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Possible Involvement of Lipoic Acid in Binding Protein-Dependent Transport Systems in *Escherichia coli*

GILBERT RICHARME

Institut Jacques Monod, Université Paris VII, 75251 Paris Cedex 05, France

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We describe the properties of the binding protein dependent-transport of ribose, galactose, and maltose and of the lactose permease, and the phosphoenolpyruvate-glucose phosphotransferase transport systems in a strain of *Escherichia coli* which is deficient in the synthesis of lipoic acid, a cofactor involved in α-keto acid dehydrogenation. Such a strain can grow in the absence of lipoic acid in minimal medium supplemented with acetate and succinate. Although the lactose permease and the phosphoenolpyruvate-glucose phosphotransferase are not affected by lipoic acid deprivation, the binding protein-dependent transports are reduced by 70% in conditions of lipoic acid deprivation when compared with their activity in conditions of lipoic acid supply. The remaining transport is not affected by arsenate but is inhibited by the uncoupler carbonylcyanide-*m*-chlorophenylhydrazone; however the lipoic acid-dependent transport is completely inhibited by arsenate and only weakly inhibited by carbonylcyanide-*m*-chlorophenylhydrazone. The known inhibitor of α-keto acid dehydrogenases, 5-methoxyindole-2-carboxylic acid, completely inhibits all binding protein-dependent transports whether in conditions of lipoic supply or deprivation; the results suggest a possible relation between binding protein-dependent transport and α-keto acid dehydrogenases and shed light on the inhibition of these transports by arsenicals and uncouplers.

Bacterial transports are usually divided into three classes according to the mode of energization that they use for concentration of solutes. For the phosphotransferase systems, an energy-rich phosphate bond in the form of phosphoenolpyruvate is implicated as an energy donor; the transported molecule is phosphorylated as it is translocated in the interior of the cell (26). For the lactose permease system, the proton-motive force has been shown to drive concentrative transport in the form of a proton-lactose symport across the membrane (34, 45, 46, 50). For the energization of binding protein-dependent transport systems, controversial propositions have been advanced. The arsenate sensitivity of these transports, along with their requirement for a source of ATP, be it oxidative or glycolytic, have been generally interpreted to indicate that binding protein-dependent transport systems are driven by ATP (2, 3, 7, 24, 49); however, this interpretation has been questioned by results showing a lack of correlation between ATP levels and binding protein-dependent transports under certain conditions (27, 32), and several studies have shown that a membrane potential may be required for binding protein-dependent transport (33, 41). In more recent studies, acetylphosphate has been implicated in the energization of binding protein-dependent transports (17). In recent studies, I have shown that arsenite (G. Richarme, submitted for publication) and 5-methoxyindole-2-carboxylic acid (G. Richarme, submitted for publication) strongly inhibit the binding protein-dependent transports. Since 5-methoxyindole-2-carboxylic acid is known as an inhibitor of the lipoamide dehydrogenase component of α-keto acid dehydrogenases in animal cells (12, 35) and of the lipoamide dehydrogenase activity of bacterial cells (Richarme, submitted for publication), we have investigated the properties of three binding protein-dependent transport systems in a lipoic acid-deficient strain of *Escherichia coli* K-12 supplemented or deprived of lipoic acid; (arsenite, which is a general inhibitor of thiol functions, is a strong inhibitor of α-keto acid dehydrogenases as a consequence of its interaction with the lipoic acid cofactor [37]). The properties of lactose permease and of phosphoenolpyruvate-glucose phosphotransferase are presented in parallel.

MATERIALS AND METHODS

Bacterial strains. *E. coli* W1485lip2 (14), a lipoic acid auxotroph of *E. coli* K-12, and its parent W1485 were generous gifts of J. R. Guest, University of Sheffield, United Kingdom. The lipoic acid-deficient strain does not aerobically grow at all in minimal glucose medium unless supplemented either with 4 mM of acetate and succinate or 5 ng of lipoic acid per ml. The activity of the pyruvate dehydrogenase complex and that of the α-ketoglutarate dehydrogenase complex from the lipoic acid-deficient strain grown in minimal medium supplemented with lipoic acid are similar to the activities observed in the parental strain, and the activities of these complexes from the lipoic acid-deficient strain grown in minimal medium supplemented with acetate and succinate are undetectable (14). Results similar to those described in this study were obtained when 2 mM cAMP was added to the growth medium to overcome the catabolite repression. The transport activities were somewhat higher (around twofold), and similar inhibitions of transport were observed with arsenicals, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), and 5-methoxyindole-2-carboxylic acid and as a consequence of lipoic acid deprivation.

Lipoic acid deprivation or supply. The *lip* mutant was grown aerobically at 37°C with 0.4% glucose as the carbon source in M-63 minimal salts medium (29) supplemented with 4 mM each of acetate and succinate and with the appropriate inducers, 1 mM D-ribose, 1 mM D-fucose, 1 mM D-maltose, and 0.2 mM isopropyl-β-D-thiogalactopyranoside. In the exponential phase of growth, chloramphenicol was added to a final concentration of 50 μg/ml, and the bacterial culture was divided in two batches: One batch was further incubated aerobically for 1 h at 37°C with a supplement of lipoic acid (5 ng/ml), and the other was further

incubated in the same conditions without any addition. Bacteria from these two batches are referred to as bacteria supplemented with or deprived of lipoic acid, respectively.

