RESPIRATORY CONTROL AND K^+ TRANSPORT IN SUBMITOCHONDRIAL PARTICLES

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Respiratory control as measured by stimulation of respiration by an uncoupler has been demonstrated in certain submitochondrial particles after treatment with the energy transfer inhibitor, oligomycin (1). Similarly, respiratory control may be induced in ASU-particles by treatment with DCCD, dicyclohexylcarbodiimide (2). The latter system has provided means by which the earliest stages of energy conservation in highly resolved submitochondrial particles may be examined.

In the present study it was found that submitochondrial particles are capable of energy-linked K^+ transport and that this expenditure of energy causes the release of respiratory control induced by DCCD. Evidence is also provided that respiratory control can be achieved in highly resolved submitochondrial particles by means of purified coupling factors without addition of oligomycin or DCCD.

RESULTS AND DISCUSSION

We have examined the capacity of permeability modifying antibiotics (3,4) to release DCCD-induced respiratory control in ASU-particles oxidizing DPNH. In agreement with observations from several laboratories, it was found that valinomycin in the presence of relatively low concentrations of K^+ uncouples oxidative phosphorylation in intact mitochondria (3,5) but not in submitochondrial particles (6-8). However, at very high K^+ concentrations a small uncoupling effect by valinomycin has been reported (9). It was demonstrated, however, that valinomycin markedly uncouples submitochondrial particles in the presence of NH_4^+ (10). As shown in Table I valinomycin released DCCD-induced respiratory control in submitochondrial particles provided NH_4^+ was present. Under identical conditions K^+, Rb^+ or Cs^+ did not substitute for NH_4^+. In the presence of nigericin which alone or together with K^+ had little or no effect, valinomycin released the respiratory control. The dependency of this

effect on K^+ is also shown in Table I. Uncoupling dependent upon the combination of K^+, valinomycin and nigericin has been demonstrated in chromatophores from Rhodospirillum rubrum (11) and in submitochondrial particles (10, 12).

TABLE I

EFFECT OF CATIONS AND ANTIBIOTICS UPON RESPIRATORY CONTROL

Addition(s)	O_2 Consumption (mμatoms/min/mg prot.) - Valinomycin	+ Valinomycin	Ratio +Val/-Val
$Tris^+$	112	132	1.2
K^+	109	124	1.1
Rb^+	126	136	1.1
Cs^+	107	129	1.2
NH_4^+	146	522	3.6
K^+	125	141	1.1
$Tris^+$ + Nigericin	72	99	1.4
K^+ + Nigericin	118	438	3.7

ASU-particles were incubated at 0-4°C. for 10 minutes with 70 n-moles DCCD/mg and then suspended in medium consisting of 0.25 M sucrose, 20 mM Tris-acetate (pH 7.5) and 1 mM DPNH at a final concentration of 0.36 mg/ml. Chloride salts of the indicated cations were added at final concentrations of 20 mM and valinomycin and nigericin, when included, were present at final concentrations of 4×10^{-7}M and 2×10^{-7}M, respectively. Oxygen was measured polarigraphically in a total volume of 1.2 ml at 22°C.

This similarity between submitochondrial particles and bacterial chromatophores extends also to the directionality of K^+ transport as indicated by the results summarized in Figure 1.

Submitochondrial particles treated with DCCD accumulated a limited amount of K^+ which was released on addition of valinomycin. Nigericin stimulated K^+ uptake, which was reversed or prevented by valinomycin. Both the spontaneous and nigericin-stimulated K^+ ion movements were found to depend upon the anion added. NO_3^- was particularly effective in stimulating K^+ transport. As in intact mitochondria, these movements of K^+ were energy-linked since they were prevented by rotenone (0.2 μg/mg) or FCCP, trifluoromethoxycarbonylcyanide-phenylhydrazone (0.76 μM). It should be noted that in intact mitochondria,

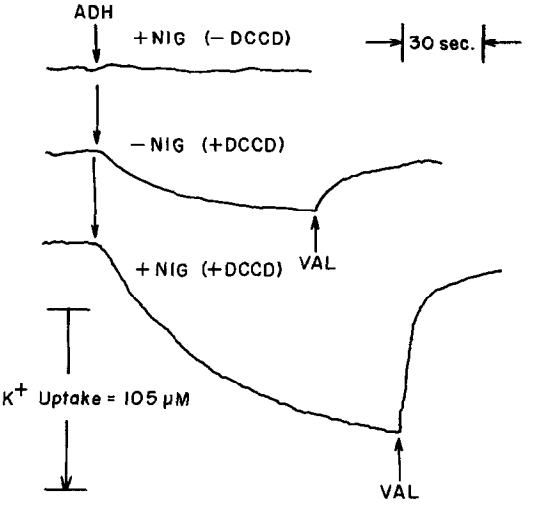

Figure 1. A-particles incubated 30 minutes with 35 n-moles DCCD/mg at 0-4° C. were employed at a final concentration of 3.2 mg/ml. The medium contained 0.25 M sucrose, 20 mM Tris-NO_3 (pH 7.5), 0.2 mM KCl, 0.5 mM DPN and 8.7 mM ethanol in a final volume of 3 ml at 22° C. Respiration was activated by addition of yeast alcohol dehydrogenase, ADH (8.7 µg/ml). Net changes in K^+ ion were determined by means of the Beckman 39047 cation electrode connected through a Corning expanded scale pH Meter to an Esterline Angus recorder. When included, nigericin, NIG (8×10^{-7} M) was added before activation of respiration; valinomycin, VAL (6.4×10^{-7} M) was added as indicated.

