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Binding protein-dependent transports in 2-oxo acids dehydrogenase mutants of *Escherichia coli*

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The binding protein-dependent transport of ribose, galactose and maltose are reduced in several 2-oxo acids dehydrogenase mutants of *Escherichia coli*. The results suggest an implication of the pyruvate dehydrogenase complex and to a lesser extent of the 2-oxoglutarate dehydrogenase complex in the energization of these transport systems.

Several recent observations suggest that the 2-oxo acid dehydrogenases [1,2] are implicated in binding protein-dependent transport [3] in *Escherichia coli*: the binding protein-dependent transport of ribose, galactose and maltose are strongly reduced as a consequence of lipoic acid deprivation of a mutant deficient in lipoic acid synthesis [11]; they are inhibited by the inhibitor of 2-oxo acid dehydrogenases, 5-methoxyindole-2-carboxylic acid [5], and lipoamide dehydrogenase activities related to the binding protein-dependent transports of galactose and maltose have been detected in toluenized bacteria [6]. The major 2-oxo acid dehydrogenases in *E. coli* are the pyruvate dehydrogenase complex and the 2-oxoglutarate dehydrogenase complex [1,2,7]. The pyruvate dehydrogenase complex catalyses the conversion of pyruvate coenzyme A and NAD^+ to acetyl coenzyme A carbon dioxyde and NADH; it contains multiple copies of three enzymatic components: pyruvate dehydrogenase (EC 1.2.4.1, *aceE* gene product), dihydrolipoamide acetyl-transferase (EC 2.3.1.12, *aceF* gene product), which contains covalently bound lipoic acid, and lipoamide dehydrogenase (EC 1.6.4.3 *lpd* gene product); the *ace* genes constitute an operon with *aceE,F* polarity and there is a separate promoter for the *lpd* gene. The 2-oxoglutarate dehydrogenase complex catalyses the conversion of 2-oxoglutarate coenzyme A and NAD^+ to succinyl coenzyme A carbon dioxide and NADH; it contains multiple copies of three enzymatic components; 2-oxoglutarate dehydrogenase (EC 1.2.4.2, *sucA* gene product), dihydrolipoamide succinyl transferase (EC 2.3.1.61, *sucB* gene product) which contains covalently bound lipoic acid, and lipoamide dehydrogenase (EC 1.6.4.3, *lpd* gene product); (the *lpd* gene product is common to both dehydrogenase complexes). The *suc* genes constitute an operon with *suc A,B* polarity. We describe in this study the transport activities of several 2-oxo acid dehydrogenase mutants, for ribose galactose and maltose which are substrates of three high-affinity-binding protein-dependent transport systems.

The bacterial strains were from the Dr. J.R.

[a] Deprived of lipoic acid.

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Guest collection and were kindly provided by Dr. J.R. Guest from Sheffield University and by Dr. B. Bachmann: W1485 lip2(F^+, lip-2), its parent W1485(F^+) [8], JRG 590 (Hfr, azi-7, Δ(nadC-aceF)72, relA1, spoT, thi-1), its parent Hfr 3000 (Hfr, thi-1, relA1, spoT1) [9]l, JRG 153 (F^-sucB9), its parent W3110 (F^-) [10], ac10 (F^+, aceF10, mel-1, supF58), its parent Y-mel (mel-1, supF58) [9,11], JRG 253 (aceE64, sucA35, trpA9761, trpR47, gal-25, ic1R7) [12], JRG 465 (sucA8, trpA9761, TrpR47, gal-25, ic1R7) [10], their parent WGA (trp9761, trpR47, gal-25, ic1R7) [12], JRG 301 (F^-, lpd-1, trpA58, trpE61) and its parent T3A58 (F^-, trpA58, trpE61) [12].

The strains were grown in 63 minimal medium [13] containing 0.4% glucose as carbon source and supplements as required [9–12]. Results similar to those described in this study (except that transport activity were several-fold higher) were obtained when 2 mM cAMP was added to the growth medium to overcome catabolite repression. Ribose, galactose and maltose transport measurements were made as previously described [4] in 63 minimal medium containing 0.4% glycerol, 10 μg/ml B1 vitamin B_1 and 4 mM each of acetate and succinate (in order to minimize the effects of the 2-oxo acids dehydrogenase mutations on metabolism), at a concentration of sugar of 2 μM (at this low concentration, ribose galactose and maltose are transported almost exclusively through their respective binding protein-dependent transport systems). We observed a 90% inhibition of galactose transport in the presence of competing amounts (up to 10 mM) of beta-methyl galactose which specifically inhibits galactose transport through the beta methyl-galactoside permease [14]. Transport of methylthiogalactoside and experiments with carbonylcyanide *m*-chlorophenylhydrazone were made as previously described [4]. ATP determinations were made as described in Ref. 4 and calculations have been made assuming 130 μg cell protein per ml of bacteria at an absorbance of 1 at 600 nm. We have checked that the levels of expression of the binding protein-dependent transport systems were not affected by the 2-oxo acid dehydrogenase mutations by measuring (as described in Ref. 15) the ribose galactose and maltose binding activities of osmotic shock fluids prepared from each strain by the osmotic shock procedure of Nossal and Heppel