Transport measurements. The bacteria were washed twice at 0°C with minimal medium M-63 containing 0.2% glycerol and 40 μM vitamin B_1 (unless otherwise stated); they were resuspended in the same medium supplemented with 4 mM each of acetate and succinate and kept on ice. The washed bacteria were incubated 10 min at 22°C before each transport measurement. Ribose, galactose, and maltose transport activities were assayed at 22°C in a volume of 1 ml containing the equivalent of 50 μg of cell protein per ml. D-[^{14}C]ribose (60 μCi/μmol), D-[^{14}C]galactose (250 μCi/μmol), or D-[^{14}C]maltose (36 μCi/μmol) was added to the assay mixture to a concentration of 2 μM. A 200-μl sample of the assay mixture was removed from each flask at 15, 30, 45, and 60 s after the addition of the radioactive substrate, filtered on cellulose ester filters (pore size, 0.45 μm; Millipore HAWP), washed three times with 1 ml of the transport medium, and counted for radioactivity. The transport of methylthiogalactoside was assayed in an assay volume of 5 ml containing the equivalent of 70 μg of cell protein per ml. [^{14}C]methylthiogalactoside (2 μCi/μmol) was added to the assay mixture to a concentration of 1 mM. Samples (1 ml) of the assay were removed from the flask at 15, 30, 45, and 60 s after addition of the radioactive substrate, filtered, washed, and counted as described above. The transport of methyl-α-glucoside was assayed in a volume of 2 ml containing the equivalent of 70 μg of cell protein per ml. [^{14}C]methyl-α-glucoside (1 μCi/μmol) was added to the assay mixture to a concentration of 0.3 mM. Samples (500 μl) of the assay mixture were removed from the flask at 0.5, 2, and 4 min after the addition of the radioactive substrate, filtered, washed, and counted as described above. Proline transport was assayed in an assay volume of 400 μl containing the equivalent of 70 μg of cell protein per ml, in the presence of 50 μg of chloramphenicol per ml. [^{14}C]proline (50 μCi/μmol) was added to a final concentration of 10 μM. A 100-μl sample of the assay mixture was removed from the flask at 15, 30, and 60 s, after the addition of the radioactive substrate, filtered, washed, and counted as described above. A low concentration of ribose and galactose (2 μM) was chosen for the transport measurements, since at this concentration, ribose and galactose are transported mainly through their respective binding protein-dependent transport systems (4, 19). In fact, we observed an 85% inhibition of galactose transport in the presence of competing amounts (up to 10 mM) of β-methylgalactose, which specifically inhibits galactose transport through the β-methylgalactoside permease (4).

Experiments with CCCP. Cells washed with minimal medium M 63 containing 0.2% glycerol and 40 μM vitamin B_1 were incubated for 10 min at 22°C in the presence of 20 μM CCCP before transport measurements.

Experiments with arsenicals. For the experiments with arsenicals, the cells were washed twice at 0°C with 50 mM Tris-hydrochloride (pH 7.2) containing 0.2% glycerol and 40 μM vitamin B_1, resuspended in the same buffer supplemented with 4 mM each of acetate and succinate, and kept on ice. The washed bacteria were incubated for 10 min at 22°C in the presence of sodium arsenate or arsenite at the indicated concentrations before transport measurements.

Experiments with 5-methoxyindole-2-carboxylic acid. The cells washed in M-63 minimal medium containing 0.2% glycerol and 40 μM vitamin B_1 were incubated for 10 min at 22°C in the presence of 5-methoxyindole-2-carboxylic acid before transport measurements.

ATP determination. The reaction mixtures were identical to those used for ribose transport except that 120 μg of cell protein was used in a 1-ml volume. After 10 min of incubation at 22°C, the reactions were terminated by the addition of 300 μl of ice-cold 10% perchloric acid. The acid samples were neutralized with 5 N KOH and centrifuged before use. The extracts were assayed for ATP by a luciferin luciferase assay. To a vial containing 100 μl of 100 mM Tris maleate buffer (pH 7.2), 40 μl of luciferin luciferase and 20 μl of the experimental perchloric extract were added, and the chemiluminescence was measured in a LKB-Wallac luminometer 1250.

Materials. D-[^{14}C]galactose, D-[^{14}C]methylthiogalactoside, and L-[^{14}C]proline were from C.E.A., France; D-[^{14}C]maltose and D-[^{14}C]ribose were from ICN; D-[^{14}C]methyl-α-glucoside was from Amersham Corp. D-L α-lipoic acid was from Sigma Chemical Co. All other chemicals were reagent grade and were obtained from Sigma or from Merck & Co., Inc. The ATP-monitoring reagent was obtained from LKB-Wallac.