valinomycin stimulates the uptake of K^+ and nigericin catalyzes the release of accumulated K^+ (13). This apparent reversal of membrane polarity supports the proposal that submitochondrial particles are "inside out" (1) and is in line with the observations that the direction of energy-linked H^+ ion translocation in submitochondrial particles is opposite to that of intact mitochondria (14, 15)

Induction of respiratory control in submitochondrial particles by energy transfer inhibitors is therefore accompanied by restoration of K^+ transport. It has been proposed that nigericin acts by stimulating exchange between K^+ and H^+ ions (13). Since respiration in submitochondrial particles has been shown to result in an inward flux of H^+ it appears that nigericin stimulates K^+ uptake via exchange with internal H^+ ion. Thus the uncoupling effect of valinomycin in conjunction with nigericin can be explained in terms of an energy-dependent cyclic cation transport (12, 13). The uncoupling effect of valinomycin plus NH_4^+ in the absence of nigericin is seen as a similar cyclic cation transport facilitated by the spontaneous penetration by NH_3 (16) into the particles where it combines with H^+ to form NH_4^+ which is extruded in the presence of valinomycin.

These observations may be interpreted in terms of the chemiosmotic coupling

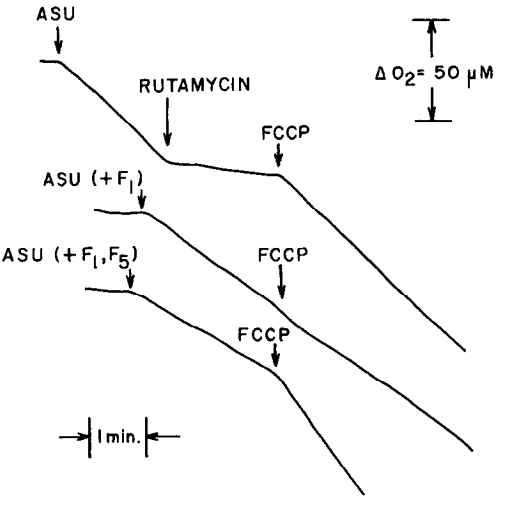

Figure 2. The conditions were the same as described in the legend to Table I. Rutamycin (4.5 μg/mg) and FCCP (4 μM) were added as indicated. When treated with coupling factors, the ASU-particles were incubated with F_1 (18) (115 μg/mg) or F_1 + F_5 (19) (90 μg/mg) for 20 minutes at room temperature.

mechanism of Mitchell (17) in which it is proposed that H^+ ion transported into submitochondrial particles generates a membrane potential and pH gradient. If it is assumed that nigericin catalyzes an electrically neutral exchange of K^+ for H^+, this exchange would collapse the pH gradient without effecting the membrane potential (11, 17). Therefore, the nigericin-stimulated K^+ uptake should not cause uncoupling. Similarly, spontaneous NH_3 uptake would result in a collapse of the pH gradient by neutralization of internal H^+ ion (forming NH_4^+ inside) without effecting the membrane potential. Valinomycin would uncouple in either case by conducting K^+ or NH_4^+ outward as charged complexes (13) thereby collapsing the membrane potential.

Whether cyclic cation transport per se or simultaneous collapse of a membrane potential and pH gradient is the basis of uncoupling by the antibiotics, there is apparently a close relationship between the release of respiratory control and ion transport in submitochondrial particles.

In an attempt to related these observations to physiological respiratory control in the absence of energy transfer inhibitors, the influence of purified coupling factors upon the respiration of submitochondrial particles has been examined. In Figure 2, respiratory control in ASU-particles treated with rutamycin (cf. ref. 1 for oligomycin effects) is compared to similar effects obtained by addition of F_1 (18) and F_5 (19). Treating ASU-particles with F_1 resulted

in a partial inhibition of DPNH oxidation which was not relieved by FCCP. After treatment with both F_1 and F_5 respiration was further inhibited but was now stimulated by FCCP. That this respiratory control required both F_1 and F_5 is indicated by the results shown in Figure 3. The ratio of the rates of respiration in the presence to those in the absence of FCCP is plotted as a function of the quantity of coupling factor added. No significant respiratory control was obtained with either F_1 or F_5 alone whereas the control ratio rose to 2.2 with increasing F_5 levels in the presence of saturating amounts of F_1. Heating F_1 or F_5 (5 minutes at 70°) abolished their activity.

