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An increase of the energy coupling capacity of submitochondrial particles by lanthanides

V.G. Grivennikova, E.V. Gavrikova, A.D. Vinogradov*

Department of Biochemistry, School of Biology, Moscow State University, 119899 Moscow, Russian Federation

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Abstract

NADH and succinate oxidase activities of inside-out submitochondrial particles treated with excess oligomycin are inhibited by lanthanides (La^{3+} and Dy^{3+}). Both inhibition by oligomycin and oligomycin *plus* lanthanides are completely relieved by an uncoupler. The respiratory control measured as the stimulation of NADH or succinate oxidation caused by the addition of uncoupler to the oligomycin-treated particles is thus increased in the presence of lanthanides. The coupling effect of lanthanides is completely prevented and rapidly reversed by excess EDTA. La^{3+} increases the extent of the aerobic energy-linked succinate-supported NAD^+ reduction catalyzed by the oligomycin-treated submitochondrial particles. Lanthanides seem to be useful tools to increase the energy coupling capacity of the submitochondrial particles.

Key words: Lanthanide, Energy coupling; Submitochondrial particle

1. Introduction

Respiratory control as measured by the stimulation of respiration by an uncoupler has been demonstrated in certain submitochondrial particles (SMP) treated with the F_0-directed energy transfer inhibitors such as oligomycin [1,2], DCCD [3], chloromercurinitrophenol [4] or with a number of 'coupling factors' derived from the resolved mitochondrial preparations [5,6]. The ability of oligomycin and DCCD to induce the respiratory control provides a simplified system to analyze the kinetics and thermodynamics of the respiratory chain components in the energized state, i.e. when free energy of the intramolecular redox reactions within the individual complexes is in apparent equilibrium with $\Delta\bar{\mu}_{H^+}$. It is anticipated that some of the redox components directly involved in the vectorial H^+-translocation may only be detected in such an energized state. For example, rapidly relaxing Complex I-associated ubisemiquinone(s) is only seen in tightly coupled SMP [7].

One general problem in studies with the resolved SMP is that even in the presence of oligomycin or DCCD the preparations are never absolutely coupled. The reasons for that are not clear: the heterogenity of inside-out SMP, proton leakage through the phospholipid bilayer, nonspecific protein-mediated proton conductivity and possible slip in operation of the proton pumps may contribute to the respiration in the presence of the energy-transfer inhibitors. Search for the factors which would increase the energy coupling capacity of SMP thus seems to be worthwhile. In this report we will show that lanthanides (LA^{3+} and Dy^{3+}) are capable of increasing the respiratory control and stimulation of the energy-linked reverse electron transfer in the oligomycin-treated SMP.

2. Materials and methods

Bovine heart submitochondrial particles were prepared as described [8] and stored in liquid nitrogen. NADH oxidase activity was measured spectrophotometrically as a decrease of absorption at 340 nm ($\varepsilon_{340,mM}$ = 6.22). Succinate oxidase was measured with an oxygen electrode. Protein content was determined by the biuret procedure [9].

BSA (Fraction V), oligomycin and CCCP were from Sigma (USA), $LaCl_3 \cdot 6H_2O$ and $DyCl_3 \cdot 4H_2O$ were from Alfa Products (USA), NADH was from Reanal (Hungary), Tris was from Serva (Germany). Other chemicals were of the purest grade commercially available.

3. Results

In agreement with the classical observations [1,2] oligomycin at a concentration inhibiting more than 90% of ATPase activity of SMP causes an inhibition of respiration with NADH as the substrate, and this inhibition is relieved by an uncoupler (Fig. 1, trace A). The respiration in the presence of oligomycin was further decreased by $LaCl_3$ and the uncoupler caused an activation of NADH oxidase up to the level observed without lanthanide (Fig. 1, trace B). The net result caused by lanthanide was an increase of the oligomycin-induced respiratory control. Table 1 summarizes data illustrating this phenomenon. In different batches of SMP routinely prepared in this laboratory [8] the respiratory control ratios as measured by stimulation of respiration by an uncou-

*Corresponding author. Fax: (7) (095) 939 3955.

