ENERGY LINKED NICOTINAMIDE ADENINE DINUCLEOTIDE TRANSHYDROGENASE IN A MUTANT OF *ESCHERICHIA COLI* K12 LACKING MEMBRANE Mg^{2+}–Ca^{2+}-ACTIVATED ADENOSINE TRIPHOSPHATASE

Baruch I. KANNER and David L. GUTNICK
Department of Microbiology, Tel Aviv University, Tel Aviv, Israel

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1. Introduction

A number of reports have been published on the energy dependent transhydrogenase reaction in membrane fragments of *E.coli* [1–5]. The energy for this reaction has been shown to be derived either from respiration or from ATP [4,5]. One approach to the study of these energy linked reactions in bacteria is the isolation of mutants defective in various stages in energy transduction. Recently, mutants of *E.coli* K12, termed "Unc", which appear to lack membrane Mg^{2+}–Ca^{2+}-stimulated ATPase, have been isolated [6, 7]. Furthermore, no ATP driven transhydrogenase activity in membrane preparations from an UncA mutant was detected [8]. This report presents a comparative study of both the respiratory driven and ATP driven nicotinamide adenine dinucleotide transhydrogenase reactions in membrane particles from a similar mutant [7]. The mutant particles were found to be defective in ATPase and to lack the ATP-driven transhydrogenase. In addition, vesicles from the mutant retained their ability to catalyze the respiratory driven transhydrogenase.

* Abbreviations: TTFB: 4,5,6,7-tetrachloro-2-trifluoromethylbenzimadazole. DCCD: N,N'-dicyclohexylcarbodiimide.

2. Materials and methods

The point mutant, N_{144}, was selected as a neomycin resistant derivative of *E.coli* K12 strain A428 (F^-, Pro^-, Lac_1^-, T_6^r, Gal_2^-, Ara^-, His^-, Xyl^-, Man^-, B_1^-, Str^r), unable to utilize Krebs cycle intermediates as sole sources of carbon and energy. The isolation and partial characterization of N_{144} has been described elsewhere [7]. Cells of mutant and parent were grown batchwise to late log phase in 12.5 l carboys in Davis mineral salts medium [9], supplemented with 0.5% glucose, 50 μg each of L-histidine and L-proline per ml and 1 μg per ml vitamin B_1. The cultures were aerated by bubbling at a rate of 30 l per min and the cells harvested at late log phase in a Sorvall RC2B centrifuge fitted with a continuous flow apparatus. The preparation of crude extract and subsequent isolation of the membrane particles were according to Fisher et al. [4].

The respiration and ATP driven reduction of NADP by NADH, as well as the non-energy linked reduction of acetyl-pyridine NAD by NADPH were measured according to the procedures of Fisher et al. [4] and Kaplan [10] using 0.4–1.0 mg particle protein. The activity of the ATP driven transhydrogenase reaction was determined by subtracting the specific activity of the reaction in the absence of ATP from that in the presence of ATP. The activity of the ATP driven reaction could also be determined by adding ATP following exhaustion of the oxygen in the cuvette [5].

Table 1
Transhydrogenase activity in membrane particles of strains A428 (parent) and N_{144} (mutant).

Strain	(μmoles NADPH/min/mg protein		(μmoles acetyl pyridine NADH/min/mg protein)
	Respiration driven	ATP driven	Non-energy linked
A428	16.2	11.6	93.2
N_{144}	15.9	−2.2	86.0

The respiration driven transhydrogenase [4,5] was measured in a reaction mixture containing 50 μmoles Tris sulfate pH 7.8, 700 μmoles sucrose, 0.3 μmoles NAD, 2 mg bovine serum albumin, 10 μmoles $MgCl_2$, 90 μmoles ethanol, and membrane particles (0.4–1.0 mg protein). Sixty micrograms crystalline yeast alcohol dehydrogenase was added and after incubation for 3 min the transhydrogenase reaction was initiated by the addition of 1.5 μmoles NADP. The final volume was 3 ml. The increase in absorbance at 340 nm was followed in a Gilford Multiple Sample recording spectrophotometer at 37°. The ATP driven transhydrogenase reaction was assayed in a similar manner except that 6 μmoles ATP was added with NADP to initiate the reaction. The specific activity of the ATP driven reaction was calculated by subtracting the specific activity of the respiration-driven from that of the ATP + respiration driven. The non-energy linked transhydrogenase [5,10] was measured in a mixture containing 100 μmoles potassium phosphate pH 6.5, 1.5 μmoles 3-acetyl pyridine NAD, 1 μmole KCN, and membrane particles (0.4–0.8 mg protein) in a total volume of 3.0 ml. The reaction was initiated with the addition of 1.5 μmoles NADPH and the increase in absorbance at 375 nm recorded at 20°.