TABLE I

TRANSPORT ACTIVITIES IN VARIOUS STRAINS

Sugar transport activities are expressed as the ratio of the transport activities measured in the mutants and in their respective parental strains. The transport activities of the parental strains for ribose, galactose, maltose and for ribose in the presence of carbonyl cyanide *m*-chlorophenylhydrazone are (values in nmol/min per 10^9 cells): W1485: 2.5, 0.1, 6.1, 1.5; WGA: 1.2, 0.06, 4.2, 0.5; Hfr3000: 1.3, 0.12, 5.3, 0.8; W3110: 2.1, 0.14, 2.1, 0.5; T3A58: 1.3, 0.08, 5.2, 0.4. The activities of the alpha ketoacid dehydrogenase complexes and of the *aceE, aceF, sucA, sucB, lpd* gene products measured in ultrasonic extracts from strain grown in glucose minimal medium (with supplements as required) have been taken from Refs 8–10 and 12. The answer yes or no indicates that the activity of the mutant strain is similar to the activity of the parental strain or is reduced to a very low level (generally less than 1% of the activity of the parental strains).

Strain phenotype	W1485lip26 [a] Ace^- Suc^-	JRG253 Ace^- Suc^-	JRG590 Ace^-	JRG153 Suc^-	JRG301 Ace^- Suc^-
Pyruvate dehydrogenase complex activity	no	no	no	yes	no
2-Oxoglutarate dehydrogenase complex activity	no	no	yes	no	no
aceE and *aceF* gene products activity	no	no	no	yes	yes
sucA and *sucB* gene products activity	no	no	yes	no	yes
lpd gene product activity	yes	yes	yes	yes	no
Ribose transport	0.25	0.30	0.25	0.9	0.5
Galactose transport	0.35	0.15	0.25	0.9	1.2
Maltose transport	0.40	0.40	0.60	0.8	1.4
Ribose transport in the presence of 20 μM carbonyl cyanide *m*-chlorophenylhydrazone	0.05	0.00	0.3	0.7	0.8

[16]. The results are shown in Table I.

In strain W1485 lip2 (this mutant is deficient in lipoic acid synthesis and possesses no activity of the pyruvate dehydrogenase complex or of the 2-oxoglutarate dehydrogenase complex [8] when grown in the absence of lipoic acid with a supply of acetate and succinate), the ribose galactose and maltose transport activities are 0.25, 0.35 and 0.40 of the transport activities of the parental strain. (Further incubation of strain W 1485 lip 2 with lipoic acid in the presence of chloramphenicol as described in Ref. 4 leads to transport activities similar to that of the parental strain. This shows that the lip mutation does not affect significantly induction of the genes involved in these transport studies).

In strain JRG 253, (this double amber mutant in the *aceE* and in the *sucA* genes possesses no significant activity of the pyruvate dehydrogenase complex or of the 2-oxoglutarate dehydrogenase complex and has reduced lipoamide dehydrogenase activity [12]) ribose galactose and maltose transport activities are 0.30, 0.15, 0.40 of the transport activities of the parental strain. The similitude between these values and those obtained in the lipoic-acid-deficient mutant suggests that the implication of lipoic acid in binding protein-dependent transports is related to its function in the 2-oxo acid dehydrogenase complexes. It will be shown further that the significant residual transport activities of these mutants are reduced to a very low value under conditions where the protonmotive force is abolished. In strain JRG 590, an *AceE* and *aceF* deletion mutant with no detectable pyruvate dehydrogenase complex activity but normal 2-oxoglutarate dehydrogenase complex activity [9], ribose galactose and maltose transport activities are 0.25, 0.25 and 0.6 of the transport activities of the parent strain. Similar decreases of transport activities were obtained in strain *ac10* [11] (an *aceF* mutant with no activity of the pyruvate dehydrogenase complex [9]).

