the incubation medium, decrease in absorbance was observed after a certain lag-time, but in the absence of P_i there was little absorbance change. Furthermore, the P_i transport inhibitor KIH-201 almost completely prevented the absorbance change in the presence of P_i.

We next examined the effect of Ruthenium red, an inhibitor of Ca^{2+} uptake via the Ca^{2+}-uniporter [40], on the uncoupling action of Cd^{2+}, because the uncoupling action of Cd^{2+} was similar in some respects to that of Ca^{2+}. As shown in Fig. 9A, Ruthenium red inhibited the induction of uncoupling by Cd^{2+} when added before Cd^{2+}. However, it had no effect on the uncoupling of Cd^{2+} (Fig. 9B), when added during uncoupling by Cd^{2+}, as observed with kidney mitochondria [43].

Discussion

The P_i-dependent uncoupling by Cd^{2+} has been studied extensively [15–19,22–28], although the effect of P_i transport during uncoupling has not been exam-

ined. In most cases, uncoupling by Cd^{2+} has been interpreted to be due to the inhibitory effect of Cd^{2+} on the activity of F_B by binding with a dithiol site of the protein from the matrix side of the mitochondria [25–28]. However, from our present results this modification seems not to be the trigger of uncoupling.

We found that the uncoupling by Cd^{2+} caused enhancement of P_i uptake via the P_i/H^+ symporter in a manner such that the uptake of P_i increased linearly with the uncoupling of respiration. The inhibitory effect of KIH-201 on the uncoupling increased with increase in the concentration of KIH-201 in a similar manner as that observed in its inhibition of P_i uptake into mitochondria and the ΔpH across the mitochondrial membrane was not changed by uptake of Cd^{2+}. These results showed that uncoupling is due to the transport of P_i into mitochondria via the P_i/H^+ symporter, and excluded the possibility that the stimulation of the P_i/H^+ symporter by the concentration gradient of P_i or H^+ [41] is directly responsible for induction of uncoupling. In contrast to the uncoupling by Cd^{2+}, uptake of P_i is inhibited in the uncoupling by SF 6847, as in uncoupling by other protonophoric uncouplers [42]. This inhibition has been interpreted as due to a decrease in ΔpH, which governs H^+ transport via the P_i/H^+ symporter [42].

The finding that addition of ruthenium red before Cd^{2+} inhibited the induction of Cd^{2+}-uncoupling suggests that Cd^{2+} penetrates into mitochondria via the Ca^{2+}-uniporter. However, the possibility that the cycling of Cd^{2+} across the mitochondrial membrane via the Ca^{2+}-uniporter and Ca^{2+}/H^+-antiporter caused uncoupling, as in the uncoupling action of Ca^{2+} can be ruled out by the following findings: (i) Ruthenium red had no effect on the uncoupling by Cd^{2+} when it was added after induction of uncoupling by Cd^{2+}. (ii) The ΔpH was not changed by uptake of Cd^{2+} by respired mitochondria in the presence of KIH-201 to inhibit the P_i/H^+ symporter. (iii) The presence of P_i was indispensable for induction of Cd^{2+} uncoupling. (iv) A certain period was necessary before induction of full release of respiration, whereas uptake of Cd^{2+} occurred in accordance with the lag-time before induction of respiratory release, being independent of the presence of P_i in the incubation medium and the energy state of the mitochondria.

It is also possible that transport of P_i facilitates the transfer of Cd^{2+} into the mitochondria and that this transfer causes dissipation of $\Delta\Psi$, regardless of whether or not the transferred Cd^{2+} recycles across the mitochondrial membrane. However, about 90% of the added Cd^{2+} was taken up by mitochondria before induction of uncoupling and the amount of bound Cd^{2+} was insensitive to change in the energy state of mitochondria. Thus, this possibility can also be ruled out.

It is possible that H^+ efflux through the respiratory chain is directly coupled with H^+ influx in combination with influx of P_i through the P_i/H^+ symporter, keeping the ratio of J_{P_i} to J_0 at 4.38 (cf. Eqn. 1). If we assume that almost all the extruded H^+ coupled with electron transfer from succinate to O_2 is transported into the matrix space of uncoupled mitochondria via the P_i/H^+ transporter keeping $H^+/O = 6$, and that the transports of both $H_2PO_4^-$ and HPO_4^{2-} are coupled with H^+, the ratios of P_i^-/O and P_i^{2-}/O would be 6 and 3, respectively. It is interesting to note that the value of J_{P_i}/J_0 (= 4.38) is the average of these P_i^-/O and P_i^{2-}/O values. The reason for the stoichiometry of J_{P_i}/J_0 should be studied further. In view of these results, uncoupling of Cd^{2+} is suggested to be due to enhancement of H^+ influx via the P_i/H^+ symporter.

From the present results, we propose as a mechanism of Cd^{2+}-uncoupling that Cd^{2+} penetrates into the mitochondria via the Ca^{2+}-uniporter, and then causes some conformational change of the P_i/H^+ symporter by reacting with an SH group(s) from the matrix side of the mitochondrial membrane. This conformational change results in stimulation of the influxes of H^+ and P_i, and the transported H^+ acts as a trigger for uncoupling. However, it is not clear at present why P_i associated H^+ influx is activated, even though $\Delta\mu_{H^+}$ generated by respiration is dissipated.

As the features of the uncoupling actions of the cyanine dyes tri-S-C_4(5) and tri-S-C_7(5) [7,8], the (*o*-phenanthroline)$_2$-Cu^{2+} complex [29], phenylarsine oxide [24], crystal violet [9] and Ca^{2+} [21] are very similar to those of uncoupling by Cd^{2+}, acceleration of P_i uptake by mitochondria is also expected to play a key role in induction of these uncoupling actions. The mechanism of co-transport of P_i and H^+ mediated by the P_i/H^+ symporter under these uncoupling conditions is important, and studies on this mechanism are under way.

