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ENERGY REQUIREMENT FOR BIOSYNTHESIS OF DNA IN *ESCHERICHIA COLI*

ROLE OF MEMBRANE-BOUND ENERGY-TRANSDUCING ATPase (COUPLING FACTOR)

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SUMMARY

A mutant of *Escherichia coli* missing energy-transducing ATPase and known to be defective in a variety of membrane functions from earlier studies (Yamamoto, T. H., Mével-Ninio, M. and Valentine, R. C. (1973) Biochim. Biophys. Acta 314, 267–275; Thipayathasana, P. and Valentine, R. C. (1974) Biochim. Biophys. Acta 347, 464–468; Mével-Ninio, M. and Yamamoto, T. (1974) Biochim. Biophys. Acta 357, 63–66) has been found to be blocked for anaerobic DNA synthesis. The rate of anaerobic DNA synthesis in the mutant, measured as radioactive adenine incorporation into the alkali-resistant fraction of whole cells, is about 1/6 the rate of DNA synthesis in the wild type culture under similar conditions. Addition of NO_3^- or O_2 restores DNA biosynthesis in the mutant. The entry of radioactive adenine is not appreciably affected in the mutant by anaerobiosis. It is concluded that coupling factor plays a role in some step(s) of DNA biosynthesis.

INTRODUCTION

Membrane-associated energy-transducing ATPase (also (Mg^{2+}, Ca^{2+})-ATPase or coupling factor) of *Escherichia coli* has recently been implicated through the study of mutants missing ATPase (*unc* A) as essential for a variety of membrane functions including oxidative phosphorylation [1, 2], ATP-dependent transhydrogenation of pyridine nucleotides [3], anaerobic active transport of amino acids [4, 5] and sugar [6], anaerobic motility [7] and anaerobic growth [4, 8]. A number of the anaerobic functions missing in the *unc* A mutants have been found to be restored by NO_3^- [4, 7] which serves as terminal electron acceptor during anaerobic respiration. These studies have led us to consider the possible role of this crucial membrane enzyme in DNA biosynthesis, a process that many workers consider to be somehow associated with the cell membrane (see ref. 9 for a general review). Experiments on DNA synthesis in a mutant missing ATPase activity are reported in this communication.

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MATERIALS AND METHODS

Organism and growth conditions

The mutant (our laboratory strain *unc*-17) used in this work was derived from *E. coli* K12 (W3110) as described previously [4]. Stock cultures of the wild type and the mutant were maintained aerobically on agar plates of Davis minimum salts medium [10] supplemented with 0.4 % glucose. The cells were grown in M-9 medium [11] supplemented with 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.17 μM ferrous citrate and 0.45 % glucose.

To start an experiment a liquid cell culture, previously grown to early exponential phase of growth (about 20 Klett units) and stored at 4 °C, was diluted with an equal volume of fresh medium and grown to a cell density of 70 Klett units.

NO_3^--grown cells were cultured anaerobically (filled stationary tubes) in similar medium supplemented with 0.2 % $NaNO_3$. When necessary, cells were freed of NO_3^- by two cycles of centrifugation (about 10 000 $\times$ *g* for 10 min at room temperature) with washing medium lacking NO_3^-. After each experiment the culture was checked for revertants capable of growing on succinate as a carbon source (presumably wild type) by streaking a loopful of cells onto Davis minimum salts medium agar plates supplemented with 0.4 % succinate.

P1 transduction

For mapping the *unc-17* lesion a lysate of the generalized transducing phage P1 was grown on the *unc* mutant as donor with an *ilv*$^-$ auxotroph serving as recipient. Handling of phage and the procedure for performing P1 crosses were as described by Miller [11]. Recombinants for *ilv*$^+$ were scored for the *unc* phenotype by spot testing on medium containing succinate.

