Oxidative Phosphorylation in *Escherichia coli* K12

AN UNCOUPLED MUTANT WITH ALTERED MEMBRANE STRUCTURE

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1. A new mutant strain (AN228) of *Escherichia coli* K12, unable to couple phosphorylation to electron transport, has been isolated. The mutant allele (*unc-405*), in strain AN228, was found to map near the *uncA* and *uncB* genes at about minute 74 on the *E. coli* genome. 2. A transductant strain (AN285) carrying the *unc-405* allele is similar to the *uncA* and *uncB* mutants described previously in that it is unable to grow on succinate, gives a low aerobic yield on limiting concentrations of glucose, has a normal rate of electron transport, is unable to couple phosphorylation to electron transport, and lacks ATP-dependent transhydrogenase activity. 3. Strain AN285 (*unc-405*) is similar to an *uncA* mutant, but different from an *uncB* mutant, in that it is unable to grow anaerobically in a glucose–mineral–salts medium, and membrane preparations do not have Mg^{2+} -stimulated adenosine triphosphatase activity. 4. Strain AN285 (*unc-405*) does not form an aggregate analogous to the membrane-bound Mg^{2+} -stimulated adenosine triphosphatase aggregate found in normal cells. In this respect it differs from strain AN249 (*uncA*[−]), which forms an inactive membrane-bound Mg^{2+} -stimulated adenosine triphosphatase aggregate.

Mutant strains of *Escherichia coli* in which oxidative phosphorylation is affected have been isolated in several laboratories (Butlin *et al.*, 1971; Kanner & Gutnick, 1972*a,b*; Butlin *et al.*, 1973). Some of these strains have lost Mg-ATPase* activity as have other mutant strains (Schairer & Haddock, 1972; Simoni & Shallenberger, 1972) that have been used to study active transport but have not been examined for the ability to carry out oxidative phosphorylation.

Mutant strains of *E. coli* K12 carrying mutations in either the *uncA* or *uncB* genes and unable to couple electron transport to the esterification of phosphate have been examined in detail (Butlin *et al.*, 1971, 1973). Fractionation of the membranes from the *uncA* and *uncB* mutants, combined with reconstitution studies (Cox *et al.*, 1973*a,b*), has allowed a working scheme to be proposed for the function of the proteins coded for by the *uncA* and *uncB* genes (Cox *et al.*, 1973*b*).

The present paper describes a mutant strain of *E. coli* K12 which resembles an *uncA* mutant in lacking Mg-ATPase activity, but differs from an *uncA* mutant in that there appears to be an alteration in membrane structure.

* Abbreviation: Mg-ATPase, Mg^{2+} -stimulated adenosine triphosphatase.

Experimental

Materials

Chemicals. Chemicals generally were of the highest purity available commercially and were not further purified. Lactate dehydrogenase and hexokinase were supplied by Sigma Chemical Co., St. Louis, Mo., U.S.A., and glutathione reductase and alcohol dehydrogenase were from Calbiochem (Australia) Pty. Ltd., Carlingford, N.S.W., Australia.

Organisms. All the bacterial strains used were derived from *E. coli* K12 and are described in Table 1.

Methods

Media and growth of organisms. The medium used was that described by Monod *et al.* (1951) as medium 56. Growth supplements were added as sterile solutions to the sterilized mineral salts base to give the following final concentrations: L-amino acids, 0.2mM; thiamin, 0.2μM; 2,3-dihydroxybenzoate, 40μM; glucose, 30mM.

Growth yields of various strains on media containing limiting glucose were measured as turbidities in a Klett–Summerson colorimeter as described by Cox *et al.* (1970). For growth under anaerobic conditions,

Table 1. *Strains of E. coli K12 used*

Genes coding for enzymes in various biosynthetic pathways are denoted as follows: *met*, methionine; *ilv*, isoleucine-valine; *arg*, arginine; *ent*, enterochelin. The *uncA* and *uncB* genes code for proteins required in the coupling of phosphorylation to electron transport.

