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The phosphate potential generated by membrane fragments of *Azotobacter vinelandii*

Respiring rat-liver mitochondria[1-3] and illuminated chloroplasts[4] in State 4 are able to maintain in the suspending medium a 'phosphate potential' of 17.0–17.5 kcal/mole*. The phosphate potential is defined as $\Delta G' = \Delta G'_0 + 1.36 \log [ATP]/[ADP][P_i]$, where $\Delta G'_0$ is the standard free-energy change of the reaction ADP + $P_i \rightleftharpoons$ ATP. As is to be expected, submitochondrial particles, that show no respiratory control and a substantial ATPase activity, are unable to maintain these high phosphate potentials (E. C. SLATER AND E. A. LEM-HEGGELUND, unpublished experiments).

Membrane fragments prepared from *Azotobacter vinelandii* resemble sub-mitochondrial particles in having a low P:O ratio (0.4–0.6 with NADH) and showing only a slight stimulation of respiration by ADP[8], but differ in possessing a very low ATPase activity. It therefore became of interest to determine the phosphate potential that can be generated by these poorly phosphorylating preparations.

Incubations were carried out in centrifuge tubes containing 2.0 ml of a reaction mixture consisting of 25 mM Tris–HCl buffer, 1 mM EDTA, 10–15 mM substrate, and $MgCl_2$, ADP, ATP and P_i (as Na_2HPO_4) as given in the legends to the figures and tables. The final pH was 8.1. Air was bubbled through the solution during the incubation. The reaction was started by addition of 0.1–0.2 mg (protein) phosphorylating particles[9] and stopped at various times by addition of $HClO_4$ to 3 %. After removal of protein by centrifugation, and neutralization and precipitation of perchlorate with KOH, ADP and P_i were determined in the supernatant as described in refs. 10 and 11, respectively. The concentration of ATP was measured with the method described in ref. 12, except in Expt. 2 of Table I, where it was assumed to be equal to the initial concentration *plus* the disappearance of ADP (which was close to the disappearance of P_i). The calculated $\Delta G'$ is not sensitive to small errors in the ATP concentration. Corrections were made for the slight hydrolysis of ATP between acidification and neutralization, as determined in a control experiment.

Fig. 1 shows that within about 15 min with NADH as substrate, a steady state is reached with a very low concentration of ADP. The results of this and of experiments with other substrates are summarized in Table I. In previous measurements with rat-liver mitochondria[1-3], no allowance had to be made for binding of ATP, ADP or P_i to Mg^{2+}, since Mg^{2+} was not added. Azotobacter membranes, however, do not catalyse oxidative phosphorylation in the absence of added Mg^{2+}. The concentrations of unbound Mg^{2+} were calculated from the concentrations of ATP, ADP and P_i, by making use of the binding constants reported by PHILLIPS *et al.*[7].

The last line of Table I shows that, despite their low P:O ratio, Azotobacter membranes are able to generate a phosphate potential of about 17 kcal/mole, of the same order as that found with respiring mitochondria or illuminated chloroplasts, and much higher than in submitochondrial particles. The potential is the same with

* Previously published values were based on a value for $\Delta G'_0$ at pH 7.5 and 25° of 9.1 kcal per mole (ref. 5), and the variation of $\Delta G'_0$ with pH given by GEORGE AND RUTMAN[6]. These values have now been recalculated, on the basis of a new value for $\Delta G'_0$ (10.8 at pH 7.5 and 25°) published by PHILLIPS *et al.*[7].

lactate as substrate as with malate or NADH, even though lactate is oxidized much more slowly than malate or NADH[13,14].

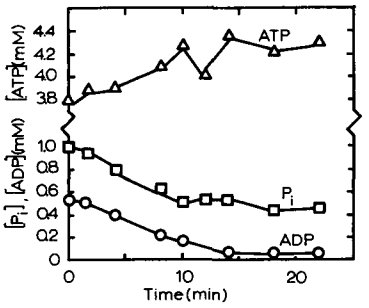

Fig. 1. Time-course of phosphorylation of ADP by P_i after addition of Azotobacter particles (0.26 mg) to 25 mM Tris–HCl buffer, 1 mM EDTA, 7.5 mM NADH, 5 mM $MgCl_2$, 3.8 mM ATP, 0.55 mM ADP and 1.0 mM P_i at 25°. The final pH was 8.1.