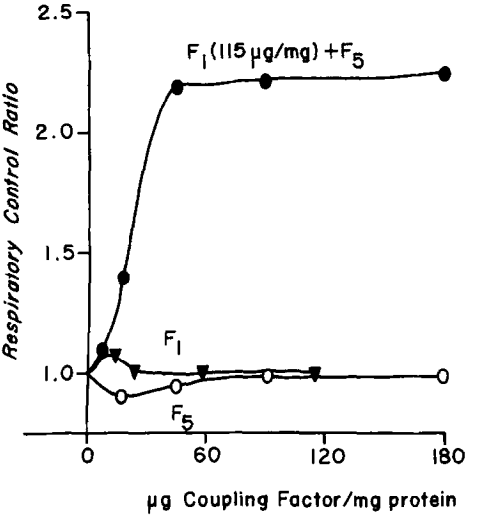

Figure 3. The conditions were the same as described in the legend to Figure 2 and the indicated quantities of F_1 and F_5 incubated with ASU-particles for 20 minutes at room temperature.

Although the maximum degree of respiratory control obtainable thus far with F_1 and F_5 was less than that elicited with energy transfer inhibitors (Figure 2) it should be pointed out, that in order to obtain high P:O ratios in these particles additional coupling factors (F_2 and F_3) are required (20). Stimulation of respiration by ADP and Pi has not as yet been demonstrated which may also indicate a need for additional factors. Since the F_5 preparation still contained considerable amounts of F_3 an exact delineation of the coupling factor requirement cannot be made at this time.

In any case, it is apparent from these experiments and from observations that the energy-dependent enhancement of fluorescence of 8-anilino-1-naphthalene

sulfonate (21) is lost in resolved submitochondrial particles but can be restored by addition of coupling factors (22), that the process of energy conservation can be restored to a large extent to highly resolved particles by addition of natural mitochondrial components. Whether the effect of coupling factor in these experiments is structural (2) rather than catalytic is under investigation.

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REFERENCES

1. Lee, C. P. and Ernster, L., BBA Library (Elsevier, Amsterdam) 7, 1218 (1966).
2. Racker, E., Fed. Proceed., 26, 609 (1967).
3. Moore, C. and Pressman, B. C., Biochem. Biophys. Res. Commun., 15, 562 (1964).
4. Graven, S. N., Estrada-O., S. and Lardy, H. A., Proc. Nat. Acad. Sci., 53, 1076 (1965).
5. Harris, E. J., Cockrell, R. and Pressman, B. C., Biochem. J., 99, 200 (1966).
6. Harris, E. J., Hofer, M. and Pressman, B. C., Biochem., 6, 1348 (1967).
7. Smith, E. H. and Beyer, R. E., Arch. Biochem. Biophys., 122, 614 (1967).
8. Beyer, R. E., Brinker, K. R. and Crankshaw, D. L., Canad. J. Biochem., 47, 117 (1969).
9. Papa, S., Tager, J. M., Guerrieri, F. and Quagliariello, E., Biochim. Biophys. Acta, 172, 184 (1969).
10. Cockrell, R. S., Fed. Proceed., 28, 472 (1969).
11. Jackson, J. B., Crofts, A. R. and von Stedingk, L.-V., Europ. J. Biochem., 6, 41 (1968).
12. Montal, M., Chance, B., Lee, C. P. and Azzi, A., Biochem. Biophys. Res. Commun., 34, 104 (1969).
13. Pressman, B. C., Harris, E. J., Jagger, W. S. and Johnson, J. H., Proc. Nat. Acad. Sci., 58, 1949 (1967)
14. Mitchell, P. and Moyle, J., Nature, 208, 1205 (1965).
15. Chance, B. and Mela, L., J. Biol. Chem., 242, 830 (1967).
16. Chappell, J. B. and Haarhof, K. N. in E. C. Slater, Z. Kaniuga and L. Wojtczak, (Eds.) Biochemistry of Mitochondria, Academic Press, London, 1967, p. 75.
17. Mitchell, P., Chemiosmotic Coupling and Energy Transduction, Glynn Research, Bodwin, Cornwall (1968).
18. Penefsky, H. S., Pullman, M. E., Datta, A. and Racker, E., J. Biol. Chem., 235, 3330 (1960).
19. Fessenden-Raden, J. M. and Racker, E., Fed. Proceed., 27, 297 (1968).
20. Racker, E., J. Gen. Physiol., in press (1969).
21. Azzi, A., Chance, B. and Radda, G. K., Proc. Nat. Acad. Sci., in press (1969).
22. Penefsky, H. S. and Datta, A., Fed. Proceed., 28, 663 (1969).