Strain	Relevant genetic loci	Other information
AB3311	<i>metB</i> ⁻	Hfr Reeves
AN228	<i>metB</i> ⁻ , <i>unc-405</i>	Derived from strain AB3311 after treatment with <i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitroso-guanidine
AN248	<i>ilvC</i> ⁻ , <i>argH</i> ⁻ , <i>entA</i> ⁻	Butlin <i>et al.</i> (1973)
AN285	<i>unc-405</i> , <i>argH</i> ⁻ , <i>entA</i> ⁻	Isolated after transduction with strain AN228 as donor and strain AN248 as recipient
AN259	<i>argH</i> ⁻ , <i>entA</i> ⁻	Butlin <i>et al.</i> (1973)
AN283	<i>uncB401</i> , <i>argH</i> ⁻ , <i>entA</i> ⁻	Butlin <i>et al.</i> (1973)
AN249	<i>uncA401</i> , <i>argH</i> ⁻ , <i>entA</i> ⁻	Cox <i>et al.</i> (1973b)

cultures were incubated in an atmosphere of H₂+CO₂ (95:5) as described previously (Butlin *et al.*, 1971).

Cells used for membrane preparations were grown in 7-litre New Brunswick fermenters as described by Cox *et al.* (1970).

Preparation and fractionation of cell membranes. The preparation and fractionation of membranes was carried out as described previously (Cox *et al.*, 1973a). Briefly, washed cells were disintegrated by using a Sorvall Ribi cell fractionator and the 'membranes' were separated by ultracentrifugation and resuspended in a 0.1M-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid (TES) buffer system (pH 7.0) containing 0.02M-magnesium acetate, 0.25M-sucrose and 0.25mM-ethanedioxybis(ethylamine)-tetra-acetate (EGTA). The membranes were then washed with 50mM-Tris-HCl buffer (pH 7.4) and subsequently subjected to two low-ionic-strength washes with 5mM-Tris-HCl buffer (pH 7.4) containing 0.25mM-EDTA and 0.5mM-dithiothreitol. After centrifugation at 160000g for 2h the supernatant was decanted and then concentrated by using a Diaflo XM-50 ultrafilter to give the '5mM-Tris wash'. The pellet was resuspended in the 5mM-Tris buffer system to give the 'membrane residue'.

Protein concentrations were determined by using Folin's phenol reagent (Lowry *et al.*, 1951) with bovine serum albumin (fraction V; Sigma Chemical Co., St. Louis, Mo., U.S.A.) as standard.

Transduction technique. The technique used for transduction experiments, in which the generalized transducing bacteriophage PIKC was used, is described by Pittard (1965).

Measurement of the non-energy-linked transhydrogenase activity. Reduction of NAD⁺ by NADPH was assayed by coupling the reaction to the NADH-dependent reduction of pyruvate by lactate dehydrogenase and measuring the decrease in *E*₃₄₀. Details of the technique used are described by Cox *et al.* (1971).

Measurement of the energy-linked transhydrogenase activities. The techniques used were based on those described by Fisher & Sanadi (1971) and Ernster & Lee (1967). Experimental details are given by Cox *et al.* (1973b).

Assay of Mg-ATPase. The method used to assay Mg-ATPase activity is described by Cox *et al.* (1973a).

Oxidative phosphorylation. The technique used for the determination of P/O ratios is described by Cox *et al.* (1973a).

Column chromatography. Column chromatography was carried out by using Agarose (Biogel A-5m, 200-400 mesh; Bio-Rad, Richmond, Calif., U.S.A.) and eluting with 5mM-Tris-HCl buffer, pH 7.4, containing 0.5mM-dithiothreitol and 0.25mM-EDTA as described previously (Cox *et al.*, 1973a).

Results

Mapping of the *unc-405* allele

Mutants unable to grow on succinate but able to grow on glucose as sole source of carbon (Suc⁻ phenotype) were screened to detect strains giving low aerobic growth yields in mineral-salts media containing limiting concentrations of glucose. Strain AN228 gave a low aerobic growth yield and was therefore examined in detail. This selection technique has been used previously (Butlin *et al.*, 1971, 1973) to isolate strains carrying the *uncA401* or *uncB401* alleles. Both these mutant alleles are co-transducible with the *ilv* genes at about minute 74 on the *E. coli* genome. Co-transduction experiments were therefore carried out to determine whether the mutation causing the Suc⁻ phenotype in strain AN228 also mapped near the *ilv* genes. The generalised transducing bacteriophage, PIKC, was grown on strain AN228 and used in co-transduction experiments with strain AN248 (*ilvC*⁻). Of the *ilvC*⁺ transductants examined, 34 out of 102 were unable to grow on succinate and the *ilvC* gene was therefore

Table 2. Comparison of some properties of strain AN285 (*unc-405*) with the normal strain AN259

Details are given in the Experimental section. Assays of enzymic activities were carried out on membrane preparations.