RESULTS

Transport of ribose, galactose, maltose, methylthiogalactoside, and methyl-α-glucoside in bacteria supplemented with or deprived of lipoic acid. The bacteria were supplemented with or deprived of lipoic acid as described above. For transport measurements, they were resuspended in minimal salts medium containing glycerol and supplemented with acetate and succinate to minimize the metabolic effects of lipoic acid deprivation. The results are shown in Fig. 1A, 2A, 3A, 4A, and 5. As compared with bacteria supplemented with lipoic acid, bacteria deprived of lipoic acid showed a transport activities of 0.25 for ribose, 0.35 for galactose, 0.4 for maltose, 0.95 for methylthiogalactoside, and 1.0 for methyl-α-glucoside. All three sugars transported through binding protein-dependent transport systems (ribose [47], galactose [4], maltose [21]) were transported at a reduced rate in lipoic acid-deprived cells whereas methylthiogalactoside transport and methyl-α-glucoside transport were not affected by lipoic acid deprivation. The parental strain W1485 showed the same transport activities in conditions of lipoic acid deprivation or supply; these activities are similar to the transport activities of strain W1485lip2 in conditions of lipoic acid supply (data not shown). Since a direct requirement for ATP has been suggested for binding protein-dependent transport (2, 3), we suspected that the reduced transport activities for ribose, galactose, and maltose of cells deprived of lipoic acid might be due to a lowering of ATP levels caused by lipoic acid deprivation. We measured the intracellular ATP levels of the lipoic acid-deficient strain supplemented or deprived of lipoic acid. A value of 1.2 nmol of ATP per mg of cell protein was found in conditions of lipoic acid deprivation compared with 1.3 nmol in conditions of lipoic acid supply. This very low effect of lipoic acid on ATP levels is in agreement with the lack of involvement of lipoic acid in oxidative phosphorylation in *E. coli* (42).

Arsenate and arsenite inhibitions of ribose, galactose, maltose, and methylthiogalactoside transport in bacteria supplemented with or deprived of lipoic acid. The arsenate sensitivity of binding protein-dependent transport systems has been demonstrated for ribose (7), galactose (43, 50), and maltose (9) transport. Since arsenate is known to deplete ATP levels (22), the arsenate inhibition of the binding protein-dependent transport systems has been generally interpreted to mean that they are driven by ATP (2, 3, 7, 9, 50). However, in a recent study (Richarme, submitted for

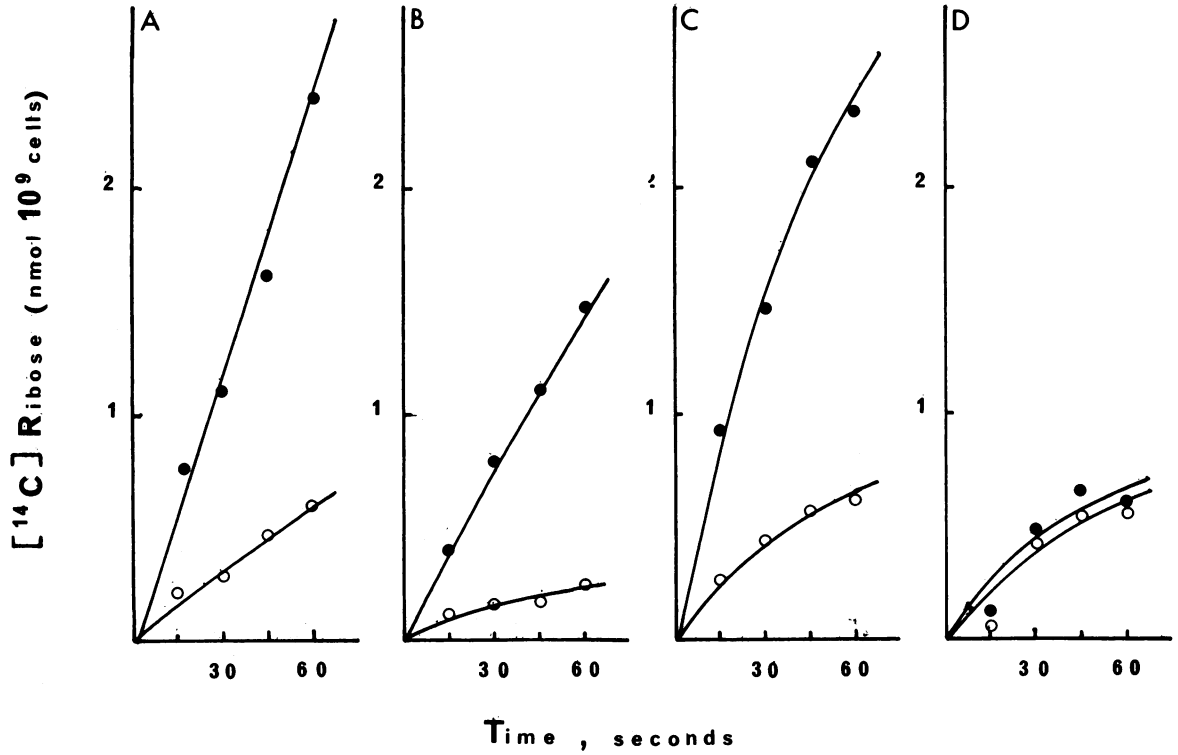

FIG. 1. Ribose transport. (A) Ribose transport in lipoic acid-deficient strain W1485lip2 supplemented with (●) or deprived of (○) lipoic acid as described in the text. (B) Same as (A) in the presence of 20 μM CCCP as described in the text. (C) Same as (A) with replacement of medium M-63 by 50 mM Tris-hydrochloride (pH 7.2). (D) Same as (C) in the presence of 1 mM arsenate as described in the text.

