RECONSTITUTION OF ENERGY-DEPENDENT TRANSHYDROGENASE IN ATPase-NEGATIVE MUTANTS OF ESCHERICHIA COLI

P.D. Bragg and C. Hou
Department of Biochemistry
University of British Columbia
Vancouver 8, B.C., Canada

Received December 21,1972

SUMMARY

The aerobic-driven and ATP-driven energy-dependent transhydrogenase activities of membrane particles from two different Ca^{2+}, Mg^{2+}-activated ATPase-negative mutants of E. coli were examined. The activities were low or absent in one of the mutants (DL-54). Reconstitution of the aerobic-driven reaction could be obtained by addition to particles from this mutant of DCCD or of a coupling factor prepared from the parent strain. The coupling factor also restored the ATP-driven reaction. In the other mutant (N144) the aerobic-driven activity was unimpaired, and was not affected by DCCD or by the coupling factor. The difference between the two mutants could be rationalized if the coupling factor ATPase had both a stabilizing and an enzymic function.

INTRODUCTION

There have been several reports of mutants of E. coli which lack Ca^{2+}, Mg^{2+}-activated ATPase activity (1-5). These mutants are defective in oxidative phosphorylation and in the ATP-driven energy-dependent transhydrogenase reactions (1-3). Active transport of amino acids and the energy-dependent transhydrogenase reaction driven directly by oxidation are not affected (3,6). Thus, it seems likely that these mutants are unable to carry out the terminal transphosphorylation reaction of oxidative phosphorylation, but are still able to form the high energy state or intermediate required both for this process and for active transport and energy-dependent transhydrogenation.

We have shown that removal of the Ca^{2+}, Mg^{2+}-activated ATPase from membrane particles of wild-type strains by a washing procedure results in the loss of both transhydrogenase activities, and that

addition of an almost homogeneous preparation of this enzyme completely restores both reactions (7). Recently, a mutant (DL-54) of E. coli has been described (5) which lacks Ca^{2+}, Mg^{2+}-activated ATPase activity but differs from another ATPase-negative mutant (6) in being somewhat defective in active transport of amino acids. Thus, DL-54 behaves in a similar manner to our membrane particles which have been stripped of the ATPase in being less effective in forming the energized state.

In the present paper we show that DL-54 has lower energy-dependent transhydrogenase activities than the parent strain, and that this deficiency may be remedied by addition of an ATPase preparation ("coupling factor") from the parent strain. DL-54 differs from another ATPase-negative mutant (N144) which has the modified ATPase firmly bound to the membrane. In DL-54 the modified ATPase is less firmly bound to the membrane than in the parent strain. Our results suggest that the Ca^{2+}, Mg^{2+}-activated ATPase, besides acting as a transphosphorylating enzyme, has a role in stabilizing the high energy state or intermediate.

METHODS

E. coli ML308-225 (wild-type) and DL-54 (Ca, Mg-activated ATPase-negative), and E. coli WS 1 (wild-type) and N 144 (Ca, Mg-activated ATPase-negative) were generous gifts from Drs. R.D. Simoni and D.L. Gutnick, respectively. The first two strains were grown on a minimal salts-glucose (0.4%) medium containing 12 μM ferric citrate (8), and the latter two strains were cultured on a similar medium but supplemented with histidine (50 mg/l), proline (50 mg/l) and thiamine (1 mg/l). The cells were harvested in the late exponential phase and converted to washed respiratory particles as previously described (7). The washed particles were suspended in, and dialysed overnight against, 1 mM Tris-HCl buffer,

pH 7.5, containing 0.5 mM EDTA, 0.1 mM dithiothreitol, and 10% glycerol (7). The dialysed particle suspension was centrifuged (120,000 x g; 2 hr) to yield a pellet of "stripped" particles. The supernatant fluid was recentrifuged to remove residual particulate material and then concentrated to about one-tenth of its volume by ultrafiltration through a PM 10 membrane (Amicon Corp.) to yield the "coupling factor" (protein, 14 mg/ml) (7).

Protein, Ca^{2+}-activated ATPase, aerobic- and ATP-driven energy-dependent transhydrogenase activities were measured exactly as described previously (8). The activites of the energy-dependent transhydrogenases were corrected for that of the energy-independent transhydrogenase. The stripped particles were suspended in the medium used before (8). When coupling factor or dicyclohexylcarbodiimide (DCCD) was added to the particles they were included during the preincubation period. DCCD was added as a solution in ethanol.

In experiments to measure the distribution of Ca^{2+}-activated ATPase activity between particulate and supernatant fractions the cells were converted to respiratory particles and supernatant fractions as previously described (8) except that the low-speed centrifugation step was omitted.