In strain JRG 153 (a *sucB* mutant with no significant 2-oxoglutarate dehydrogenase complex activity but normal pyruvate dehydrogenase complex activity) ribose galactose and maltose transport activities are 0.9, 0.9 and 0.8 of the activities of the parental strain. Similar decreases of transport activities were obtained in strain JRG 465, a *sucA* mutant which possesses no activity of the 2-oxoglutarate dehydrogenase complex [10]. The feeble reduction of transport activities observed in the 2-oxo-glutarate dehydrogenase mutants contrasts with the 75% reduction of ribose and galactose transport observed in the pyruvate dehydrogenase mutants and suggests a more important implication of pyruvate dehydrogenase than 2-oxoglutarate dehydrogenase in ribose galactose and maltose transport. In strain JRG301 [12] (this strain contains a mutation in the *lpd* gene product, and soluble extracts from this strain possess no lipoamide dehydrogenase activity and no activity of the pyruvate dehydrogenase complex and of the 2-oxoglutarate dehydrogenase complex [12]), the ribose galactose and maltose transport activities are 0.5, 1.2 and 1.4 of the parental activity. The strong galactose and maltose transport activities of this *lpd* mutant may be related to the occurence of a significant lipoamide dehydrogenase activity in toluenized cells of this mutant [6] (which is not detectable in ultrasonic extracts [6,12]). Whether this lipoamide dehydrogenase activity is due to the mutated *lpd* gene product, or is a component of the binding protein-dependent transport systems as suggested in Ref. 6 or is as yet an undetermined lipoamide dehydrogenase (branched chain keto acid dehydrogenases may possess specific lipoamide dehydrogenases as it has been shown in *Pseudomonas putida* [17]) remains to be clarified.

We have measured the transport activities of the keto acid dehydrogenase mutants for methylthiogalactoside (a substrate of the lactose permease): these activities were similar to the transport activities of the parental strains (not shown).

A significant residual transport activity for ribose galactose and maltose is observed in the lipoic acid-deficient strain grown in the absence of lipoic acid and in the double amber mutant JRG 253, although in these conditions there is no activity of the pyruvate dehydrogenase complex or the 2-oxoglutarate dehydrogenase complex [8,12]. We have already suggested that this residual transport activity may rely on the membrane potential [4]. We have verified this hypothesis by measuring ribose transport activities of the 2-oxo acids dehydrogenase mutants in the presence of 20 μM carbonylcyanide *m*-chlorophenylhydrazone a pro-

ton conductor which abolishes the protonmotive force in *E. coli*. As shown in Table I the ribose transport activities in the presence of carbonylcyanide *m*-chlorophenylhydrazone of strain W1485 lip2 (deprived of lipoid acid), JRG 253 (the double amber mutant in *aceE* and *sucA*), JRG 590 (the *aceE-aceF* deletion mutant with no pyruvate dehydrogenase complex activity), JRG 465 (the *sucA* mutant with no 2-oxoglutarate dehydrogenase complex activity) and JRG 301 (*lpd* mutant) are 0.05, 0.00, 0.3, 0.7 and 0.8 of their respective parental activity (measured in the presence of 20 μM carbonylcyanide *m*-chlorophenylhydrazone), respectively. The results obtained with carbonylcyanide *m* chlorophenylhydrazone suggest that in the absence of a protonmotive force ribose transport relies almost completely on enzymatic activities of the pyruvate dehydrogenase complex and (to a lesser extent) on enzymatic activities of the 2-oxoglutarate dehydrogenase complex. Since treatment of bacterial cells with carbonylcyanide *m*-chlorophenylhydrazone is likely to reduce ATP levels [18] and, since ATP has been suggested as a possible source of energy for binding-protein-dependent transports, we measured the ATP levels in the absence and in the presence of carbonylcyanide *m*-chlorophenylhydrazone in strains W 1485 lip2 and JRG 253; we found that carbonylcyanide *m*-chlorophenylhydrazone (20 μM) reduced ATP level by less than 30% in both strains to 1.6 and 1.4 nmol of ATP/mg of cell protein, respectively; this slight reduction can not explain the complete reduction of ribose transport observed in these strains in the presence of the uncoupler. This suggests that transport inhibition by carbonylcyanide *m*-chlorophenylhydrazone is not linked to ATP depletion. Furthermore, the low-transport activity in the mutants is not correlated to a decrease of ATP levels in these strains as compared to the parental strains (not shown).