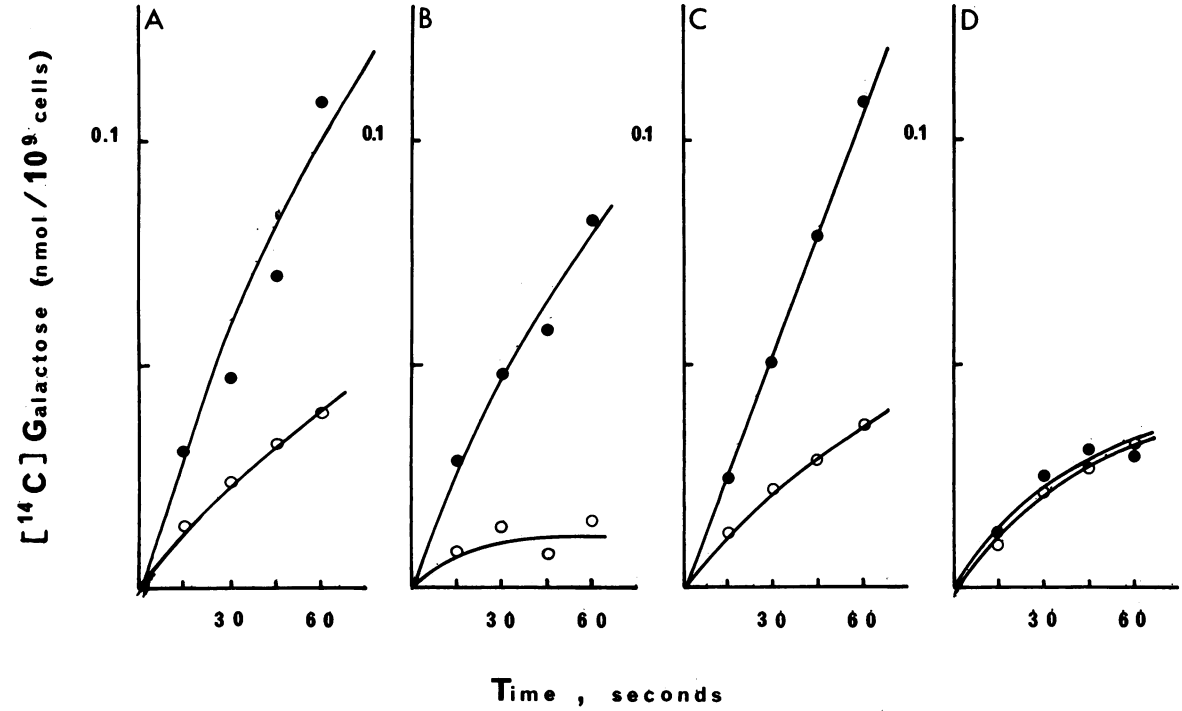

FIG. 2. Galactose transport. (A) Galactose transport in lipoic acid-deficient strain W1485lip2 supplemented with (●) or deprived of (○) lipoic acid as described in the text. (B) Same as (A) in the presence of 20 μM CCCP as described in the text. (C) Same as (A) with replacement of medium M-63 by 50 mM Tris-hydrochloride (pH 7.2). (D) Same as (C) in the presence of 1 mM arsenate as described in the text.

