UNCOUPLING AND CHARGE TRANSFER IN SUBMITOCHONDRIAL PARTICLES

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SUMMARY

Loss of respiratory control can be obtained in submitochondrial particles when a combination of K^+, nigericin and valinomycin, or NH_4Cl and valinomycin, is present. When permeant anions such as NO_3 or tetraphenylboron are present, valinomycin is not required in either case. It is suggested that valinomycin acts by permitting an electrophoretic efflux of K^+ or NH_4^+, and that the penetration of permeant anions into the particle can substitute for this efflux in the uncoupling effect. Comparisons are made between these results obtained in SMP and similar effects described in chromatophores and chloroplasts.

We have recently reported that submitochondrial particles (SMP) are still capable of energy-linked K^+ transport when treated with a combination of ion-transporting antibiotics (1), i.e., a nigericin-dependent loading of K^+ in the particle, followed by a valinomycin-mediated efflux of the K^+ taken up. This movement of K^+ across the coupling membrane results in uncoupling. Similar observations have also been reported from several other laboratories (2,3,4). In particular, Cockrell and Racker (2), have found that these ion movements are dependent upon the anion used, NO_3^- being the most active in stimulating K^+ transport. Both Papa et al. (5) and Cockrell and Racker (2) have reported uncoupling of SMP with a combination of NH_4Cl and valinomycin.

These two conditions of uncoupling in SMP suggest that the effect of valinomycin is to discharge an electrical gradient created by the accumulation of the charged-species (K^+ or NH_4^+, respectively) in the particle. If this were true, the role of valinomycin could be substituted by a permeant anion that would be transported electrophoretically, hence neutralizing the positive electric gradient, and resulting in uncoupling (6).

METHODS: Submitochondrial particles derived by sonic disruption of beef heart mitochondria were prepared as previously reported (7). Oxygen consumption was measured by conventional polarographic methods.

Abbreviations used: (E-) SMP, (EDTA) submitochondrial particles. FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone. NaTPB, sodium tetraphenyl boron.

RESULTS: Lee and Ernster (8) observed that SMP oxidizing NADH exhibit an oligomycin induced respiratory control, which is released by uncouplers. As shown in Fig. 1 addition of KNO_3 and nigericin, or NH_4NO_3 stimulated the respiration of an oligomycin-treated preparation. Furthermore, preincubation of SMP with KNO_3 and nigericin or NH_4NO_3 results in an

