Energization of the transport systems for arabinose and comparison with galactose transport in *Escherichia coli*

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(Received 22 June 1981/Accepted 18 September 1981)

1. Strains of *Escherichia coli* were obtained containing either the AraE or the AraF transport system for arabinose. AraE⁺,AraF⁻ strains effected energized accumulation and displayed an arabinose-evoked alkaline pH change indicative of arabinose-H⁺ symport. In contrast, AraE⁻,AraF⁺ strains accumulated arabinose but did not display H⁺ symport. 2. The ability of different sugars and their derivatives to elicit sugar-H⁺ symport in AraE⁺ strains was examined. Only L-arabinose and D-fucose were good substrates, and arabinose was the only inducer. 3. Membrane vesicles prepared from an AraE⁺,AraF⁺ strain accumulated the sugar, energized most efficiently by the respiratory substrates ascorbate + phenazine methosulphate. Addition of arabinose or fucose to an anaerobic suspension of membrane vesicles caused an alkaline pH change indicative of sugar-H⁺ symport on the membrane-bound transport system. 4. Kinetic studies and the effects of arsenate and uncoupling agents in intact cells and membrane vesicles gave further evidence that AraE is a low-affinity membrane-bound sugar-H⁺ symport system and that AraF is a binding-protein-dependent high-affinity system that does not require a transmembrane protonmotive force for energization. 5. The interpretation of these results is that arabinose transport into *E. coli* is energized by an electrochemical gradient of protons (AraE system) or by phosphate bond energy (AraF system). 6. In batch cultures the rates of growth and carbon cell yields on arabinose were lower in AraE⁻,AraF⁺ strains than in AraE⁺,AraF⁻ or AraE⁺,AraF⁺ strains. The AraF system was more susceptible to catabolite repression than was the AraE system. 7. The properties of the two transport systems for arabinose are compared with those of the genetically and biochemically distinct transport systems for galactose, GalP and MglP. It appears that AraE is analogous to GalP, and AraF to MglP.

The occurrence of a transport system for L-arabinose in *Escherichia coli* was first described by Isaacson & Englesberg (1964) and Novotny & Englesberg (1966). The system was induced only by L-arabinose. D-Fucose, D-xylose and D-galactose were found to inhibit arabinose uptake competitively, and transport was sensitive to the uncoupling agent 2,4-dinitrophenol. The structural gene(s) for the transport system, designated *araE*, occurred at about 61 min on the *E. coli* chromosomal map (Heffernan *et al.*, 1976; Bachmann & Low, 1980; Macpherson *et al.*, 1981).

A second transport system for arabinose was

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identified by Schleif (1969) and by Brown & Hogg (1972), specified by the gene *araF*. It differs from the system coded by *araE* in the involvement of a periplasmic binding protein [the only functional component of the system identified so far (Hogg & Hermodson, 1977; Quijcho & Pflugrath, 1980)] and in its separate gene locus at 44.5 min (Heffernan *et al.*, 1976; Hogg, 1977) on the *E. coli* chromosomal map.

Both the AraE and AraF transport systems utilized cellular metabolic energy for accumulating the sugar, chemically unmodified, to a higher concentration inside the organism than in the medium (Novotny & Englesberg, 1966; Brown & Hogg, 1972). The nature of energization of such active transport has been examined for several bacterial sugar-transport systems (reviewed by Silhavy *et al.*, 1978; Rosen & Kashket, 1978). Two mechanisms have been characterized to date. The chemiosmotic

mechanism (Mitchell, 1963, 1970) proposes that an electrochemical gradient of protons (protonmotive force) across the cell membrane, generated by respiration or ATP hydrolysis, is utilised to drive sugar-H⁺ symport systems. Evidence has been obtained that uptake of the sugars lactose (West, 1970; West & Mitchell, 1972, 1973), galactose and arabinose (Henderson, 1974; Henderson & Skinner, 1974; Henderson *et al.*, 1975, 1977) and xylose (Lam *et al.*, 1980) occurs by this type of mechanism in *E. coli*. Alternatively, a glycolytic product, possibly ATP (Berger, 1973; Berger & Heppel, 1974) or acetyl phosphate (Hong *et al.*, 1979), may be used to energize sugar transport. For example, the methyl galactoside-transport system, MglP, is energized by this mechanism (Wilson, 1974*b*; Henderson *et al.*, 1977; Daruwalla, 1979).

Although some evidence was obtained that the transport of arabinose in AraE⁺,AraF⁺ cells of *E. coli* is energized by a protonmotive force (Henderson, 1974; Henderson & Skinner, 1974; Henderson *et al.*, 1975), it was not established whether one or both systems operated by this mechanism.

The main purpose of the present investigation was to characterize the mechanism of energization of each AraE and AraF transport system in intact cells and membrane vesicles. In pursuing this aim, similarities between the AraE and AraF systems and the two active transport systems for galactose, GalP and MglP, became apparent. The relationship of these findings to the need for *E. coli* to adopt

differently energized transport mechanisms in response to changes in sugar concentration in the environment is discussed

Experimental

Organisms and growth conditions

The strains of *E. coli* used and their relevant properties are listed in Table 1. The procedures for maintaining strains and preparing inocula were described by Henderson *et al.* (1977). All incubations for growth were performed at 37°C in a Gallenkamp Orbital Incubator shaking at 250 rev./min, or in a linear shaker at 200 strokes/min (type SSB4; Grant Instruments, Cambridge, U.K.). Large-volume cultures were obtained by adding a 1% nutrient broth inoculum to defined minimal salts medium in conical flasks (Henderson *et al.*, 1977). The culture volume was less than two-fifths of the flask volume, to ensure adequate aeration of the culture.

