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LACK OF INVOLVEMENT OF LIPOIC ACID IN MEMBRANE-ASSOCIATED ENERGY TRANSDUCTION IN ESCHERICHIA COLI

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SUMMARY

Oxidative phosphorylation, active transport of proline, aerobic- and ATP-driven proton translocation and transhydrogenation of $NADP^+$ by NADH, occurred in lipoic acid-deficient cells or vesicles of a lipoic acid auxotroph of E. coli, W1485 lip 2. Addition of lipoic acid had little effect on these processes. Tributyltin chloride, which has been proposed to inhibit oxidative phosphorylation by reaction with lipoic acid (Cain et al., Biochem. J. (1977) 166, 593), was an effective inhibitor of aerobic and ATP-dependent proton translocation and transhydrogenation in lipoic acid-deficient vesicles from this organism. Our results do not support the proposal of Partis et al. (FEBS Lett. (1977) 75, 47) that lipoic acid is involved in the energy transducing processes associated with the membrane of E. coli.

INTRODUCTION

In an important series of papers Griffiths and coworkers have provided substantial evidence for the involvement of lipoic acid in oxidative phosphorylation in mitochondria and in E. coli (1-8). They propose that lipoic acid provides a link between the respiratory chain and the ATP synthetase complex, and plays a key role in the ATP-synthesizing and ATP-utilizing reactions catalyzed by the ATPase complex. An important piece of evidence in support of Griffiths' hypothesis is the demonstration of lipoic acid-dependent oxidative phosphorylation in inverted membrane vesicles of a lipoic acid auxotroph, E. coli W1485 lip 2 (6). Further support for the hypothesis is provided by the finding that dibutylchloromethyltin chloride, which has a similar mode of action to other trialkyltin inhibitors of oxidative phosphorylation, binds covalently to lipoic acid in submitochondrial particles or ATP synthetase complexes (2,4).

Abbreviations: CCCP, carbonylcyanide m-chlorophenylhydrazone; DCCD, N,N'-dicyclohexylcarbodiimide; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate.

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We have examined in the lipoic acid auxotroph E. coli W1485 lip 2 the dependence on lipoic acid of oxidative phosphorylation, and of several other reactions (active transport, aerobic- and ATP-driven proton translocation and transhydrogenation of $NADP^+$ by NADH) which measure the generation of the energized state of the membrane by substrate oxidation or ATP hydrolysis. We have found that lipoic acid is not required for these processes. Moreover, tributyltin chloride was an effective inhibitor of aerobic- and ATP-driven proton translocation and transhydrogenation in lipoic acid-deficient vesicles.

METHODS

E. coli W1485 lip 2 (9), a lipoic acid auxotroph of E. coli K-12, was a generous gift of Dr. J.R. Guest, University of Sheffield, England. Cells were grown aerobically at 37°C in a minimal medium containing 0.2% glucose, with or without the addition of lipoic acid (9) or of both sodium acetate (4 mM) and sodium succinate (4 mM). The cells were harvested in the late exponential phase of growth, washed twice with either 0.05 M potassium phosphate buffer, pH 6.6 (for transport studies) or 0.05 M HEPES-KOH buffer, pH 7.8, containing 10 mM $MgCl_2$. Inverted membrane vesicles were prepared from HEPES-washed cells as previously described (10). For the measurement of oxidative phosphorylation, membrane vesicles were prepared as described by Partis et al. (6) except that the vesicles were sonicated briefly (three 15 sec periods using a Branson Model W185D sonic oscillator equipped with a microprobe) and used without further purification on a sucrose density gradient. "Right-side out vesicles" for transport studies were prepared by the method of Kaback (11). Transport of [^{14}C]proline, proton translocation by the quenching of the fluorescence of 9-aminoacridine, energy-dependent transhydrogenation, ATPase and oxidase activities were measured as described previously (10,12,13). Oxidative phosphorylation was assayed in a glucose-hexokinase trap system by the disappearance of inorganic phosphate or the formation of glucose-6-phosphate as described by Partis et al. (6), or by the measurement of ATP synthesis using the luciferase assay (14). In the latter experiments oxygen uptake was measured simultaneously with ATP formation using a Gilson oxygraph.