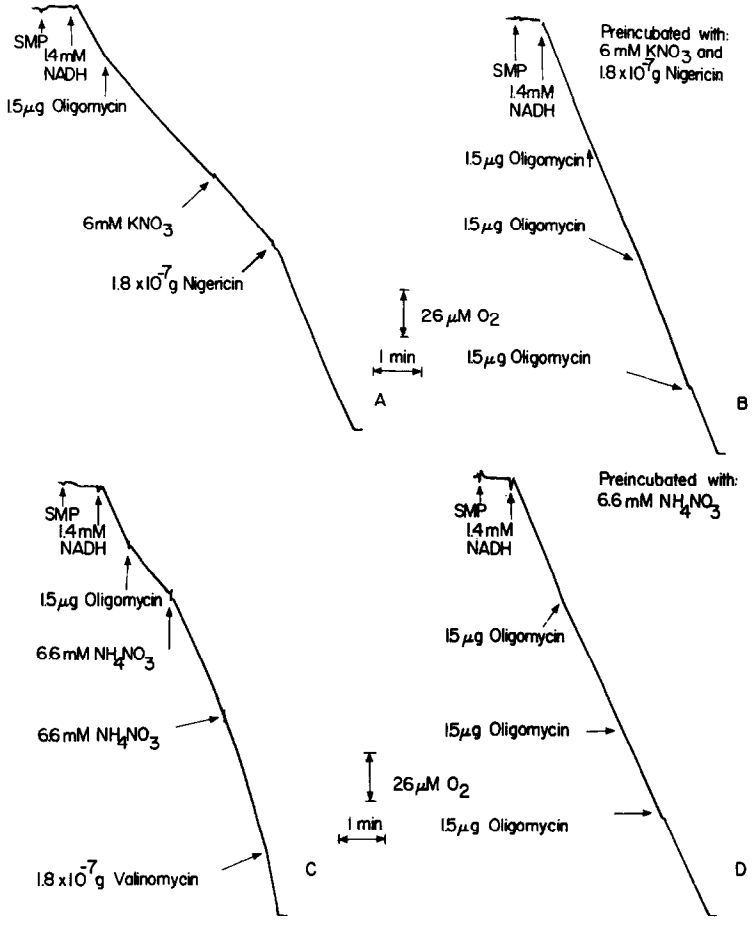

Figure 1. Effect of KNO_3 + nigericin (A and B) and NH_4NO_3 (C and D) on the oligomycin-induced "respiratory control." The reaction mixture contained 0.18 mg protein per ml of E-SMP, 0.25 M sucrose, 0.05 M Tris-acetate, pH 7.4. Final volume: 3.3 ml. Temperature, 25°C.

inability of oligomycin to inhibit (couple) respiration. A similar situation results if KNO_3 and nigericin or NH_4NO_3 are replaced by a typical uncoupler such as FCCP.

Mueller and Rudin (9) and Skulachev et al. (10) have reported voltage-

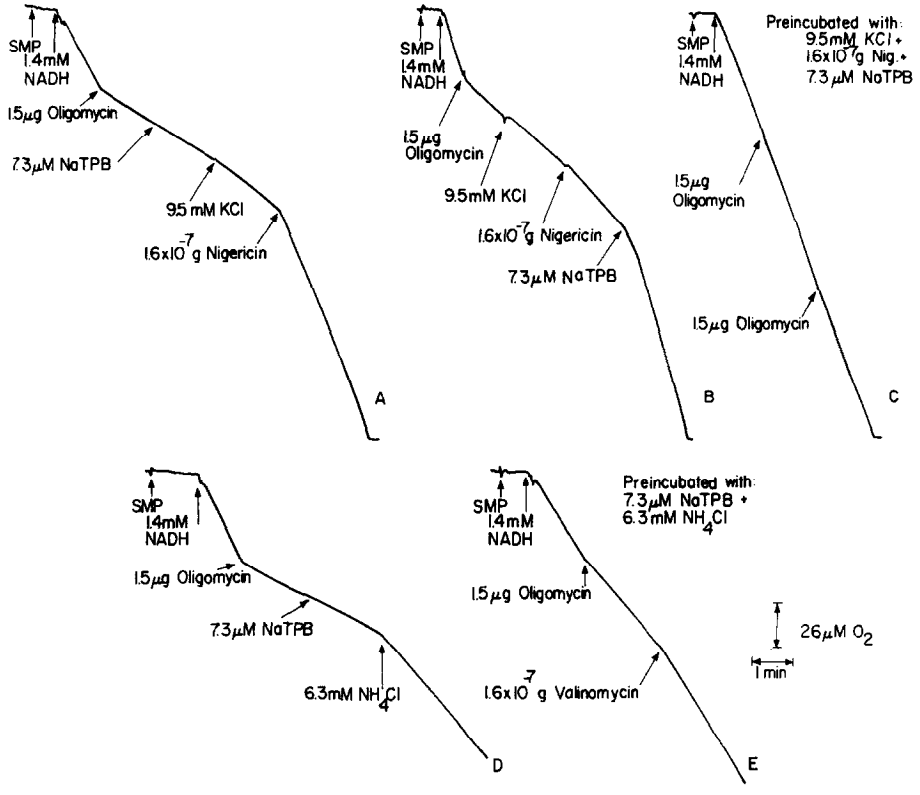

Figure 2 Effect of NaTPB + KCl + nigericin (A, B and C) and NaTPB + NH_4Cl (D and E) on the oligomycin-induced "respiratory control." The reaction mixture contained 0.13 mg protein per ml of E-SMP, 0.25 M sucrose, 0.05 M Tris-acetate, pH 7.4. Final volume: 3.3 ml. Temperature, 25°C.