Abbreviations: SMP, submitochondrial particles; BSA, bovine serum albumin; DCCD, *N*, *N'*-dicyclohexylcarbodiimide; F_0, membrane sector of the mitochondrial F_0-F_1 ATPsynthase; Complex I, NADH-ubiquinone oxidoreductase (EC 1.6.99.3); CCCP, carbonylcyanide-*m*-chlorophenylhydrazone.

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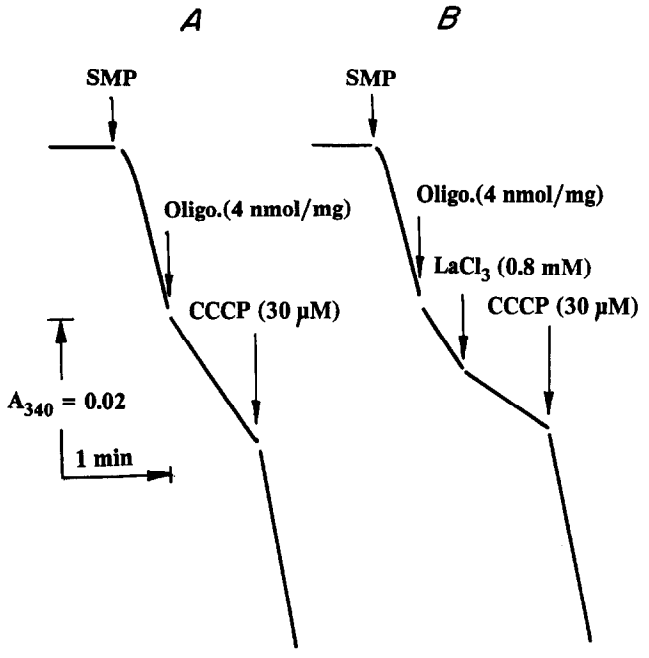

Fig. 1. Effect of $LaCl_3$ on the oligomycin-induced respiratory control. The assay cuvette (2 ml) contained 0.2 M sucrose, 50 mM Tris-acetate (pH 7.5), BSA (1 mg/ml) and 0.1 mM NADH. The reaction was initiated by the addition of submitochondrial particles (12 μg/ml). Further additions are indicated.

pler after treatment with oligomycin are 3–8 with NADH and 3–5 with succinate. Both La^{3+} and Dy^{3+} increased respiratory control in loosely coupled SMP with either NADH (Exp. 1 and 2) or succinate (Exp. 3). It should be noted that high concentrations of succinate (20 mM) prevented the coupling effect of La^{3+} (Exp. 1, sample 3) presumably due to chelation of the lanthanide cation [10]. The same phenomenon was observed with EDTA which completely abolished the coupling effect of La^{3+} (Fig. 2) and Dy^{3+} (Table 1, Exp. 2, sample 3). Nevertheless, the coupling effect of La^{3+} was observed with succinate when concentration of the latter was decreased to 1 mM (Table 1, Exp. 3). The coupling effect of La^{3+} was concentration-dependent (saturation at ~0.9 mM) and completely reversed by a slight molar excess of EDTA (Fig. 2).

The observations reported may be interpreted in terms of the coupling effect of lanthanides on energy conservation in the respiratory chain. Therefore, it might be expected that the energy-linked reactions catalyzed by SMP would be stimulated by lanthanides. In an attempt to relate the inhibitory effect of La^{3+} and Dy^{3+} on respiration to their coupling capacity, the aerobic succinate-supported reverse electron transfer was examined. In agreement with our previous data [8] oligomycin-treated SMP catalyzed NAD^+ reduction at the expense of energy produced by the succinate oxidation (Fig. 3). With limited NAD^+ the steady-state mass action ratio of 14 for NAD^+/NADH couple was established. The addition of La^{3+} caused an additional reduction of NAD^+ (NAD^+/NADH ratio equal to 6 was reached) which was completely reversed by 10 mM malonate or by an uncoupler (not shown). The results obtained unambiguously suggest that lanthanides increase $\Delta\bar{\mu}_{H^+}$ across the coupling membrane without any inhibitory effect on the respiratory chain.