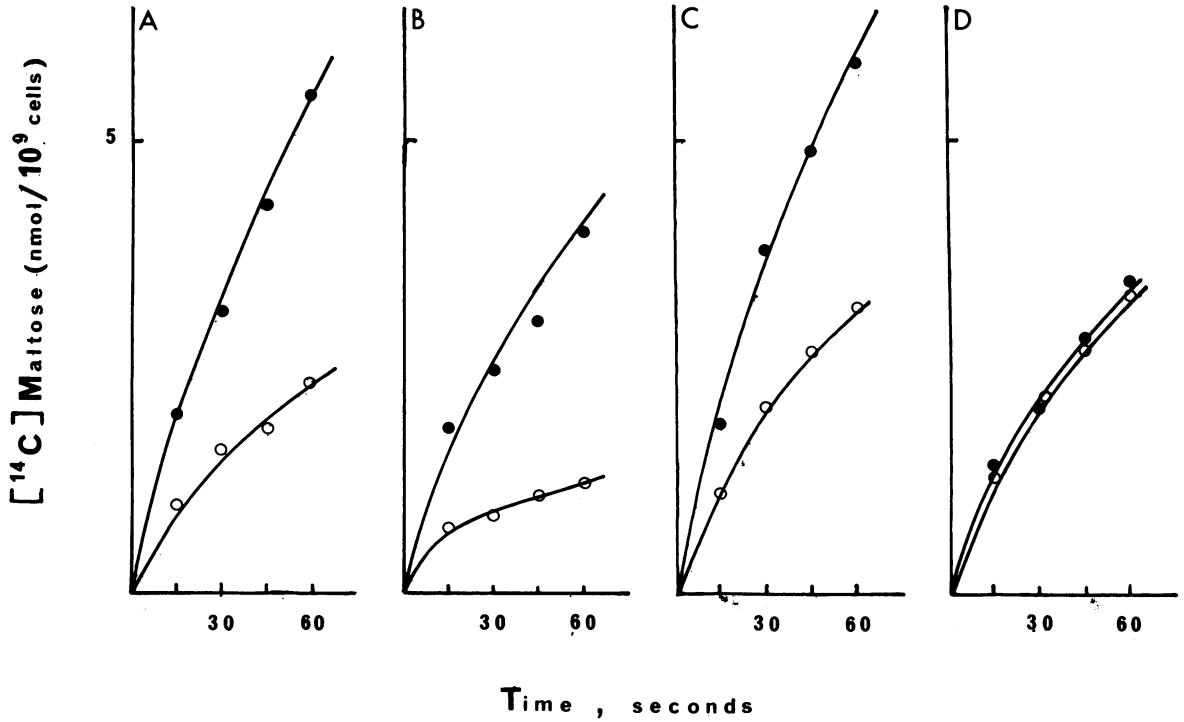

FIG. 3. Maltose transport. (A) Maltose transport in lipoic acid-deficient strain W1485lip2 supplemented with (●) or deprived of (○) of lipoic acid as described in the text. (B) Same as (A) in the presence of 20 μM CCCP as described in the text. (C) Same as (A) with replacement of medium M-63 by 50 mM Tris-hydrochloride (pH 7.2). (D) Same as (C) in the presence of 10 mM arsenate as described in the text.

publication), I have shown that arsenate inhibition of ribose transport occurs before significant ATP depletion and that arsenite, which is known as an inhibitor of thiol functions (23, 44), is at least as potent as arsenate for the inhibition of ribose transport; I have furthermore suggested, on the basis of a reversal of arsenite and arsenate inhibitions of ribose transport by the dithiol 2-3-dimercaptopropanol, that these inhibitions might affect a thiol structure, with arsenate acting possibly after in vivo reduction into arsenite (11, 44). The effect of arsenate on ribose, galactose, maltose, and methylthiogalactoside transport in bacteria supplemented with or deprived of lipoic acid is shown in Fig. 1C and D, 2C and D, 3C and D, and 4C and D. In bacteria supplemented with lipoic acid, arsenate produces an inhibition of the binding protein-dependent transport systems which is very similar to the arsenate inhibition observed by others; in our experiments, arsenate produced an inhibition of ribose, galactose, and maltose transport of 80, 70, and 45%, respectively, compared with 90, 70, and 25% inhibition observed by others (7, 9, 43). In bacteria deprived of lipoic acid, the transport activity of binding protein-dependent transports is not inhibited by arsenate. Furthermore, the arsenate inhibition in cells supplemented with lipoic acid is similar to the inhibition caused by lipoic acid deprivation; this similitude, as well as the absence of arsenate inhibition in cells deprived of lipoic acid, suggests that a lipoic acid-dependent reaction might be the main target for the arsenate inhibition in binding protein-dependent transport. Arsenate produced a slight inhibition of methylthiogalactoside transport, in accordance with previous results (8). Arsenite (1 mM) produced the same effects as arsenate on ribose, galactose, maltose, and methylthiogalactoside transport in cells deprived of or supplemented with lipoic acid (not shown), suggesting that both inhibitors may act on the same target.

CCCP inhibition of ribose, galactose, maltose, and methylthiogalactoside transport. The proton conductor CCCP has been shown to produce a strong inhibition of transport coupled to a proton gradient such as for lactose (1, 40) or proline (2) transport. It produces a consistent though less pronounced inhibition on binding protein-dependent transport systems (7, 9, 49), suggesting that a membrane-energized state, in addition to other processes, might be involved in these transport systems. The effect of CCCP on ribose, maltose, galactose and methylthiogalactoside transport in bacteria supplemented or deprived of lipoic acid is shown in Fig. 1A and B, 2A and B, 3A and B, and 4A and B. In bacteria supplemented with lipoic acid, CCCP produced an inhibition of ribose, galactose, and maltose transport of 39, 33, and 33%, respectively, very similar to the inhibitions observed by others with uncouplers (50, 40, and 40%, respectively) (7, 9, 43). In bacteria deprived of lipoic acid, CCCP inhibited ribose, galactose, and maltose transport by 74, 85, and 69%, respectively (the amount of sugar accumulated between 15 and 60 s was considered for these calculations). If we consider the part of transport which is strictly dependent on lipoic acid supply (i.e., the difference between transport activities in conditions of lipoic acid supply and deprivation) the CCCP inhibition of this lipoic acid-dependent transport is 30, 0, and 15% for ribose, galactose, and maltose, respectively. These results suggest that the part of transport dependent on lipoic acid supply is relatively unaffected by CCCP and that the lipoic acid-independent transport is more inhibited by CCCP.