DL-α-Lipoic acid, substrates and coenzymes were obtained from Sigma Chemical Company. 9-Aminoacridine hydrochloride, tri-n-butyltin chloride, and [^{14}C]proline were supplied by Aldrich Chemical Company, Alpha Inorganics, and Amersham-Searle Corporation, respectively.

RESULTS

The lipoic acid auxotroph, E. coli W1485 lip 2, can be grown aerobically in the absence of lipoic acid with glucose as carbon source provided that lysine plus methionine or acetate plus succinate are also supplied (9). The lipoic acid-deficient cells used in our experiments were grown with the latter substrates. They were always checked for the presence of revertants by plating on glucose media with and without lipoic acid or acetate plus succinate. Further-

TABLE 1

Effect of lipoic acid on oxidative phosphorylation in inverted vesicles from lipoid acid-deficient cells.

Substrate	-Lipoic acid			+Lipoic acid		
	O_2 uptake	ATP synthesis	P:O	O_2 uptake	ATP synthesis	P:O
D-lactate	91.2	38.0	0.42	88.4	38.4	0.43
Succinate	31.2	13.4	0.43	29.4	13.8	0.47
NADH	145.3	63.1	0.43	153.0	68.7	0.45

Oxygen uptake and ATP synthesis have been corrected for values obtained in the absence of substrate and are expressed as ng atoms or nmoles/min/mg protein, respectively. Substrates were present at 10 mM final concentration. The concentration of lipoic acid was 0.67 mM.

more, the cells used in each experiment were examined for their inability to oxidize α-ketoglutarate in the absence of added lipoic acid.

Partis et al. (6) found that oxidation of NADH, succinate and D-lactate was not coupled to ATP formation in inverted vesicles from lipoic acid-deficient cells of the same strain of E. coli used in our studies. ATP formation was observed only if the vesicles were supplemented with lipoic acid. In contrast with these results, we have found that oxidative phosphorylation with these three substrates is not dependent on the presence of lipoic acid (Table 1). Our P:O values are of similar magnitude to those obtained by other workers using inverted vesicles prepared from wild-type cells (14,15). ATP formation was inhibited completely by 25 μM CCCP, an uncoupler of oxidative phosphorylation, and by 70% with 0.1 mM DCCD, an inhibitor of the ATP synthetase complex (16). In Table 1 ATP formation was measured by the sensitive luciferase assay (14). In other experiments, ATP formation was determined by phosphate uptake or by the formation of glucose-6-phosphate (6). In a series of seven separate experiments using a variety of assay methods to measure ATP formation we could not detect a requirement of lipoic acid for oxidative phosphorylation.