It was shown in the separate experiments that neither Ruthenium red (30 nM) nor Ca^{2+} (1 mM) and another polivalent cation spermine (200 μM) had any significant effects on respiration and the reverse electron transfer catalyzed by the oligomycin-treated SMP. Lanthanides were unable to decrease respiration of the tightly coupled SMP having the respiratory control ratio of 7–9 in the presence of oligomycin with NADH as a substrate.

4. Discussion

It is obvious that an artificial improvement of energy conservation in the respiratory chain by lanthanides provides a useful tool for studies on the mechanisms of $\Delta\bar{\mu}_{H^+}$ generation. It should be pointed out that the use of lanthanides is somewhat limited by their 'inconvenient' chemistry in aqueous solutions (limited solubility at neutral pH, strong tendency to complex formation, low sol-

Table 1
Effect of La^{3+} and Dy^{3+} on the respiratory control ratio in submitochondrial particles

	NADH oxidase[a] (μmol/min per mg)		Respiratory control ratio
	−CCCP	+30 μM CCCP	
Exp. 1			
1. Control[b]	0.25	0.86	3.5
2. +0.5 mM $LaCl_3$	0.16	0.81	5.0
3. +0.5 mM $LaCl_3$ +20 mM succinate	0.29	0.96	3.4
Exp. 2			
1. Control[b]	0.38	1.06	2.8
2. +0.2 mM $DyCl_3$	0.21	0.91	4.3
3. +0.2 mM $DyCl_3$ +0.5 mM EDTA	0.37	1.06	2.7
	Succinate oxidase[c] (μmol/min per mg)		
Exp. 3			
1. Control[b]	0.15	0.40	2.7
2. +1.2 mM $LaCl_3$	0.07	0.35	5.0

[a] Measured spectrophotometrically at 340 nm as described in Fig. 1.
[b] Different batches of SMP treated with oligomycin (4 nmol/mg) to induce the respiratory control [2] were used.
[c] Succinate oxidase was measured with an oxygen electrode in a mixture containing 0.2 M sucrose, 50 mM Tris-acetate (pH 7.5), BSA (1 mg/ml), 1 mM succinate and 5 μM malonate (introduced with particles from the 'activation' medium [8]). The reaction was initiated by the addition of SMP (0.3 mg/ml).

ubility products with many anions) [11]. Our attempts to use lanthanides for coupling the ATP-dependent reactions have failed due to an immediate precipitation of their phosphate salts. Nevertheless, the results presented in the previous section show that with some precautions the lanthanides can be sued for the restoration of energy conservation in loosely coupled SMP.

The mechanism of the lanthanides-induced coupling remains to be established. Among several possibilities, one seems to deserve brief discussion. Lanthanides are known to be the powerful inhibitors of the mitochondrial Ca^{2+}-uniport system [12,13]. It has been shown that Ca^{2+}-uniporter contributes significantly to the proton leakage in rat liver mitochondria, presumably due to the lack of absolute specificity of the Ruthenium red-sensitive cation transport system [14,15]. the lanthanide-induced coupling may be caused by the inhibition of the proton leakage through the Ca^{2+}-uniporter. The absence of the Ruthenium red-induced coupling and much higher concentrations of lanthanides needed to increase the respiratory control (this paper) as compared to their efficiency in Ca^{2+} transport inhibition [16] may be explained by the opposite polarity of the coupling membrane in intact mitochondria and SMP. If Ca^{2+} uniporter is asymmetrically arranged in mitochondria with the Ruthenium red-sensitive gate for Ca^{2+}, H^+ or lanthanides located on the outer surface of the inner membrane, the lack of the proton leakage sensitivity to Ruthenium red and low efficiency of lanthanides are expected in inside-out SMP. The other mechanisms of the lanthanide-induced effect such as phospholipid and/or protein immobilization cannot be excluded. The latter explanation has been offered for the inhibitory effect of La^{3+} on the slow phase of proton transfer during the bacteriorhodopsin photocycle [17].