Assay for DNA synthesis

The DNA was labeled with tritiated adenine; adenine was chosen as label because of extensive previous studies of adenine transport in *E. coli* [12]. For aerobic experiments 1 μCi of [2-^{3}H]adenine (67 μCi/mol, New England Nuclear) diluted in 25 μg of cold adenine was added to 5 ml of exponentially growing cells in a 125-ml erlenmeyer flask incubated at 30 °C. Aliquots of 0.5 ml of the radioactive cells were removed and added to tubes containing 0.5 ml of 0.5 M NaOH and the samples were digested for 16 h at room temperature to hydrolyze the labelled RNA fraction. To collect the radioactive DNA the samples were first neutralized with 50 % trichloroacetic acid at 0 °C, filtered on Millipore cellulose nitrate pads (0.45 μm) and washed three times on the filter with 5 ml of 5 % trichloroacetic acid. For counting, the filters were dried and counted in toluene liquid scintillation fluid.

For anaerobic DNA labelling experiments, 5 ml of exponentially growing cells were placed in a 25-ml flask fitted with a rubber serum stopper. The flask was made anaerobic by evacuating and flushing several times with argon via a syringe needle through the stopper. A positive pressure of argon was maintained in the vessel to compensate for the volume of the samples removed, a procedure which avoids air contamination during sampling. To start the experiment, radioactive adenine (as above) was added via a small syringe through the stopper; aliquots were removed by syringe and processed as above.

Uptake of adenine

Radioactive adenine was used to measure active transport of this purine into the cells. The experimental procedure was identical to the one described above for DNA synthesis, except that radioactive samples were collected on Millipore pads directly without alkali digestion or trichloroacetic acid precipitation; cells containing radioactive adenine were washed on the filter 3 times with 5 ml of 0.5 M LiCl.

RESULTS

Mapping of unc-17

Previous workers [13] have shown by P1 transductional analysis that *unc* lesions map in a cluster on the *E. coli* chromosome near the isoleucine-valine (*ilv*) biosynthetic operon. It was therefore important to map *unc-17*, the mutant used for the following experiments on DNA biosynthesis. Transduction analysis reveals that *unc-17* is closely linked to *ilv* with 120 of 200 ilv^+ recombinants simultaneously receiving the *unc-17* phenotype in P1-mediated crosses with *unc-17* as donor and a ilv^-, unc^+ strain as recipient, a cotransduction frequency of 60 %.

Anaerobic block of DNA synthesis in an ATPase mutant

The first experiments are concerned with the relative rates of DNA biosynthesis in a coupling factor mutant and the wild type strain (Fig. 1). In Fig. 1 DNA synthesis is shown under aerobic and anaerobic conditions for both strains. Note that the rate of DNA synthesis in the mutant is strongly inhibited by anaerobiosis, being about 1/6 the rate of the wild type under similar conditions. The arrow of Fig. 1b refers to the time of exposure of the anaerobic flask to O_2, restoring aerobic conditions for DNA biosynthesis in the mutant. After exposure to O_2 the rate of DNA biosynthesis is restored to the aerobic rate, showing that the cells have not been irreversibly damaged by the anaerobiosis. As shown in Fig. 1b, the rate of aerobic DNA synthesis is

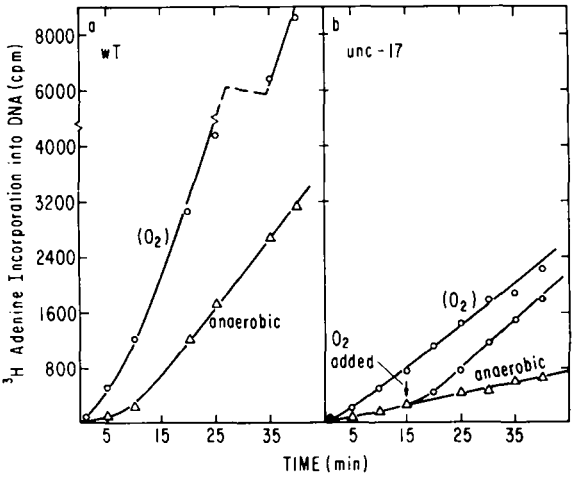

Fig. 1. Block of anaerobic DNA biosynthesis in a mutant defective for ATPase. ○, aerobic conditions; △, anaerobic conditions. Experimental details are given in Materials and Methods. Cell quantities were the same for wild type (wT) and mutant.

also considerably reduced in the mutant. ATPase may contribute significantly to DNA synthesis aerobically. Similar results have been reported for active transport of proline in this mutant [4].