Test	Strain	
	AN259	AN285 (<i>unc-405</i>)
Aerobic growth in succinate-mineral salts medium	+	—
Aerobic growth in glucose-mineral-salts medium	+	+
Anaerobic growth in glucose-mineral-salts medium	+	—
Growth yield on limiting (4mM) glucose (turbidity, Klett units)	195	120
NADH oxidase (ng-atoms of O/min per mg of protein)	1150	1240
P/O ratio with D-lactate as oxidizable substrate	0.12	<0.005
Mg-ATPase (μ mol of P_i released/min per mg of protein)	0.21	0
Non-energy-linked transhydrogenase (nmol of NADH formed/min per mg of protein)	119	93
ATP-dependent transhydrogenase (nmol of NADPH formed/min per mg of protein)	72	3
Electron-transport-dependent transhydrogenase (nmol of NADPH formed/min per mg of protein)	93	192

co-transducible with the mutation causing the Suc⁻ phenotype at a frequency of about 33%. The mutant allele was designated *unc-405*. One of the transductants (AN285) carrying the *unc-405* allele was isolated and used in the experiments described below. The mutant strains AN249 (*uncA*⁻), AN283 (*uncB*⁻) and the normal strain AN259 are similarly derived from strain AN248 by transduction and are used for comparison with strain AN285 (*unc-405*).

Comparison of some characteristics of the normal strain AN259 and the mutant strain AN285 (unc-405)

The growth characteristics of strain AN285 (*unc-405*) differ from the normal strain in that AN285 is unable to grow aerobically on succinate as sole source of carbon or in a glucose-mineral-salts medium under anaerobic conditions, and has a lower growth yield on limiting glucose under aerobic conditions (Table 2). Strain AN285 (*unc-405*) therefore resembles strain AN249 (*uncA*⁻) rather than strain AN283 (*uncB*⁻) in that the latter strain is able to grow in a glucose-mineral-salts medium under anaerobic conditions.

Membranes prepared from strain AN285 (*unc-405*) also differ from membranes prepared from the normal strain in that there is no detectable phosphorylation with D-lactate as oxidizable substrate, Mg-ATPase activity is absent, and the ATP-dependent transhydrogenase is inactive (Table 2). Electron transport, the non-energy-linked transhydrogenase and the electron-transport-dependent transhydrogenase activities, however, appear to be normal (Table 2). Thus the membranes prepared from strain AN285 (*unc-405*) have similar properties to membranes from a strain carrying the *uncA401* allele (Butlin *et al.*, 1971; Cox *et al.*, 1971, 1973b). Membranes prepared from strain AN283 (*uncB*⁻)

are different in that the Mg-ATPase activity in such membranes is normal (Cox *et al.*, 1973b).

Reconstitution of the ATP-dependent transhydrogenase activity in membranes from AN285 (unc-405)

The ATP-dependent transhydrogenase activity in membrane preparations from a strain carrying the *uncA401* allele could not be reactivated by solubilized Mg-ATPase unless the membranes had been washed with low-ionic-strength buffer (Cox *et al.*, 1973a). The reactivation of this activity in membranes and membrane residues prepared from strains AN249 (*uncA*⁻) and AN285 (*unc-405*) by solubilized Mg-ATPase from the normal strain was therefore tested. As found previously for strains carrying the *uncA401* allele, the ATP-dependent transhydrogenase in membranes from strain AN249 (*uncA*⁻) could not be reactivated by solubilized Mg-ATPase (Table 3). However, in contrast, the ATP-dependent transhydrogenase activity in membranes from strain AN285 (*unc-405*) was reactivated by the addition of solubilized Mg-ATPase (Table 3). The specific activities of both the non-energy-linked and the ATP-dependent transhydrogenase activities increased in the membrane residue owing to the loss of protein during the washing procedure. However, the ratio of the reconstituted ATP-dependent transhydrogenase activity to the non-energy-linked transhydrogenase activity is the same in the membranes and the membrane residue derived from strain AN285 (*unc-405*) (Table 3). It would therefore seem likely that a polypeptide aggregate corresponding to the Mg-ATPase aggregate found in membranes from normal cells is not present in membranes prepared from strain AN285 (*unc-405*).

Table 3. *Effects of partially purified Mg-ATPase on the ATP-dependent transhydrogenase activities of membranes and membrane residues from strains AN249 (uncA⁻) and AN285 (unc-405)*

Membrane and membrane residues (0.5–1.1 mg of protein) were assayed for ATP-dependent transhydrogenase activity before and after the addition of solubilized Mg-ATPase (about 1 mg of protein) and for non-energy-linked transhydrogenase activity without the addition of solubilized Mg-ATPase. Details are given in the Experimental section.