TABLE I

MEASUREMENT OF PHOSPHATE POTENTIAL WITH DIFFERENT SUBSTRATES

The incubations were carried out as described in the text. The temperature was 25°. In Expt. 2 the final concentration of ATP and in Expt. 3 the final concentration of P_i were calculated from the initial concentrations, and the disappearance of ADP.

	Expt.		
	1	*2*	*3*
Substrate	NADH	L(+)-Malate	D(−)-Lactate
$MgCl_2$ added (mM)	5.0	6.0	6.0
pMg*	4.5	2.8	3.5
Initial concentrations (mM)			
ATP	3.80	2.86	4.40
ADP	0.55	0.17	0.21
P_i	1.00	1.07	0.90
Final concentrations (mM)			
ATP	4.20	3.00	4.59
ADP	0.045	0.03	0.02
P_i	0.45	0.91	0.71
$'K'^{**} \times 10^{-5}$ (M^{-1})	2.07	1.10	3.23
$\Delta G'_0$ (kcal/mole)***	10.3	10.1	9.9
$\Delta G'$ (kcal/mole)	17.6	17.0	17.4

* Calculated from binding constants of Mg^{2+} with ADP, ATP and P_i given in Table I of PHILLIPS *et al.*[7].

** $[ATP_{tot}]/[ADP_{tot}] \cdot [P_{i\ tot}]$.

*** Calculated by interpolation from Fig. 2 of PHILLIPS *et al.*[7].

The phosphate potential found with mitochondria is consistent with the known P:O ratios and the conclusion that State 4 is close to thermodynamic equilibrium. The high values found with Azotobacter membranes, despite the low P:O ratios, may, at first sight, seem surprising. If, however, we assume that the low P:O ratios are

due to the presence in these membrane fragments of a non-phosphorylating respiratory pathway, operating independently of a conventional phosphorylating pathway[15], the high phosphate potential suggests that also in Azotobacter membranes thermodynamic equilibrium is approached, comparable with State 4 in mitochondria.

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1 R. S. COCKRELL, E. J. HARRIS AND B. C. PRESSMAN, *Biochemistry*, 5 (1966) 2326.
2 E. C. SLATER, in L. ERNSTER AND Z. DRAHOTA, *Mitochondria — Structure and Function*, Academic Press, London and New York, 1969, p. 205.
3 E. C. SLATER, in S. PAPA, J. M. TAGER, E. QUAGLIARIELLO AND E. C. SLATER, *The Energy Level and Metabolic Control in Mitochondria*, Adriatica Editrice, Bari, Italy, 1969, p. 255.
4 R. KRAAYENHOF, *Biochim. Biophys. Acta*, 180 (1969) 213.
5 T. BENZINGER, C. KITZINGER, R. HEMS AND K. BURTON, *Biochem. J.*, 71 (1959) 400.
6 P. GEORGE AND R. J. RUTMAN, *Progr. Biochem. Biophys. Chem.*, 10 (1960) 1.
7 R. C. PHILLIPS, P. GEORGE AND R. J. RUTMAN, *J. Biol. Chem.*, 244 (1969) 3330.
8 L. J. M. EILERMANN, H. G. PANDIT-HOVENKAMP AND A. H. J. KOLK, *Biochim. Biophys. Acta*, 197 (1970) 25.
9 H. G. PANDIT-HOVENKAMP, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 10, Academic Press, New York, 1967, p. 152.
10 H. ADAM, in H.-U. BERGMEYER, *Methoden der Enzymatischen Analyse*, Verlag Chemie, Weinheim/Bergstr., 1962, p. 573.
11 B. E. WAHLER AND A. WOLLENBERGER, *Biochem. Z.*, 329 (1958) 508.
12 W. LAMPRECHT AND I. TRAUTSCHOLD, in H.-U. BERGMEYER, *Methoden der Enzymatischen Analyse*, Verlag Chemie, Weinheim/Bergstr., 1962, p. 543.
13 C. W. JONES AND E. R. REDFEARN, *Biochim. Biophys. Acta*, 113 (1966) 467.
14 H. G. HOVENKAMP, *Biochim. Biophys. Acta*, 34 (1959) 485.
15 H. G. PANDIT-HOVENKAMP, L. J. M. EILERMANN AND A. H. J. KOLK, in J. M. TAGER, S. PAPA, E. QUAGLIARIELLO AND E. C. SLATER, *Electron Transport and Energy Conservation*, Adriatica Editrice, Bari, 1970, in the press.

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