NO_3^- restoration of anaerobic DNA synthesis

Experiments in this section are concerned with the restoration of anaerobic DNA synthesis by NO_3^- which functions as a terminal electron acceptor during anaerobic respiration. All the experiments are done anaerobically using cells adapted for anaerobic growth on nitrate. As in the preceding experiments (Fig. 1) the DNA synthesis in the mutant is strongly inhibited under anaerobic conditions (Fig. 2) without NO_3^-. In this experiment, the rate of anaerobic DNA synthesis of the mutant is about 1/12 the rate of wild type under similar conditions (extrapolated from the linear portion of the curves of Fig. 2); but addition of NO_3^- to the anaerobic mutant cells stimulates the anaerobic DNA synthesis by a factor of 6.5 (Fig. 2). Note that, in the mutant as well as in the wild type, the anerobic rates of DNA synthesis in the presence of NO_3^- as electron acceptor are close to the rates observed with O_2 as terminal acceptor (Fig. 1a, Fig. 2b).

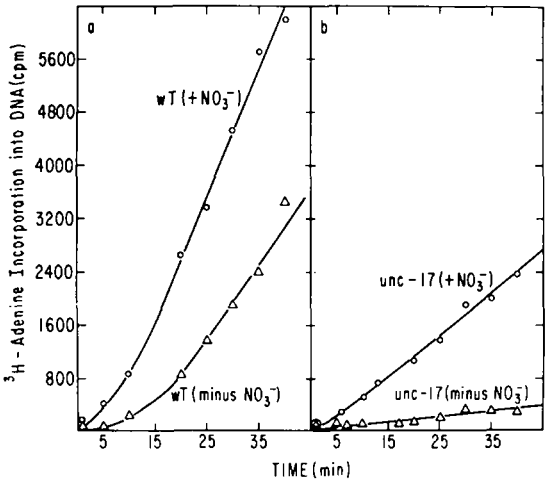

Fig. 2. Restoration of anaerobic DNA synthesis by NO_3^-. Mutant and wild type (wT) cells were grown anaerobically on NO_3^- medium and freed of exogenous NO_3^- as described in Materials and Methods. The quantity of cells used was the same in both experiments.

Adenine uptake

Hochstadt-Ozer and Stadtman [12] showed earlier that adenine transport in *E. coli* involved the conversion of adenine to AMP, a reaction catalyzed by adenine phosphoribosyltransferase and requiring *P*-Rib-*PP* as energy source. From this work, it seems improbable that the membrane-bound (Mg^{2+}, Ca^{2+})ATPase is involved in adenine transport. In conformation of this idea we have found that anaerobic transport is not appreciably affected in the mutant. As seen from Fig. 3, the level of anaerobic adenine uptake in the mutant was 85 % that of the wild type, in contrast to anaerobic DNA synthesis which is depressed by more than 90 % (Fig. 2b). Thus it seems unlikely that adenine transport is responsible for the block in anaerobic DNA synthesis.

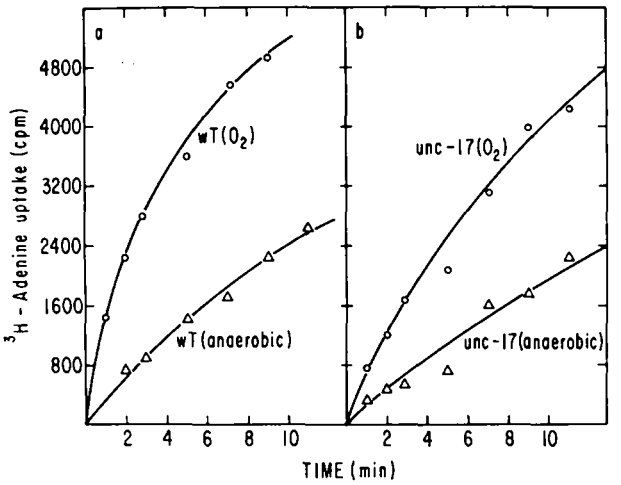

Fig. 3. Aerobic (○) and anaerobic (△) adenine uptake. Assay conditions are described in Materials and Methods. wT, wild type.

