RESPIRATORY CONTROL IN MEMBRANE PARTICLES FROM *MICROCOCCUS DENITRIFICANS*

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1. Introduction

The generally accepted [1–3] criterion for establishing the existence of respiratory control is the demonstration of an increased rate of oxygen uptake on the addition of ADP, followed by a return to a slow rate on exhaustion of the ADP, this slow rate being capable of restimulation by a further addition of ADP. Respiratory control has been demonstrated with mitochondria from both animals [4, 5] and plants [2], and its equivalent in photosynthesis (photosynthetic control) has been observed in isolated pea chloroplasts [3]. Respiratory control has not however been demonstrated with preparations from bacteria, although increased rates of oxygen uptake by the inclusion of phosphate or phosphate-acceptor have been reported [6–9]. The presence of respiratory control in intact bacteria has been indicated indirectly by the stimulation of the respiratory rates of whole cells on the addition of uncouplers of oxidative phosphorylation [10–12]. In this paper we describe the control exerted by ADP over the rate of oxygen uptake by membrane particles isolated from *Micrococcus denitrificans*, and the release of this respiratory control by the uncoupler of mitochondrial oxidative phosphorylation carbonylcyanide *m*-chlorophenylhydrazone (CCCP). Oxidative phosphorylation in similarly prepared particles has already been described [13]: NADH-dependent oxygen uptake was coupled to phosphorylation with a P/O ratio of 1.5, and the rate of oxygen uptake was decreased when either ADP or inorganic phosphate were omitted from the reaction mixture.

2. Materials and methods

M. denitrificans was maintained, grown and harvested, and the membrane particles prepared, as described previously [13]. Washed cells were incubated with lysozyme in 0.5 M sucrose, sedimented by centrifugation, and osmotically lysed by resuspension in 10 mM tris acetate, pH 7.3; the particles were sedimented, washed and resuspended in 10 mM tris acetate, pH 7.3 containing 5 mM Mg acetate. In the present, but not in the previous study [13], 1 mM ATP was present during lysis. Protein was determined by a modification [13] of the biuret method. Oxygen uptake was measured with a Clark-type oxygen electrode (Rank Brothers, Bottisham, Cambs., England) calibrated by allowing the particles to completely oxidise standard aliquots of NADH [14]. ADP was assayed enzymatically [14]. CCCP (Calbiochem) was added in 3 μl of acetone solution.

3. Results

Fig. 1a shows that, in the presence of inorganic phosphate, addition of ADP caused an increase in the rate of oxygen uptake, which subsequently returned to a rate similar to that observed before ADP addition. A second addition of ADP restimulated the slow rate of oxygen uptake. In accordance with the nomenclature of Chance and Williams [5] we term the rate of oxygen uptake during phosphorylation the state 3 rate, and the rate after exhaustion of the ADP the state 4 rate.

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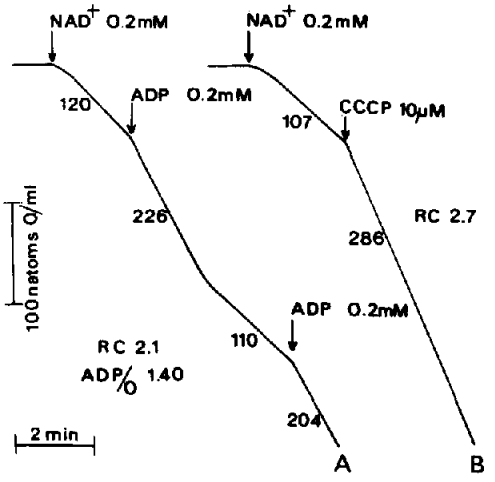

Fig. 1. Effect of ADP and CCCP on the rate of oxygen uptake by particles prepared from *M. denitrificans*. The reaction medium contained 50 mM tris acetate, pH 7.3, 5 mM Mg acetate, 15 μl ethanol, 12 units dialysed alcohol dehydrogenase, and 0.1 ml of particle suspension containing 0.63 mg protein. In addition, A contained 3.3 mM sodium phosphate, pH 7.3. Total volume was 3 ml; temperature, 30°. The numbers along the traces are natoms O/min. Respiratory control ratios, RC, were calculated as in table 1. The ADP/O ratio was calculated by the procedure of Chance and Williams [4].

Table 1
ADP/O ratios and respiratory control ratios of particles prepared from *M. denitrificans*.

Preparation No.	ADP/O ratios	Respiratory control ratios	
		ADP($\frac{\text{state 3}}{\text{state 4}}$)	CCCP($\frac{+\text{CCCP}}{-\text{CCCP}}$)
1	1.31	1.79	2.66
2	1.35	1.77	2.74
3	1.40	2.06	2.59
4	1.34	1.93	2.54
5	1.27	1.75	2.50

ADP/O ratios and ADP-induced respiratory control ratios were obtained under the experimental conditions of fig. 1a. CCCP-induced respiratory control ratios were obtained under the conditions of fig. 1b. The preparations contained 1.0–1.7 mg protein per 0.1 ml added to the reaction mixture.

Addition of CCCP also increased the rate of oxygen uptake (fig. 1b).

Table 1 shows the ADP/O ratios and respiratory control ratios calculated from our results by the conventional procedure [4]. Although AMP was present in the ADP solution at a concentration of approximately 10% that of the ADP, the AMP content was neglected in the calculation of the ADP/O ratios since AMP did not substitute for ADP in stimulating either the initial rate or the state 4 rate of oxygen uptake and additional AMP did not affect the ADP/O ratios. Furthermore, the particles were shown to lack appreciable adenylate kinase activity when assayed by the method of Naik and Nicholas [15].

4. Discussion

The results presented in this report are interpreted as demonstrating the presence of respiratory control in particles prepared from *M. denitrificans*. The ADP/O ratios are in agreement with the P/O ratios determined previously [13] by measuring the incorporation of $^{32}P_i$ into an esterified form during NADH-dependent oxygen uptake.

From the results of preliminary experiments we have identified the direction of the proton movements associated with (a) electron transport between NADH and oxygen by the particles, and (b) the respiration of endogenous substrates by intact cells. Electron transport by the particles caused the pH of the medium to increase; respiration by the intact cells caused the pH of the medium to decrease (see [16]). Our studies [17] of valinomycin-induced uncoupling [18] of respiration by the particles confirm that the orientation of the particle membrane is the opposite to that of the membrane in the intact cell. This orientation of the particle membrane has the practical advantage of making the ATPase and NADH dehydrogenase freely accessible to their respective substrates in the reaction medium.

In subsequent publications [17] we shall report more fully on the properties of the oxidative phosphorylation system of these particles, and on uncoupling by membrane-active antibacterial compounds.

Acknowledgement

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