As shown in Fig. 4, the transport of methylthiogalactoside in cells deprived of lipoic acid was strongly inhibited by CCCP. The CCCP inhibition of methylthiogalactoside accumulation in whole cells has been previously reported (1, 40) and is in agreement with the proposal that a proton gradient

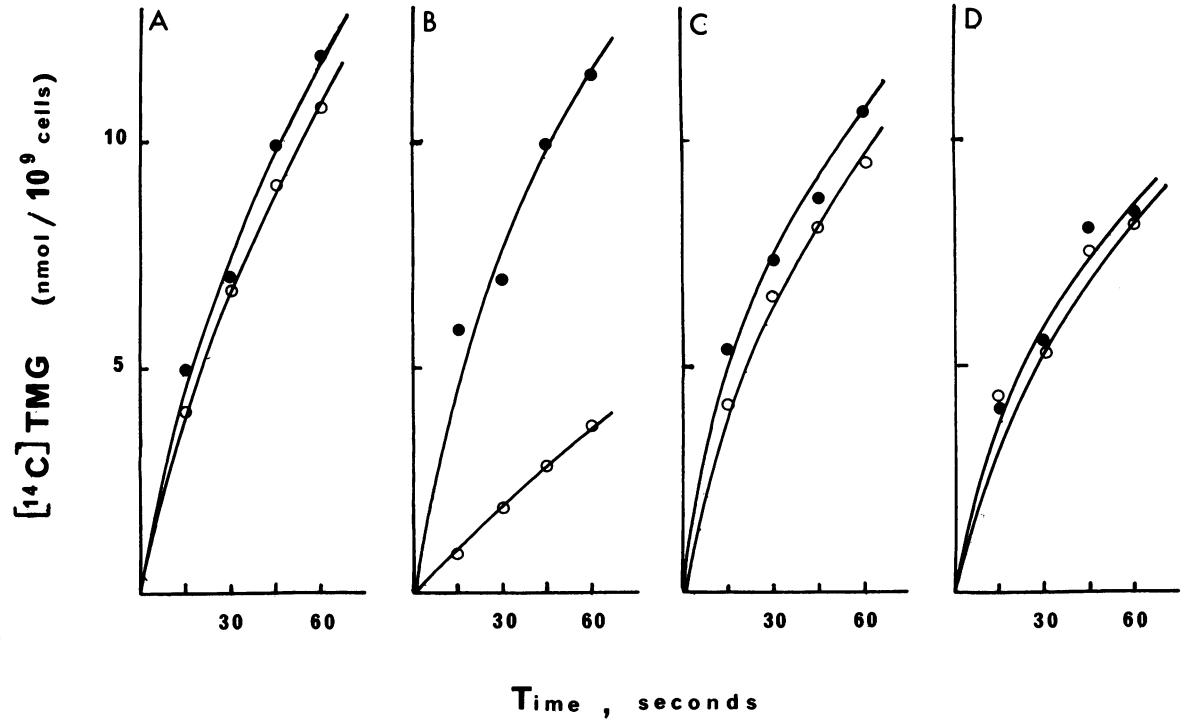

FIG. 4. Methylthiogalactoside transport. (A) Methylthiogalactoside transport in lipoic acid-deficient strain W1485lip2 supplemented with (●) or deprived of (○) lipoic acid as described in the text. (B) Same as (A) in the presence of 20 μM CCCP as described in the text. (C) Same as (A) with replacement of medium M-63 by 50 mM Tris-hydrochloride (pH 7.2). (D) Same as (C) in the presence of 1 mM arsenate as described in the text. The methylthiogalactoside transport system described in this study was inducible by isopropyl-β-D-thiogalactopyranoside, and more than 90% inhibited by 2 mM nonradioactive lactose, thus indicating that this transport is effected through the lactose permease (42).

must be established across the membrane for active methylthiogalactoside accumulation in *E. coli* (5). In bacteria supplemented with lipoic acid, 20 μM CCCP reproducibly gave no inhibition of methylthiogalactoside accumulation. This result is in contradiction with the observed inhibition of β-galactoside accumulation by uncouplers in whole cells (5, 30, 40). Despite the fact that I obtained CCCP inhibitions of ribose and maltose transport in the lipoic acid-deficient strain supplemented with lipoic acid in accordance with the results of others (7, 9), I suspected an artifactual interference of the lipoic acid supply with the action of CCCP, which could explain this result. For that purpose, I measured the effect of CCCP on the transport of proline in the lipoic acid-deficient strain supplemented with lipoic acid: an 85% inhibition of proline transport (not shown) was observed at a 20 μM CCCP concentration in accordance with the CCCP inhibition of proline transport observed in whole cells by others (2, 3), thus demonstrating the capacity of CCCP to inhibit a proton substrate symport in the lipoic acid-deficient strain supplemented with lipoic acid. Furthermore, I have checked the hypothesis that CCCP inhibits the membrane potential of cells supplemented with lipoic acid to the same extent as cells deprived of lipoic acid by measuring the accumulation of radioactive tetraphenylphosphonium ion in bacteria. These data suggest that CCCP is not inactivated by lipoic acid. The inability of CCCP to inhibit methylthiogalactoside transport in bacteria supplemented with lipoic acid compared with its ability to inhibit proline transport in the same conditions and to inhibit methylthiogalactoside transport in bacteria deprived of lipoic acid might reflect a possibility for some relation between a reaction dependent on the presence of lipoic acid and methylthiogalactoside transport.