References

1 Hanstein, W.G. (1976) Biochim. Biophys. Acta 456, 129–148.
2 McLaughlin, S.G. and Dilger, J.P. (1980) Physiol. Rev. 60, 825–863.
3 Terada, H. (1981) Biochim. Biophys. Acta 639, 225–242.
4 Terada, H. (1990) Environ. Health Perspect. 87, 213–218.
5 Takaishi, Y., Terada, H. and Fujita, T. (1980) Experimentia 36, 550–551.
6 Kiehl, R. and Bauerlein, E. (1976) FEBS Lett. 72, 24–28.
7 Terada, H., Nagamune, H., Morikawa, N. and Ikuno, M. (1985) Biochim. Biophys. Acta 807, 168–176.
8 Shinohara, Y., Nagamune, H. and Terada, H. (1987) Biochem. Biophys. Res. Commun. 148, 1081–1086.
9 Moreno, S.N., Gadelha, F.R. and Docampo, R. (1988) J. Biol. Chem. 263, 12493–12499.
10 Garlid, K.D. and Nakashima, R.A. (1983) J. Biol. Chem. 258, 7974–7980.
11 Dabadie, P., Bendriss, P., Erny, P. and Mazat, J.-P. (1987) FEBS Lett. 226, 77–82.

12 Rottenberg, H. (1990) Biochim. Biophys. Acta 1018, 1–17.
13 Terada, H., Shima, O., Yoshida, K. and Shinohara, Y. (1990) J. Biol. Chem. 265, 7838–7842.
14 Vaghy, P.L., Matlib, M.A. and Schwartz, A. (1981) Biochem. Biophys. Res. Commun. 100, 37–44.
15 Jacobs, E.E. and Sanadi, D.R. (1955) Biochim. Biophys. Acta 17, 290–292.
16 Jacobs, E.E. Jacob, M. Sanadi, D.R. and Bradley, L.B. (1956) J. Biol. Chem. 223, 147–156.
17 Fluharty, A.L. and Sanadi, D.R. (1961) Proc. Natl. Acad. Sci. USA 46, 608–616.
18 Fluharty, A.L. and Sanadi, D.R. (1962) Biochemistry 1, 276–281.
19 Fluharty, A.L. and Sanadi, D.R. (1963) Biochemistry 2, 519–522.
20 Nicholls, D.G. and Scott, I.D. (1980) Biochem. J. 186, 833–839.
21 Nicholls, D.G. and Akerman, K. (1982) Biochim. Biophys. Acta 683, 57–88.
22 Sanadi, D.R., Hughes, J.B. and Joshi, S. (1981) J. Bioenerg. Biomembr. 13, 425–431.
23 Rasheed, B.K.A., Diwan, J.J. and Sanadi, D.R. (1984) Eur. J. Biochem. 144, 643–647.
24 Novgorodov, S.A., Kultayeva, E.Y., Yaguzhinsky, L.S. and Lemeshko, V.V. (1987) J. Bioenerg. Biomembr. 19, 191–202.
25 Sanadi, D.R. (1982) Biochim. Biophys. Acta 683, 39–56.
26 Sanadi, D.R., Pringle, M., Kantham, L. Hughes, J.B. and Srivastava, A. (1984) Proc. Natl. Acad. Sci. USA 81, 1371–1374.
27 Huang, Y., Kantham, L. and Sanadi, D.R. (1987) J. Biol. Chem. 262, 3007–3010.
28 Stephenson, G. and Sanadi, D. R. (1989) Biochem. Int. 19, 1087–1094.
29 Shinohara, Y. and Terada, H. (1987) Biochim. Biophys. Acta 890, 387–391.
30 Inayama, S., Mamoto, K., Shibata. T. and Hirose, T. (1976) J. Med. Chem. 19, 433–436.
31 Myers, D.K. and Slater, E.C. (1957) Biochem. J. 67, 558–572.
32 Gornall, A.G., Bardawill, C.J. and David, M.M. (1949) J. Biol. Chem. 177, 751–766.
33 Winkler, H.H., Bygrave, F.L. and Lehninger, A.L. (1968) J. Biol. Chem. 243, 20–28.
34 Kamo, N., Muratsugu, M. Hongoh, R. and Kobatake, Y. (1979) J. Membr. Biol. 49, 105–121.
35 Dawson, A., Klingenberg M. and Kramer, R. (1987) in Mitochondria (Darley-Usmer V.M., Rickwood, D. and Wilson, M.T., eds.), pp. 35–78, IRL Press.
36 Koike, H., Hori, H., Inayama, S. and Terada, H. (1988) Biochem. Biophys. Res. Commun. 155, 1066–1074.
37 Klingenberg, M., Grebe, K. and Appel, M. (1982) Eur. J. Biochem. 126, 263–269.
38 Ligeti, E., Brandolin, G., Dupont, Y. and Vignais, P.V. (1985) Biochemistry 24, 4423–4428.
39 Lehninger, A.L. (1974) Proc. Natl. Acad. Sci. USA 71, 1520–1524.
40 Brierley, G.P. and Jung, D.W. in Inhibitors of Mitochondrial Functions (Erccinska, M. and Wilson, D.F. eds.) pp. 305–328, Pergamon Press, Oxford.
41 Nicholls, D.G. (1978) Biochem. J. 176, 463–474.
42 Lehninger, A.L. Reynafarje, B. (1982) Curr. Topics Cell. Regul. 18, 329–341.
43 Chavez, E., Briones, R., Michel, B., Bravo, C. and Jay, D. (1985) Arch. Biochem. Biophys. 242, 493–497.