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dependent permeability changes in black lipid membranes induced by tetraphenylboron and picrate anions, and Skulachev et al. (11) regard these anions as the most permeant for SMP. Fig. 2 shows that the addition of 7.8 μM NaTPB has essentially no effect upon the oligomycin control. Addition of either KCl and nigericin or NH_4Cl, which by themselves do not affect the respiratory control (see Tables I and II), result in release of the oligomycin-inhibited respiration. Preincubation of NaTPB + KCl + nigericin, or NaTPB + NH_4Cl led to an uncoupling effect equivalent to that induced by FCCP.

The effect of various K- and NH_4- salts in the presence or absence of ionophores (12) is shown in Tables I and II, respectively. Uncoupling in the absence of valinomycin was obtained in either situation only when a permeant anion (NO_3^-, TPB^-, or picrate) was present.

TABLE I

EFFECT OF VARIOUS K- SALTS AND IONOPHORES ON THE RESPIRATORY CONTROL OF SUBMITOCHONDRIAL PARTICLES

ADDITIONS	RELEASE OF THE OLIGOMYCIN INHIBITED (coupled) NADH OXIDATION natoms O/min/mg protein				RESPIRATORY CONTROL RATIO Addition rate/ oligomycin rate			
	---	(V)*	(N)*	V+N	---	(V)	(N)	V+N
None	240	215	245	235				
KCl	275	280	345	725**	1.1	1.2	1.4	3.0**
KAc	310	255	415	1100**	1.3	1.1	1.7	4.6**
KPi	325	270	425	1200**	1.4	1.1	1.8	5.0**
KI	255	260	400	970**	1.1	1.1	1.7	4.0**
KSCN	270	250	270	460**	1.1	1.0	1.1	1.9**
KNO_3	305	290	600**	1160**	1.3	1.2	2.5**	4.9**
KCl + 51.0 µM KPicrate	295	275	700**	950**	1.2	1.1	2.9**	4.0**
KCl + 7.3 µM NaTPB	285	260	1115**	1115**	1.2	1.1	4.8**	4.8**

*V = valinomycin; N = nigericin. **Uncoupling Condition: Inability of oligomycin to inhibit (couple) NADH oxidation of SMP preincubated with the salt (± ionophores) as indicated.

Reaction Mixture: 0.15 mg protein per ml of E-SMP; 0.25 M sucrose, 0.05 M Tris-acetate, pH 7.4; 1.4 mM NADH; 0.5 µg/ml oligomycin. 9.5 mM of indicated salt, 6 x 10^{-8} g/ml valinomycin; 9 x 10^{-8} g/ml nigericin. Final volume: 3.3 ml. Temperature, 25°C.

TABLE II

EFFECT OF VARIOUS NH_4- SALTS ON THE RESPIRATORY CONTROL OF SUBMITOCHONDRIAL PARTICLES

ADDITIONS	RELEASE OF THE OLIGOMYCIN INHIBITED (coupled) NADH OXIDATION natoms O/min/mg protein		RESPIRATORY CONTROL RATIO Addition rate/ oligomycin rate	
	-VAL	+VAL	-VAL	+VAL
None	270	250		
NH_4Cl	320	740*	1.2	2.7*
NH_4Ac	285	735*	1.1	2.7*
NH_4Pi	350	810*	1.3	3.0*
NH_4I	360	750*	1.3	2.8*
NH_4NO_3	630*	1200*	2.4*	4.5*
NH_4Cl + 7.3 µM NaTPB	650*	800*	2.4*	3.3*

*Uncoupling Condition: Inability of oligomycin to inhibit NADH oxidation of SMP preincubated with the salt (±VAL), as indicated.

Reaction Mixture: 0.15 mg protein per ml of E-SMP; 0.25 M sucrose; 0.025 M Tris-HCl, pH 7.4; 1.4 mM NADH; 0.5 µg/ml oligomycin; 6.1 mM of the indicated salt, 6 x 10^{-8} g/ml valinomycin. Final volume: 3.3 ml. Temperature, 25°C.