Inhibition of ribose transport by 5-methoxyindole-2-carboxylic acid. I have previously shown that 5-methoxyindole-2-carboxylic acid is a potent inhibitor of binding protein-dependent transports although it does not affect the lactose permease and the phosphoenolpyruvate-glucose phosphotransferase. I investigated the effect of 5-methoxyindole-2-carboxylic acid on ribose transport in the lipoic acid-deficient strain supplemented with or deprived of lipoic acid; a complete inhibition of ribose transport was observed in the presence or in the absence of lipoic acid (not shown). As previously reported, 5-methoxyindole-2-carboxylic acid is a more potent inhibitor of binding protein dependent transport than arsenate or arsenite; the complete inhibition of ribose transport by 5-methoxyindole-2-carboxylic acid in conditions of lipoic acid depletion or supply suggests that this inhibition affects a reaction common to both situations. Though it is not possible to ascertain the nature of this crucial reaction implicated in the binding protein-dependent transport, it is interesting to note that a chemical oxido-reduction similar to that catalyzed by the lipoamide dehydrogenase would present the characteristics described in the present communication: the complete inhibition by 5-methoxyindole-2-carboxylic acid could be explained by its interaction with the oxidoreduction center containing a bound flavin adenine dinucleotide molecule (35); the partial inhibition caused by lipoic acid deprivation or by abolishment of the membrane potential could be due to the possibility that either lipoic acid (as it does in the reaction catalyzed by the lipoamide dehydrogenase [39]) or a mem-

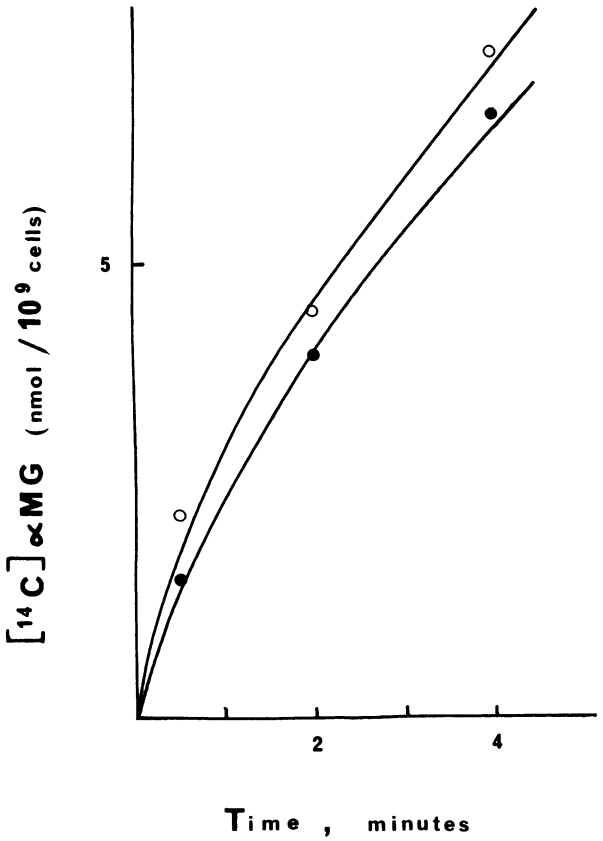

FIG. 5. Methyl-α-glucoside transport in lipoic acid-deficient strain W1485lip2 supplemented with (●) or deprived of (○) lipoic acid as described in the text.

brane potential (in a manner similar to that which was recently described for energization of the lactose permease [25]) could affect the system.

DISCUSSION

The consequences of lipoic acid deprivation of a lipoic acid-deficient strain on the activity of several transport systems is described in this study. A 60 to 80% inhibition of three binding protein-dependent transports was observed as a consequence of lipoic acid deprivation (ribose, galactose, maltose transports), whereas the transport activities of methylthiogalactoside and methyl-α-glucoside were unaffected. The intracellular pool of ATP was only slightly affected by lipoic acid deprivation, providing another example of a lack of correlation between ATP levels and the activity of binding protein-dependent transports (27, 32).