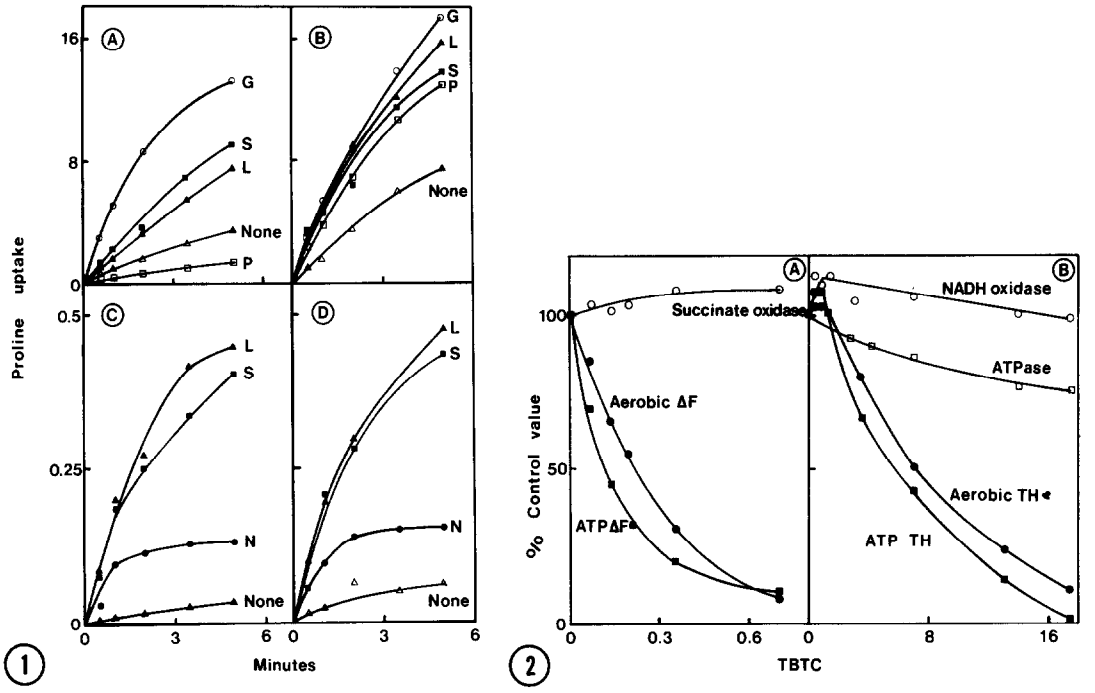

FIGURE 1. [^{14}C]Proline uptake by lipoic acid-deficient intact cells (A,B) or membrane vesicles (C,D) in the presence (B,D) or absence (A,C) of added lipoic acid (30 μM). All substrates (G, glucose; L, D-lactate; S, succinate; P, pyruvate; N, NADH) were used at a final concentration of 20 mM. The concentrations of cells and vesicles were 0.3 and 0.2 mg protein/ml, respectively. Proline uptake is expressed as nmoles/mg protein.

FIGURE 2. Effect of tri-n-butyltin chloride (TBTC) on (A) succinate oxidase (aerobic)- and ATP-dependent quenching of 9-aminoacridine fluorescence, and (B) NADH oxidase (aerobic)- and ATP-dependent transhydrogenase (TH) activities of inverted membrane vesicles from lipoic acid-deficient cells. The concentration of protein in experiments A and B was 1.82 and 0.95 mg/ml, respectively. The 100% values for succinate oxidase, NADH oxidase, ATPase, aerobic and ATP-dependent transhyrogenase activities are 134, 116, 1722, 18.4 and 9.5 nmoles/min/mg protein, respectively. The 100% value for fluorescence quenching (ΔF) is equal to 75% of the fluorescence of the system prior to quenching. The concentration of TBTC is expressed as nmoles/mg protein.

Proline transport into E. coli is dependent on the formation of an energized membrane state by substrate oxidation through the respiratory chain or by ATP hydrolysis by the ATP synthetase complex (13). The results shown in Fig. 1 demonstrate that transport of [^{14}C]proline into intact cells or vesicles from lipoic acid-deficient cells is not dependent on the presence of lipoic acid except when pyruvate or α-ketoglutarate are used as the energy source.

This is expected with the last two substrates since lipoic acid is required for their metabolism. However, proline uptake into intact cells, but not vesicles, is stimulated slightly by lipoic acid with glucose, D-lactate and succinate as substrates. This is due to a stimulation of the metabolism of these compounds by lipoic acid (Table 2). The likely mechanism for the observed increase in the rate of oxidation of glucose, D-lactate and succinate is that all of these substrates can ultimately be converted to pyruvate, the metabolism of which is then stimulated by the added lipoic acid.

Energization of the membrane of E. coli by substrate oxidation through the respiratory chain or by ATP hydrolysis by the ATP synthetase complex can be readily measured as respiration- or ATP-dependent proton translocation or transhydrogenation of $NADP^+$ by NADH (10,12,17). Respiration- and ATP-dependent transhydrogenation and proton translocation were present in inverted vesicles from lipoic acid-deficient cells (Fig. 2). Addition of 1 µmole lipoic acid did not affect these processes. In fact, transhydrogenation was inhibited by 10-20%. However, these reactions were inhibited by tributyltin chloride in lipoic acid-deficient vesicles (Fig. 2). The apparent difference between proton translocation and transhydrogenation in sensitivity to tributyltin chloride is probably due to the different ionic compositions of the media needed to demonstrate optimal activities of the reactions. Under the conditions of the reaction, ATPase and succinate and NADH oxidase activities were not significantly inhibited by tributyltin chloride. This suggests that this compound is acting as an uncoupler.