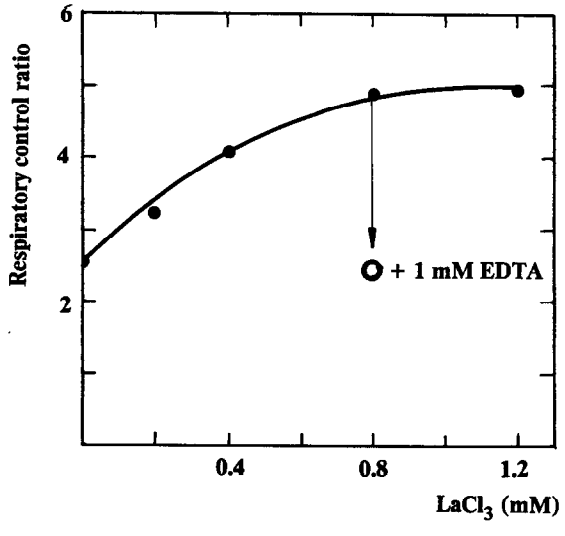

Fig. 2. An increase of the energy coupling capacity in submitochondrial particles by $LaCl_3$. The assay conditions are described in Fig. 1. The open point indicates the respiratory control ratio in the presence of 0.8 mM $LaCl_3$ and 1 mM EDTA.

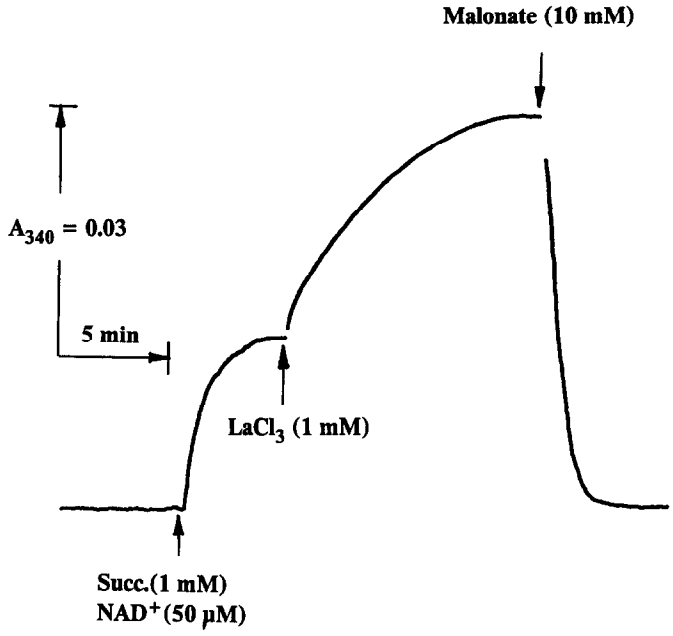

Fig. 3. Effect of $LaCl_3$ on aerobic succinate-supported NAD^+ reduction (reverse electron transfer). The reaction mixture (2 ml) contained 0.2 M sucrose, 50 mM Tris-acetate (pH 7.5), BSA (1 mg/ml) and submitochondrial particles treated with oligomycin (4 nmol/mg). SMP were pulsed with 5 μM NADH before the reaction was initiated as indicated.

Whatever the mechanism of the lanthanide-coupling effect, their capacity to increase energy conservation in the respiratory chain seems to be a useful experimental tool.

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