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OXIDATIVE PHOSPHORYLATION IN MUTANTS OF *ESCHERICHIA COLI* DEFECTIVE IN ENERGY TRANSDUCTION

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SUMMARY

Two mutants defective in oxidative phosphorylation have been isolated from *Escherichia coli* K12. The mutants lack ATP-driven transhydrogenase activity and exhibit P/NADH ratios, as measured in whole cells, of 0.04 and 0.16, respectively, as compared with a parental value of 2.1. Respiration-linked transhydrogenase was detected in membrane particles from the two strains. One of the mutants was defective in membrane (Mg^{2+}–Ca^{2+})-ATPase activity while the second strain exhibited a level of ATPase activity comparable to that in the parent. The ATPase in this latter mutant appeared to be converted to a form which is resistant to the inhibitor *N,N'*-dicyclohexylcarbodiimide.

INTRODUCTION

The isolation and partial characterization of a mutant of *Escherichia coli* K12 which lacks membrane (Mg^{2+}–Ca^{2+})-adenosine triphosphatase (ATPase) has recently been described[1,2]. In addition to the defect in ATPase, this mutant N_{I44} lacks the ATP-driven reduction of $NADP^+$ by NADH, although it exhibits respiration-driven transhydrogenase and non-energy-linked transhydrogenase activities. The isolation of a similar mutant of *E. coli* K12, termed *uncA*$^-$, which lacks ATPase and ATP-driven transhydrogenase, has been described by Cox *et al.*[3,4]. The *uncA*$^-$ mutant was reported also to have impaired oxidative phosphorylation activity in cell-free systems. Unfortunately, however, the very low P/O ratios observed even with wild-type particles make these results difficult to interpret.

A technique for measuring oxidative phosphorylation in intact cell suspensions of *E. coli* has been described by Hempfling[5,6] and applied to cells of *Azotobacter vinelandii* by Baak and Postma[7]. In both systems, P/NADH ratios of 3 were measured. In this report we present results of measurements of oxidative phosphorylation in whole cells of the parent organism A428, and two mutants of *E. coli* K12, N_{I44} and B_{V4}. In addition, results are presented of measurements of ATPase and transhydrogenase activities in particulate fractions from these strains.

Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

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MATERIALS AND METHODS

Strains

Strain A428 (F$^-$, *pro*$^-$, *lac*$_1^-$, T_6^R, *gal*$_2^-$, *ara*$^-$, *his*$^-$, *xyl*$^-$, *man*$^-$, B_1^-, *str*^{R}) was used as a parent organism. The strains N_{I44} and B_{V4} were derived as single-step mutants from A428 following mutagenesis by ultraviolet irradiation. The isolation and partial characterization of N_{I44} has been described previously[1,2]. The genetic and physiological characteristics of B_{V4} will be the subject of a separate communication.

ATPase and transhydrogenase in membrane particles

Cells of mutant N_{I44} and B_{V4}, and of the parent A428, were grown in batch cultures to the late exponential phase in 12.5 l carboys in Davis mineral salts medium, supplemented with 0.5 % glucose, 50 μg each of L-histidine and L-proline per ml and 1 μg/ml vitamin B_1. The cultures were aerated by bubbling air at a rate of 30 l per min. The cells were harvested in a Sorvall RC2B centrifuge fitted with a continuous flow apparatus. Preparation of the cell-free extract and subsequent isolation of the particles were according to Fisher *et al.*[8].

The respiration and ATP-driven reduction of $NADP^+$ by NADH, as well as the non-energy-linked reduction of acetyl-pyridine NAD^+ by NADH, were measured according to the procedures of Fisher *et al.*[8,9] and Kaplan[10], respectively, using 0.4–1.0 mg particle protein in a reaction volume of 3.0 ml. ATPase in membrane particles was measured as described previously[2]. Particle protein was determined according to the method of Lowry *et al.*[11].