DISCUSSION

There are two recent reviews dealing mainly with membrane defects in ATPase mutants of *E. coli* [13, 14], so this disscussion will be limited to the role of ATPase in the anaerobic cell where it fulfills several vital functions. A literature survey of various anaerobic defects of the mutant strains is summarized in Table I. As seen from Table I, mutants missing this enzyme cannot grow anaerobically; anaerobic growth is restored by supplementing the culture with respiratory acceptors such as NO_3^- or O_2 [4]. We found previously that anaerobic active transport of proline is blocked in mutant *unc-17* [4], with addition of NO_3^- restoring this activity. Similarly it was recently observed that the flagella of an ATPase minus mutant were inactive during anaerobiosis [7], again being activated by NO_3^- or O_2. Thus, such seemingly diverse reactions as anaerobic active transport, anaerobic motility and anaerobic DNA biosynthesis, share an essential requirement for ATPase with NO_3^- restoring all of these functions. Note also that the system catalyzing ATP-dependent transhydrogenation of pyridine nucleotides shares similar properties [3, 15] although restoration by NO_3^- has not yet been reported for this reaction.

There are several possible mechanisms which might account for the anaerobic block of DNA synthesis in these mutants. For example, we have considered and ruled out the possibility that a block of anaerobic adenine uptake is responsible by showing that ATPase mutants transport adenine with only slightly depressed efficiency anaerobically. Also the rapid restoration of DNA synthesis upon addition of NO_3^- or O_2 to an anaerobic culture tends to rule out an "anaerobic shock" which might occur as the result of transferring the mutant grown aerobically to an anaerobic environment.

A deficiency in ATP supply in the mutant which could impair DNA synthesis has not been considered here since Larsen et al. [16] have shown that in similar ATPase mutants ATP levels are not appreciably affected by anaerobiosis.

It is possible that ATPase, or more specifically an energy-rich product of this

TABLE I

SUMMARY OF ANAEROBIC MEMBRANE DEFECTS OF *E. COLI* MUTANTS MISSING ATPase

N.D., not done. WT, wild type.

Property or reaction	Strain	Specific activity		
		Aerobic	Anaerobic	NO_3^-
Growth* (doubling time, min)	WT	25	40	N.D.
	ATPase-deficient mutant	33	>400	60
Active transport of proline [4] (nmol/10 min per mg dry wt cells)	WT	19.4	7.8	7.6
	ATPase-deficient mutant	4	0.5	7.5
Motility [7] (cpm/10 min per mg dry wt cells)	WT	8550	2300	5700
	ATPase-deficient mutant	8550	114	3800
DNA synthesis (Figs 1, 2) (cpm/30 min per mg dry wt cells)	WT	$56.8 \cdot 10^3$	$20 \cdot 10^3$	$45 \cdot 10^3$
	ATPase-deficient mutant	$17.4 \cdot 10^3$	$3 \cdot 10^3$	$18 \cdot 10^3$
ATP-dependent trans-hydrogenation of pyridine nucleotides (nmol NADPH formed/min per mg protein)	WT	93 [15]	77 [3]	N.D.
	ATPase-deficient mutant	116 [15]	4.2 [3]	N.D.

* Growth medium consisted of L-broth [17] supplemented with 0.5 % glucose as described in ref. 4.

enzyme derived from ATP, might be directly associated with the DNA replicative machinery. On the other hand, ATPase could be indirectly involved in DNA synthesis, being necessary for the biosynthesis, transport or maintenance of some nucleic acid precursors or some vital ions. Obviously further biochemical experiments are needed to distinguish between these mechanisms. The major conclusion of this work is that energy-transducing ATPase or coupling factor is required for anaerobic synthesis of DNA.

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