RESULTS AND DISCUSSION

Dialysis of particles from wild-type strains of *E. coli* against a low ionic strength buffer resulted in loss of both aerobic- and ATP-driven energy-dependent transhydrogenase activities. These activities could be restored by addition of almost pure Ca^{2+}, Mg^{2+}-activated ATPase (7) or, more conveniently since the enzyme is labile, by a less purified preparation of the ATPase ("coupling factor") (Figure 1) (Table I). In contrast to the parent strain (ML308-225) the ATPase-negative mutant (DL-54) had a much lower

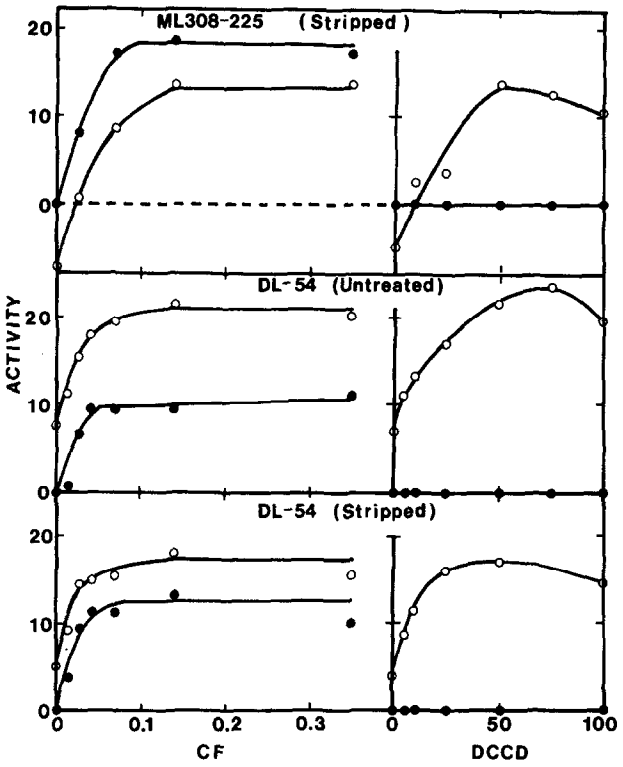

FIGURE 1. Effect of coupling factor (CF) (left) and DCCD (right) on aerobic-driven (open points) and ATP-driven (solid points) energy-dependent transhydrogenase activites. Activity is expressed as nmoles NADPH formed/min. CF or DCCD present is given as mg protein or nmoles DCCD, respectively. The amounts of stripped particles of ML308-225 and DL-54, or of untreated DL-54 were 0.24, 0.32 and 0.73 mg protein, respectively.

aerobic-driven transhydrogenase activity besides completely lacking that driven by ATP (Table I). The particles from DL-54 responded to coupling factor even when the dialysis treatment had been omitted. This latter behaviour contrasts both with that of the parent strain and also with that of E. coli NRC-482 (7) and E. coli WS 1. The aerobic-driven transhydrogenase activites of both untreated and stripped particles from DL-54 in the presence of the coupling factor was close to that of the untreated or stripped particle of the wild-type strain, respectively (Table I). It should be noted that the dialysis treatment itself resulted in an

TABLE 1. Effect of coupling factor and DCCD on energy-dependent transhydrogenase of respiratory particles

Strain	Treatment of Particles	Additions	Transhydrogenase	
			Aerobic	ATP
ML308 -225	None	-	17.5	52.7
		CF	23.4	52.7
		DCCD	30.2	12.1
	Stripped	-	0	0
		CF	57.2	78.4
		DCCD	57.2	0
DL-54	None	-	10.4	0
		CF	29.8	15.2
		DCCD	29.8	0
	Stripped	-	15.8	0
		CF	57.3	42.1
		DCCD	54.1	0
WS 1	None	-	36.9	47.3
	Stripped	-	12.9	0
		CF	51.2	80.4
N 144	None	-	33.5	0
		DCCD	32.8	0
	Stripped	-	50.8	0
		CF	49.3	0
		DCCD	48.3	0

CF, saturating level of coupling factor; DCCD, 41.7 μM. Transhydrogenase activity is expressed as nmoles NADPH formed/min/mg particle protein. The coupling factor was prepared from the respective wild-type strains.

increase in the specific activity of the supplemented systems (Table I). It is not known whether this is due to the removal of an inhibitor or to the removal of protein.