Oxidative phosphorylation in whole cells

Overnight cultures were prepared from single-colony isolates of each of the three strains and diluted 1:100 into 1 l of fresh medium. The cells were grown with reciprocal shaking at 37 °C and harvested at the late exponential phase. Cells were washed once with 50 mM phosphate buffer (pH 7.4), suspended in 200 ml phosphate buffer, starved by bubbling with air for 40 min at room temperature, washed twice with 50 mM Tris buffer (pH 7.2), and resuspended in Tris buffer to a final concentration of 10–20 mg bacterial protein per ml, according to the procedure described by Hempfling[5]. Oxidative phosphorylation was measured in whole cells according to the procedure of Hempfling[5] as modified by Baak and Postma[7]. The reaction was started by adding 0.6 ml of an anaerobic cell suspension to 0.6 ml of an air-saturated solution of 50 mM Tris buffer (pH 7.2). The reaction was allowed to proceed for 2–7 s and stopped by the addition of 0.4 ml 40 % perchloric acid. After neutralization, the concentrations of NAD^+, AMP, ADP and ATP were measured fluorimetrically according to Williamson and Corkey[12]. Cell protein was determined according to the procedure of Gornall *et al.*[13].

RESULTS

ATPase and transhydrogenase

A comparison of the ATPase in membrane particles of the parent A428 and the mutants N_{I44} and B_{V4} is shown in Table I. The most striking difference is the

TABLE I

ATPase ACTIVITIES IN MEMBRANE PARTICLES OF STRAINS A428 (PARENT), N_{I44} (MUTANT) AND B_{V4} (MUTANT) OF *E. coli* K12

The growth of the cells, preparation of particles and assay of ATPase were as described in Materials and Methods.

Strain	*ATPase (nmoles P_i released/min per mg protein)*	
	−DCCD	*+ 60 μM DCCD*
A428	254	48
N_{I44}	18	18
B_{V4}	269	254

TABLE II

TRANSHYDROGENASE ACTIVITIES IN MEMBRANE PARTICLES OF STRAINS A428, N_{I44} AND B_{V4} OF *E. coli* K12

Reaction conditions as described in Materials and Methods.

Strain	*Energy-linked transhydrogenase (nmoles NADPH/min per mg protein)*		*Non-energy-linked transhydrogenase (nmoles acetyl pyridine NADH/min per mg protein)*
	Respiration driven	*ATP driven*	
A428	19.4	28.9	93.0
N_{I44}	17.8	0	86.0
B_{V4}	15.4	0	87.0

very low activity of ATPase in the mutant N_{I44} in contrast to that measured in the other two strains. Although the specific activities of the enzyme in particles of B_{V4} and A428 are equal, the ATPase in the parental membrane was inhibited about 80% by 60 μM *N,N'*-dicyclohexylcarbodiimide (DCCD), while the inhibitor had no effect on the ATPase in B_{V4}.

The lack of ATPase activity in the membrane particles of N_{I44} is correlated with the absence of ATP-driven transhydrogenase activity as compared with the activity in particles of A428 (Table II). Of particular interest was the finding that membrane particles of the mutant B_{V4} were also devoid of ATP-driven activity, even though the ATPase in these particles is equal to that of the parent (Table I). Apparently, the defect in B_{V4} which results in the inability of the particles to utilize ATP as a source of energy in the transhydrogenase reaction is associated with the conversion of the enzyme to a form which is insensitive to DCCD. Both mutants exhibited respiration-driven transhydrogenase and non-energy-linked activities. In all strains, the respiratory-driven activity was completely inhibited by 60 μM 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole.

Oxidative phosphorylation

In order to determine the effect of the genetic lesions on the coupling process in the mutants N_{I44} and B_{V4}, oxidative phosphorylation was measured in whole cells of these strains and compared with the parental activity (Table III). It can be

TABLE III

OXIDATIVE PHOSPHORYLATION IN INTACT CELLS OF STRAINS A428, N_{I44} AND B_{V4} OF *E. coli* K12

Reaction conditions as described in Materials and Methods. The data are presented as the change in nucleotide levels when compared with zero time controls, and are the average of two independent experiments.