Arsenicals produce an inhibition of the three binding protein-dependent transports in conditions of lipoic acid supply, a result which is very similar to the arsenate inhibition observed by others. Three interesting features of our experiments with arsenicals are as follows: (i) arsenate and arsenite produce the same degree of inhibition of transport as previously reported (Richarme, submitted for publication); (ii) arsenicals produce the same degree of inhibition as lipoic acid deprivation; and (iii) arsenicals produce no inhibition in conditions of lipoic acid deprivation. These results suggest that a lipoic acid-dependent reaction could be the target of the arsenical inhibition of binding protein-dependent transport and cast doubts on the usual interpretation of the arsenate inhibition of binding protein-dependent transport as a result of a decrease (22) of ATP pools (2, 3, 7, 9). It should be recalled that arsenite produces a strong inhibition of α-keto acid dehydrogenases and is a well-known inhibitor of functions involving disulfides, with which it forms stable structures (31, 39).

CCCP produces an inhibition of the three binding protein-dependent transports in conditions of lipoic acid supply which is similar to the CCCP inhibitions obtained by others. An interesting feature of our experiments with CCCP is that although CCCP produces a moderate inhibition in cells supplemented with lipoic acid, it produces a more pronounced inhibition in cells deprived of lipoic acid. Furthermore, if we consider the transport activity which is strictly dependent on the lipoic acid supply, this value is only slightly affected by CCCP.

5-methoxyindole-2-carboxylic acid produces a complete inhibition of ribose transport in cells supplemented with or deprived of lipoic acid and therefore should affect a crucial reaction occurring in conditions of lipoic acid deprivation or supply; although it is not possible to ascertain the nature of this reaction, it is interesting to note that inhibition of the binding protein-dependent transports by arsenite, arsenate, CCCP, and 5-methoxyindole-2-carboxylic acid and inhibition as a consequence of lipoic acid deprivation could be explained if the target of these inhibitors was a reaction similar to that catalyzed by the lipoamide dehydrogenase; it is interesting to note that several observations reported by others would fit with this possibility. (i) The sequence of gene *malK* which encodes a cytoplasmic membrane protein involved in maltose transport exhibits homology to the sequence of *ndh*, the structural gene for the respiratory NADH dehydrogenase; this raises the possibility that oxido-reduction mechanisms might be implicated in energization of binding protein-dependent transports (10). (ii) Partial purification of the product of gene *malK* has shown that this protein was enriched along with four other polypeptides with apparent molecular weights of 69,000, 58,000, 32,000, and 26,000 (P. Bavoil, Ph.D. thesis, University of California, Berkeley, Calif., 1982); I have checked these molecular weights and found that they are very similar to those of several subunits of the pyruvate dehydrogenase complex: dihydrolipoyl transacetylase, 70,000 (6); and dihydrolipoyl transhydrogenase, 56,000 (6). Previous studies of dihydrolipoyl transacetylase have revealed species with molecular weights of 35,000 (13) and 26,000 (48) which could represent degradated or dissociated forms of the 70,000-molecular-weight component (48). (iii) Reconstitution of the binding protein-dependent transport of glutamine in an isolated membrane vesicle of *E. coli* requires the addition of pyruvate and NAD, suggesting a role for some metabolite of pyruvate in transport (18). (iv) Oxido-reduction processes (20) have been recently shown to control the affinities of the lactose permease (25), proline permease (25), and phosphoenolpyruvate-dependent transport systems (38), and have been recently shown to play a radical function in several biological processes (15, 16).

With respect to methylthiogalactoside and methyl-α-glucoside transports the transport activity of whole cells is not seriously affected by lipoic acid deprivation. This contrasts with the inhibition observed for the binding protein-dependent systems. The electrochemical proton gradient and the pool of phosphoenolpyruvate are probably not seriously affected by lipoic acid deprivation. The absence of CCCP inhibition of methylthiogalactoside accumulation in cells supplemented with lipoic acid is in contradiction with the known CCCP inhibition of methylthiogalactoside accu-

mulation in whole cells (5, 30, 40); however, this result could be interesting for the following reasons: (i) methylthiogalactoside accumulation is inhibited by CCCP in conditions of lipoic acid deprivation; (ii) proline accumulation is inhibited by CCCP in conditions of lipoic acid supply, indicating that CCCP is able to inhibit a transport energized by a proton-substrate symport in conditions of lipoic acid supply; (iii) binding protein-dependent transport shows the expected inhibition by CCCP in conditions of lipoic acid supply; and (iv) CCCP inhibits the membrane potential of cells supplemented with lipoic acid. For these reasons, we think that the absence of CCCP inhibition of methylthiogalactoside accumulation in cells supplemented with lipoic acid is not likely to be due to an artifactual inactivation of CCCP by lipoic acid and might suggest a possible relationship between a lipoic acid-dependent reaction and methylthiogalactoside transport. The activity of the transport protein for lactose has been recently shown to be altered by changing the redox state of the carrier, and it has been suggested that the electrochemical proton gradient would perform such a function (25). It is possible that a lipoic acid-dependent reaction could also affect the activity of the lactose permease and that in some conditions or in some strains (possibly as a result of an unknown mutation), this reaction would be important enough to allow accumulation of methylthiogalactoside in the presence of uncouplers.

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