DISCUSSION: Fig. 3 summarizes our present views on the various ion fluxes and effects described in this paper, showing the possible mechanisms

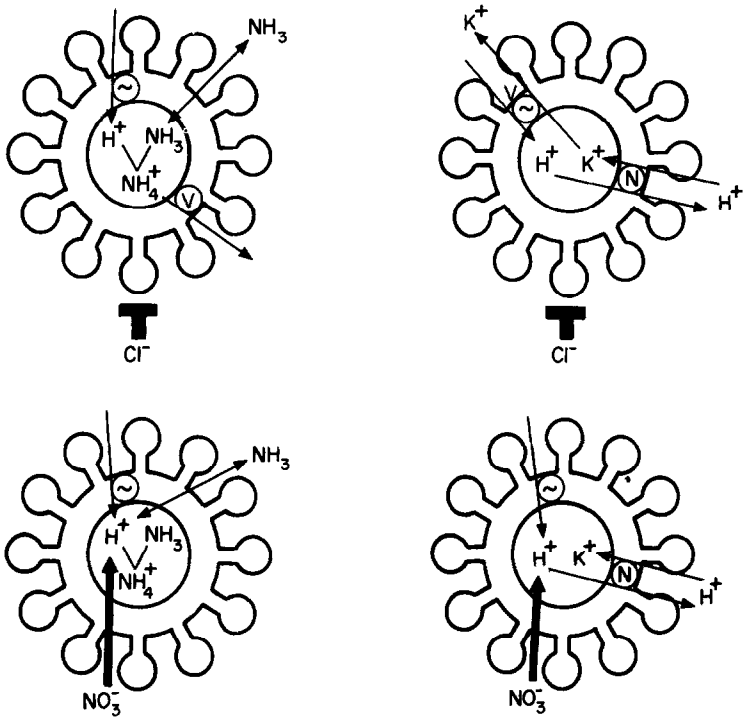

Figure 3. Mechanisms of ion fluxes and uncoupling in submitochondrial particles.
The (~) represents the H^+-translocating respiratory chain, and the (V) and (N) represent valinomycin and nigericin, respectively.

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of uncoupling in SMP in the presence and absence of permeant anions. This scheme can also apply to chromatophores (upper right) and chloroplasts (lower left and right) substituting NO_3^- for Cl^- in the latter case. Respiration (13,14) [or illumination (15,16)] results in H^+ uptake. Addition of nigericin collapses the resultant pH gradient by inducing an electrically neutral K^+/H^+ exchange (upper right)(1,2,6,12). If NH_3 is present, it penetrates the particle and associates with H^+ forming NH_4^+ and also collapses the pH gradient (2,17)(upper left). In the absence of a permeant anion, valinomycin would allow K^+ (1,2) or NH_4^+ (2) efflux down the electric gradient. When a permeant anion is present, however, it is transported electrophoretically thus collapsing the membrane potential (lower left and right).

Analogies can be drawn between the effects in SMP and observations that have been reported in chloroplasts and chromatophores. It has been generally accepted that SMP are "inside-out" with respect to intact mito-

chondria (8,18,19). This sidedness of the SMP membrane has been suggested to be equivalent to that of chloroplasts and chromatophores (19) since the direction of H^+ (13,14) and K^+ (2,20,21) movements is equivalent to that in chloroplasts (15) and chromatophores (16). Uncoupling of photophosphorylation is obtained in chromatophores (22,23) and SMP (1,2,3,4) by a combination of K^+, nigericin and valinomycin. Uncoupling in chloroplasts is obtained with KCl and nigericin (24,25) or KNO_3 and nigericin in SMP. Uncoupling is obtained with NH_4Cl in chloroplasts (17) or NH_4NO_3 in SMP. These results suggest that the intimate mechanism of uncoupling is the same for chloroplasts and SMP, but differences are due to membrane anion selectivity, the permeant anion in chloroplasts being Cl^- (17) and NO_3^- (2) or TPB^- (10,11) for SMP.

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