Strain	*ΔATP*	*−ΔADP*	*−ΔAMP*	*ΔNAD+*	*P/NADH*
	nmoles/mg protein				
A428	7.1	1.9	4.2	5.4	2.1
N_{I44}	0.44	0	0.52	6.0	0.16
B_{V4}	0.24	0	0	5.3	0.04

seen that the mutants exhibited a P/NADH ratio of only 2 and 8 %, respectively, of that of the parental strain, even though the oxidation of NADH was about the same in all strains. All of the NADH in the cells was oxidized within the first 2 s of the reaction and the values were identical over the range 2–7 s. The results in Table III indicate that both mutants B_{V4} and N_{I44} are defective in oxidative phosphorylation. The P/NADH ratio in A428 was 2.1 which is somewhat lower than the P/NADH of 3 measured in *E. coli* B, by Hempfling[5]. It is possible that this difference may be due to a difference in the two strains. Strain A428 has undergone several mutagenesis treatments and may in fact be deficient in unknown characteristics.

The results in Table IV show the effect of 10 μM carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) on ATP synthesis and NADH oxidation in whole cells A428 and N_{I44}. The synthesis of ATP in A428 was almost completely inhibited by the uncoupling agent under these conditions, although no effect was observed in the case of N_{I44}. In addition, there was no effect of FCCP on the oxidation of NADH. These results suggest that the assay in whole cells is in fact a measure of oxidative phosphorylation, and that the residual ATP synthesis in N_{I44} may be due to an enhanced substrate level phosphorylation.

TABLE IV

THE EFFECT OF FCCP ON ATP SYNTHESIS AND NADH OXIDATION IN INTACT CELLS OF STRAINS A428 AND N_{I44} OF *E. coli* K12

Reaction conditions are as described in Materials and Methods. The uncoupler FCCP (10 μM) was added to the cells during pre-incubation under anaerobic conditions.

Strain	*FCCP*	*ΔATP*	*ΔNAD+*
	(*μM*)	(*nmoles/mg protein*)	
A428	0	5.4	2.9
	10	0.2	2.8
N_{I44}	0	0.45	2.0
	10	0.40	2.0

DISCUSSION

The results presented in this report demonstrate that the mutants B_{V_4} and $N_{I_{44}}$ are defective in both oxidative phosphorylation and in ATP-driven transhydrogenase. The mutant B_{V_4} has normal ATPase activity which is resistant to DCCD, while strain $N_{I_{44}}$ is almost completely devoid of ATPase activity. It is of interest to note that each of the strains has about the same capacity to oxidize NADH in intact cells (Table III) and in membrane particles (B. I. Kanner, unpublished observations). Since strains $N_{I_{44}}$ and B_{V_4} retain their capacity to couple respiration to the energy linked reduction of $NADP^+$ by NADH, and since both mutants appear to have altered ATPase properties, the defect in oxidative phosphorylation is most likely associated with the terminal stages of ATP synthesis. Each mutant was derived from strain A428 and could revert or be transduced to growth on Krebs-cycle intermediates. Since transductants of both mutants exhibited normal activities, the deficiencies in each of the mutants can most likely be ascribed to a defect in a single function. It is of interest that the genetic lesions in $N_{I_{44}}$ and in B_{V_4} are each about 20% co-transducible with the *ilv* locus on the *E. coli* chromosome at 73.5 min[1,2]. Cox *et al.*[3] have reported a similar location for the *uncA*$^-$ mutation, which also results in a defect in ATPase. At present, it is uncertain whether the mutations associated with B_{V_4} and $N_{I_{44}}$ are located in the same cistron or in different cistrons.

Abrams *et al.*[14] have recently described the isolation of mutants of *Streptococcus faecalis* which are resistant to DCCD. The ATPase in these strains was also shown to be resistant to the inhibitor by virtue of a defect in a membrane component which confers DCCD sensitivity on the membrane-bound enzyme. It is possible that the mutant B_{V_4} is affected in a similar function. If this is so, the gene for this function lies very close to the gene for ATPase.

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