Addition of an optimum level of DCCD to the stripped particles of the parent strain or to untreated or washed particles of DL-54 stimulated the aerobic-driven transhydrogenase to the same extent

as that produced by saturating levels of the coupling factor. The ATP-driven reaction was not restored by DCCD (Figure 1; Table I). Low levels of DCCD did not affect the aerobic-drive transhydrogenase of the wild-type strains but inhibited the ATP-driven activity (Table I).

The behaviour of the ATPase-negative strain N-144 is different to that of DL-54. In agreement with the results of Kanner and Gutnick (3) this strain had aerobic-driven transhydrogenase activity equal to that of the parent strain WS 1 but lacked the ATP-driven reaction (Table I). In a similar manner to ML308-225 stripped particles from WS 1 had much lower transhydrogenase activities which were restored by addition of coupling factor. Attempts to strip the particles of the mutant N 144 did not result in any loss of transhydrogenase activity. Furthermore, addition of coupling factor or DCCD to untreated or dialysed particles of N 144 did not affect this activity. Thus the mutation which results in loss of ATPase activity in N 144 is not identical to that in DL-54.

The above results are compatible with the suggestion by Fessenden-Raden (9) that the ATPase may have a dual role. It may act both as a transphosphorylase but also may have a structural role in stabilizing the high energy intermediate or state formed during substrate oxidation *via* the respiratory chain. In the latter role modified, inactive ATPase may act just as well as the native enzyme. It has been suggested also that DCCD can partly fulfil the structural role of the ATPase (10). It is probable that the modified ATPase of N 144 is more firmly bound to the membrane than in the parent strain whereas that of DL-54 is more readily lost from the membrane. Thus, the structural role of the ATPase in stabilizing the high energy intermediate or state can still be

carried out by the inactive, firmly-bound ATPase of N-144, and neither coupling factor nor DCCD are required. In contrast, the ready loss of the modified ATPase of DL-54 results in a "leakage" of the high energy state or intermediate. Thus, both the aerobic-driven transhydrogenase and aerobic-driven amino acid transport (5) are impaired. Addition of DCCD and coupling factor will

TABLE 2. Distribution of protein and ATPase activity on cell fractionation

Strain	Fraction	Protein (mg)*	ATPase (units)*
WS 1	P	39	11.4
	S	61	2.85
N 144	P	34	0.29
	S	66	0.95
ML308-225	P	43	20.4
	S	57	3.25
DL-54	P	29	0.26
	S	71	1.14

* Values expressed per 100 mg total protein. P, particle fraction; S, supernatant fraction. Units are expressed as μmoles/min.

repair this defect, and the latter will in addition permit ATP to be used as an energy source.

Since, we postulate that ATPase is less firmly bound to the membrane in DL-54 we have examined the distribution of Ca^{2+}-activated ATPase activity in the respiratory particles and in the non-particulate fraction which results on cell fractionation (Table 2). Significant amounts of Ca^{2+}-activated ATPase activity

were not found in the soluble fraction of DL-54. Thus, the properties of membrane particles of DL-54 were not due to the loss of an active, unmodified ATPase as a result of a modification of a "binding" protein, but presumably a consequence of a structural change in the ATPase. In experiments not reported here we have shown that the coupling factor prepared from the parent strain in fact binds to stripped membrane particles from the parent strain, and to both untreated and stripped particles from DL-54.

ACKNOWLEDGEMENTS

We wish to thank Drs. D.L. Gutnick and R.D. Simoni for generous gifts of bacterial strains, and the Medical Research Council of Canada for a research grant.

REFERENCES

1. Butlin, J.D., Cox, G.B. and Gibson, F. Biochem. J. 124, 75, 1971.
2. Cox, G.B., Newton, N.A., Butlin, J.D. and Gibson, F. Biochem. J. 125, 489, 1971.
3. Kanner, B.I. and Gutnick, D.L. FEBS Letters 22, 197, 1972.
4. Schairer, H.U. and Haddock, B.A. Biochem. Biophys. Res. Commun. 48, 544, 1972.
5. Simoni, R.D. and Shallenberger, M.K. Proc. Nat. Acad. Sci. U.S.A. 69, 2663, 1972.
6. Hong, J.-S. and Kaback, H.R. Proc. Nat. Acad. Sci. U.S.A. 69, 3336, 1972.
7. Bragg, P.D. and Hou, C. FEBS Letters, in press.
8. Bragg, P.D., Davies, P.L. and Hou, C. Biochem. Biophys. Res. Commun. 47, 1248, 1972.
9. Fessenden-Raden, J.M. J. Biol. Chem. 244, 6662, 1969.
10. Racker, E. Fed. Proc. 26, 1335, 1967.