DISCUSSION

Our results do not support the hypothesis that lipoic acid is required for the energy transducing processes associated with the membrane of E. coli. We have shown that several energy-dependent processes are present in lipoic acid-deficient cells or vesicles, and are not stimulated by the addition of lipoic acid. Tributyltin chloride, which has been suggested to act as an inhibitor of oxidative phosphorylation in submitochondrial particles by

TABLE 2

Effect of lipoic acid on oxygen uptake by lipoic acid-deficient cells.

Substrate	O_2 uptake	
	-Lipoic acid	+Lipoic acid
None	3	12
Glucose (5 mM)	124	279
D-Lactate (20 mM)	40	98
Succinate (20 mM)	68	199
Pyruvate (20 mM)	4	200
α-Ketoglutarate (20 mM)	3	70

Cells were used at a concentration of 1.6 mg/ml and lipoic acid at 33.3 μM. O_2 uptake is expressed as ng atoms/min/mg protein.

interaction with lipoic acid (2,4), inhibits energy-dependent proton translocation and transhydrogenation in lipoic acid-deficient vesicles of E. coli. Its mode of action in this system seems to be that of an uncoupler since it has little effect on ATP hydrolysis or substrate oxidation.

We have no explanation of the discrepancy between our results and those of Partis et al. (6). Our preparations were routinely tested for the absence of lipoic acid by showing that oxidation of α-ketoglutarate did not occur in the absence of added lipoic acid. In some instances we also showed that lipoic acid was absent using the direct assay as modified by Griffiths (1). Vesicles were prepared by several methods including (a) that described by Partis et al. (6), (b) the procedure of Partis et al. modified by including a sonication step to cause complete inversion of the vesicles, but omitting further purification of the vesicles on a sucrose gradient, (c) direct disruption of the cell suspension in a French press (10). Oxidative phosphorylation was demonstrated with each type of preparation although method (b) was routinely used as it gave somewhat higher P:O ratios. In no instance did we find that lipoic acid was required for oxidative phosphorylation in lipoic acid-deficient vesicles.

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REFERENCES

1. Griffiths, D.E. (1976) Biochem. J. 160, 809-812.
2. Cain, K. and Griffiths, D.E. (1977) Biochem. J. 162, 575-580.
3. Griffiths, D.E., Cain, K. and Hyams, R.L. (1977) Biochem. J. 164, 699-704.
4. Cain, K., Partis, M.D. and Griffiths, D.E. (1977) Biochem. J. 166, 593-602.
5. Griffiths, D.E., Hyams, R.L., Bertoli, E. and Carver, M. (1977) Biochem. Biophys. Res. Commun. 75, 449-456.
6. Partis, M.D., Hyams, R.L. and Griffiths, D.E. (1977) FEBS Lett. 75, 47-51.
7. Griffiths, D.E., Hyams, R.L. and Bertoli, E. (1977) FEBS Lett. 74, 38-42.
8. Griffiths, D.E., Hyams, R.L. and Partis, M.D. (1977) FEBS Lett. 78, 155-160.
9. Herbert, A.A. and Guest, J.R. (1968) J. Gen. Microbiol. 53, 363-381.
10. Bragg, P.D., Davies, P.L. and Hou, C. (1972) Biochem. Biophys. Res. Commun. 47, 1248-1255.
11. Kaback, H.R. (1971) Methods Enzymol. 22, 99-120.
12. Singh, A.P. and Bragg, P.D. (1976) Eur. J. Biochem. 67, 177-186.
13. Singh, A.P. and Bragg, P.D. (1976) Biochim. Biophys. Acta 423, 450-461.
14. Mevel-Ninio, M. and Yamamoto, T. (1974) Biochim. Biophys. Acta 357, 63-66.
15. Hertzberg, E.L. and Hinkle, P.C. (1974) Biochem. Biophys. Res. Commun. 58, 178-184.
16. Roisin, M.P. and Kepes, A. (1972) Biochim. Biophys. Acta 275, 333-346.
17. Singh, A.P. and Bragg, P.D. (1977) Biochim. Biophys. Acta 464, 562-570.