Strain	Membranous preparation	ATP-dependent transhydrogenase activity (nmol of NADPH formed/min per mg of membrane protein)	Increase in ATP-dependent transhydrogenase activity (nmol of NADPH formed/min per mg of membrane protein) after the addition of Mg-ATPase	Non-energy-linked transhydrogenase activity (nmol of NADH formed/min per mg of protein)
AN249 (<i>uncA</i> ⁻)	Membranes	4	5	103
AN249 (<i>uncA</i> ⁻)	Membrane residue	0	123	180
AN285 (<i>unc-405</i>)	Membranes	3	37	93
AN285 (<i>unc-405</i>)	Membrane residue	0	94	215

Gel filtration of the 'low-ionic-strength wash' prepared from strains AN283 (uncB⁻), AN249 (uncA⁻) and AN285 (unc-405)

The results obtained from reconstitution studies using the various uncoupled mutants have suggested that strain AN283 (*uncB*⁻) forms a normal Mg-ATPase aggregate (Cox *et al.*, 1973b), strain AN249 (*uncA*⁻) forms an inactive Mg-ATPase aggregate (Cox *et al.*, 1973b) and strain AN285 (*unc-405*) does not form a Mg-ATPase aggregate (see above). To investigate this more directly, a 'low-ionic-strength wash' prepared from each of these strains was chromatographed by passage down a column containing Biogel A-5m and the protein profiles were compared (Fig. 1). The protein profile and the Mg-ATPase activity profile obtained for the 'low-ionic-strength wash' from strain AN283 (*uncB*⁻) were similar to those obtained for the normal strain AN259 (not shown) except that the Mg-ATPase specific activity (9 μ mol of P_i released/min per mg of protein) of strain AN283 (*uncB*⁻) was about six times the specific activity of the peak Mg-ATPase from the normal strain. The protein profile obtained from the 'low-ionic-strength wash' from strain AN249 (*uncA*⁻) is closely similar to those obtained for strains AN283 (*uncB*⁻) and the normal strain (AN259). However, as expected, no Mg-ATPase activity could be detected in the fractions from this column. The protein profile obtained from the chromatography of the 'low-ionic-strength wash' from strain AN285 (*unc-405*) is different from the others in that the protein peak, corresponding to the Mg-ATPase activity peak from strain AN283 (*uncB*⁻), is absent.

Discussion

Three types of mutants have not been isolated in which electron transport is uncoupled from phosphorylation. Each type of mutant has also lost the

Fig. 1. Gel filtration, on Biogel A-5m, of low-ionic-strength wash from membranes of the uncoupled strains of *E. coli*, AN283 (*uncB*⁻), AN249 (*uncA*⁻) and AN285 (*unc-405*)

●, Protein concentration; ■, Mg-ATPase activity. Strains AN249 and AN285 had no Mg-ATPase activity. Fractions (3.4 ml) were collected.

ATP-dependent transhydrogenase activity but has retained the electron-transport-dependent transhydrogenase activity. The types have been differentiated as a result of studies on membrane fractionation and reconstitution using the various mutant strains. Thus a strain carrying a mutation in the *uncB* gene has a defect in the membrane-residue fraction but forms a membrane-bound Mg-ATPase aggregate with de-repressed Mg-ATPase activity. An *uncA* mutant has a normal membrane-residue fraction but forms an inactive membrane-bound Mg-ATPase aggregate. Polyacrylamide-gel electrophoresis, under dissociating conditions, of the appropriate fractions from the gel-filtration experiments show that the polypeptide compositions of the Mg-ATPase aggregates formed by strains AN283 (*uncB*⁻) and AN249 (*uncA*⁻) are similar (G. B. Cox, F. Gibson & L. McCann, unpublished work). A mutant strain carrying the *unc-405* allele has a normal membrane-residue fraction but does not form a membrane-bound Mg-ATPase aggregate.

The *unc-405* allele has not been given a specific gene designation, as there is a possibility that the mutation concerned occurs in the *uncA* gene. Thus if the structural genes for the polypeptides of the Mg-ATPase aggregate form an operon-like arrangement then it is possible that the *unc-405* allele is an *uncA* gene carrying a polarity mutation. The lack of a membrane-bound Mg-ATPase aggregate in the membranes of *unc-405* mutants could, however, be due to any of a number of reasons and further experimentation is required before a specific gene designation can be made.

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