Protein was determined by the method of Lowry [11] using bovine serum albumin as standard. Reagents, enzymes and substrates were of the highest purity commercially available.

3. Results and discussion

A comparison of the respiration driven-, ATP driven-, and non-energy linked transhydrogenase reactions in membrane vesicles of N_{144} (mutant) and A428 (wild type) is shown in table 1. The product of the energy-linked transhydrogenase reactions was shown to be NADPH by the addition of 2 μmoles of oxidized glutathione and 1 unit of glutathione reductase, which rapidly returned the absorbance at 340 nm to the original level. In agreement with previous reports [4,5], the energy for the reduction of NADP by NADH in particles from the parent can be derived either from the oxidation of NADH or from ATP. The ATP-driven reaction could also be measured in the parental preparation by adding ATP to the cuvette after all of the oxygen had been consumed [5]. Under these conditions the specific activity was in good agreement with the values reported in table 1. It is of interest to note that whereas the mutant particles retained the ability to catalyze the respiration driven and the non-energy linked reactions, they could not utilize ATP as an energy source in the reaction. These results suggest that the mutant N_{144} is blocked in the terminal stage of the coupling process while retaining the ability to couple NADH oxidation to the generation of some high energy state or intermediate. A further indication is provided in table 2 which shows a comparison of the effects of the inhibitors TTFB* and DCCD on the respiration driven-, and ATP driven-transhydrogenase reactions in particles of mutant and parent. In agreement with previous reports [4,5], TTFB inhibited both the respiratory and ATP driven- reactions, while DCCD affected only the ATP driven-transhydrogenase. It can be seen that the transhydrogenase activity in mutant particles was inhibited by 60 μM TTFB, yet was insensitive to DCCD.

The data in table 3 indicate that the particles from the parent contain ATPase activity which was inhibited some 70% by 60 μM DCCD. Mutant particles,

Table 2
Effect of inhibitors on the energy-linked transhydrogenase in membrane particles of A428 and N_{144}.

	Activity (%) Respiration driven	ATP driven
I.		
A428	100	100
A428 + TTFB (60 μM)	16	0
A428 + DCCD (60 μM)	80	0
II.		
N_{144}	100	–
N_{144} + TTFB (60 μM)	11	–
N_{144} + DCCD (60 μM)	92	–

Conditions of the reaction were the same as in table 1. Data are expressed as percent of specific activity obtained without the inhibitor.

Table 3
Adenosine triphosphatase in membrane particles of strains A428 and N_{144}.

	Specific activity (nmoles P_i released/min/mg protein)
A428	248
A428 + DCCD (60 μM)	69
N_{144}	18
N_{144} + DCCD (60 μM)	18

Complete reaction mixture contained 50 μmoles Tris sulfate pH 7.8, 2 μmoles $MgCl_2$, membrane particles (protein 30–60 μg), and 5 μmoles ATP in a final volume of 1 ml. The reaction mixture (minus particles) was preincubated for 6 min at 37° and the reaction initiated by adding the particles. The reaction was allowed to proceed for 30 min and was terminated with 1 ml 5% trichlorocetic acid. Inorganic phosphate released was determined by the method of Fiske and Subba-Row [12].

however, were almost completely devoid of ATPase activity. In addition, no inhibition was observed of either the ATPase, or the ATP driven transhydrogenase in vesicles of A428 when particles of the mutant were mixed with similar preparations of the parent.

The results presented here indicate that the mutant N_{144} lacks ATPase activity and therefore cannot utilize ATP to drive the energy-linked nicotinamide adenine dinucleotide transhydrogenase. It is of interest that N_{144} can catalyze the respiration-driven transhydrogenase, and that this reaction can be uncoupled by TTFB.

Cells of N_{144} cannot utilize Krebs cycle intermediates as sole sources of carbon and energy. Nevertheless, they have a higher growth yield aerobically than anaerobically in the presence of limiting concentrations of glucose [7]. This difference may be explained by the ability of the mutant to couple glucose oxidation to the generation of some high energy state, or intermediate, which might be utilized to drive certain energy-requiring cellular processes.

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