The present results are a continuation of our recent studies which suggest a relation between the 2-oxoacid dehydrogenases and binding protein-dependent transport systems [4–6,19]. The pyruvate dehydrogenase complex (mutants of which show up to 75% reduction of transport) seems to be more implicated than the 2-oxoglutarate dehydrogenase complex (mutants of which give only a slight reduction of transport). The implication of 2-oxoacid dehydrogenases in binding protein-dependent transport is compatible with results obtained in other laboratories (see Discussion in Ref. 11), especially with the results of Hunt and Hong, who show energisation of glutamine transport in membrane vesicles by pyruvate and NAD [20,21]. Galactose and maltose transport are not reduced in the *lpd* mutant. However, the discovery of a lipoamide dehydrogenase activity in toluenized extracts of this mutant [6] (undetectable in ultrasonic extracts [6,12]) suggests than other lipoamide dehydrogenase activities may exist in *E. coli* in addition to the *lpd* gene product. These lipoamide dehydrogenase activities require further characterisation, and their purification is under way. The alternative implication of the protonmotive force in the energisation of binding protein-dependent transport is shown by the nearly complete inhibition of transport obtained by addition of carbonylcyanide *m*-chlorophenylhydrazone to the lipoic acid-deficient mutant or to the double mutant affecting the pyruvate and 2-oxoglutarate dehydrogenase complexes. (The inhibition of transport is not linked to partial depletion of ATP levels resulting from the carbonylcyanide *m*-chlorophenylhydrazone treatment.) The implication of the protonmotive force in binding protein-dependent transport systems has been suggested by others [18]. Involvement of the protonmotive force in binding protein-dependent transport is compatible with a possible implication of redox centers in these transport systems (suggested by the implication of lipoic acid). Membrane potential has been shown to alter the redox state of membrane components [22,23] and it is conceivable that such redox centers could be altered either by the protonmotive force or by lipoic acid and NAD. Our recent results [24] concerning the reconstitution of the binding protein-dependent transport of galactose in proteoliposomes support the conclusion that either oxidoreduction of lipoic acid and NAD or the membrane potential can energize galactose transport; they show furthermore that ATP has an inhibitory regulatory function.

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References

1 Reed, L.J. (1974) Acc. Chem. Res. 7, 40–46
2 Guest, J.R. (1978) Adv. Neurol. 22, 219–244
3 Silhavy, T.J., Ferenci, T. and Boos, W. (1978) Bacterial Transport (Rose, B., ed.), Marcel Dekker, New York
4 Richarme, G. (1985) J. Bacteriol. 162, 286–293
5 Richarme, G. (1985) Biochim. Biophys. Acta 815, 37–43
6 Richarme, G. and Heine, H.G. (1986) Eur. J. Biochem. 156, 399–405
7 Williams, C.H., Jr. (1976) The Enzymes (Boyer, P.B., ed.), p. 13 and pp. 89–173, Academic Press, New York
8 Herbert, A.A. and Guest, J.R. (1968) J. Gen. Microbiol. 53, 363–381
9 Langley, D. and Guest, J.R. (1977) J. Gen. Microbiol. 99, 263–276
10 Herbert, A.A. and Guest, J.R. (1969) Mol. Gen. Genet. 105, 182–190
11 Dietrich, J. and Henning, U. (1970) Eur. J. Biochem. 14, 258–269
12 Guest, J.R. and Greaghan, I.T. (1973) J. Gen. Microbiol. 75, 197–210
13 Pardee, A.B., Jacob, F. and Monod, J. (1959) J. Mol. Biol. 1, 165–178
14 Boos, W. (1969) Eur. J. Biochem. 10, 66–73
15 Richarme, G. and Kepes, A. (1983) Biochim. Biophys. Acta 742, 16–24
16 Nossal, N.G. and Heppel, L.A. (1966) J. Biol. Chem. 241, 3055–3062
17 Sokatch, J.R., McCully, V., Gebrosky, J. and Sokatch, D.J. (1981) J. Bacteriol. 148, 639–646
18 Plate, C.A. (1979) J. Bacteriol. 137, 221–225
19 Richarme, G. (1986) Biochem. Int. 12, 897–903
20 Hunt, A.G. and Hong, J.S. (1981) J. Biol. Chem. 256, 11988–11991
21 Hunt, A.G. and Hong, J.S. (1983) Biochemistry 22, 844–850
22 Hinckle, P. and Mitchell, P. (1970) Bioenergetics 1, 45–60
23 Robillard, G.T. and Konings, W.N. (1983) Eur. J. Biochem. 127, 597–604
24 Richarme, G. (1987) C.R. Acad. Sci. Paris 305, 55–58