To measure growth of *E. coli* in batch culture, an intermediate inoculum was prepared in minimal salts medium with glycerol (20 mM), lactate (20 mM) or arabinose (10 mM) as the carbon source. This culture was harvested, after 16–18 h growth, by centrifugation at 20 000 *g* for 7 min at 25°C, and a portion was resuspended in fresh medium to an initial cell density of about 0.1 mg dry mass/ml. It was then shaken at 37°C, and the increase in cell density was followed with time by measuring $A_{680}^{1\text{cm}}$, which is linearly related to cell mass [$A_{680} = 1.0$

Table 1. *Strains of E. coli used*

Strain	Genotype	Relevant transport phenotype	Source
<i>E. coli</i> K12 strains			
K10	Hfr prototroph	AraE ⁺ ,AraF ⁺	Laboratory stock R. W. Hogg (Department of Microbiology, Case Western Reserve University, Cleveland OH, U.S.A.)
RS1wt*	Hfr, <i>thi</i> , Hayes prototroph	AraE ⁺ ,AraF ⁺	
RS1E ⁻ *	<i>araE, thi</i>	AraE ⁻ ,AraF ⁺	
RS1E ⁻ F ⁻ *	<i>araE, araF</i>	AraE ⁻ ,AraF ⁻	
KD1	<i>araE, araF, thyA</i>	AraE ⁻ ,AraF ⁻	From RS1E ⁻ F ⁻ by trimethoprim-resistance (Daruwalla, 1979)
KD2	<i>araF</i>	AraE ⁺ ,AraF ⁻	P1kc (RS1wt) × KD1 and selection for Thy ⁺ (Daruwalla, 1979)
JM1097	<i>mglP</i>	GalP ⁺ ,MglP ⁻	Henderson <i>et al.</i> (1977)
JM1100	<i>galP, mglP</i>	AraE ⁺ ,AraF ⁺ ,GalP ⁻ ,MglP ⁻	Henderson <i>et al.</i> (1977)
JM1104	<i>galP</i>	GalP ⁻ ,MglP ⁺	Henderson <i>et al.</i> (1977)
JM559	Hfr, <i>laci</i>	AraE ⁺ ,AraF ⁺ ,LacY ^c	Lam <i>et al.</i> (1980)
<i>E. coli</i> B/r strains*			
UP1000	F ⁻ prototroph	AraE ⁺ ,AraF ⁺	Hogg & Englesberg (1969)
CW2004	<i>araE</i>	AraE ⁻ ,AraF ⁺	Brown & Hogg (1972)
CW2022	<i>araF</i>	AraE ⁺ ,AraF ⁻	Brown & Hogg (1972)
SB5314	<i>araA</i>	AraE ⁺ ,AraF ⁺	Hogg & Englesberg (1969)
SB5313	<i>araE, araA</i>	AraE ⁻ ,AraF ⁺	Hogg & Englesberg (1969)

* We are indebted to Dr. Hogg and Dr. Englesberg for the gift of these strains.

corresponds to 0.68 mg dry mass/ml (Ashworth & Kornberg, 1966; R. A. Giddens, unpublished work)].

E. coli strains for measurements of H^+ movement and radioactively labelled sugar uptakes were cultured in the same way as for the growth studies. At $A_{680} = 0.8$ – 1.0 (late exponential phase of growth) the cells were harvested, depleted of energy reserves, washed and resuspended as described previously (Henderson *et al.*, 1977).

Preparation of spheroplasts and membrane vesicles

Spheroplasts were prepared by the method of Witholt *et al.* (1976). Membrane vesicles were prepared from the spheroplasts by the procedure of Kaback (1971). Final resuspension of the vesicles was in 100 mM-potassium phosphate buffer, pH 6.5, at a concentration of 3–6 mg of protein/ml, measured by the method of Lowry *et al.* (1951), with bovine serum albumin (fraction V) as standard.

Continuous measurement of pH changes and oxygen consumption

Sugar-promoted pH changes and oxygen concentration were measured as described by Henderson *et al.* (1977), in a temperature-controlled (25°C) glass vessel of 4 ml total volume.

Measurement of transport of radioactively labelled compounds

Uptake of radioactively labelled sugars and amino acids into intact cells was followed by the method of Henderson *et al.* (1977), at 25°C. For the measurement of arabinose uptake into membrane vesicles, 0.25 ml portions of membranes were diluted to a final volume of 0.5 ml containing 10 mM-MgSO₄/50 mM-potassium phosphate buffer, pH 6.6. The suspensions were aerated at 25°C for 2.5 min. At this time phenazine methosulphate was added, followed by potassium ascorbate, to final concentrations of 0.1 mM and 20 mM respectively. At 3 min radioactively labelled arabinose was added to a final concentration of 0.2 mM. Samples (0.2 ml) were filtered through Sartorius cellulose nitrate filters (0.45 µm pore size) at timed intervals and washed immediately with two 2 ml portions of 100 mM-LiCl. The radioisotope retained in the vesicles on the filters was measured as in intact cells. A 15 s sample was used to calculate the initial rates of transport of labelled sugar.

Preparation of radioisotope-labelled binding protein

Two 50 ml cultures containing 20 mM-sodium succinate in 250 ml flasks were inoculated to 0.1 mg dry mass/ml with *E. coli* strain SB5314 grown overnight under the same conditions. Arabinose (1 mM) was included in one culture as inducer. After growth

to 0.14 mg dry mass/ml, 150 µCi of ³H-labelled amino acid mixture (TRK.440; The Radiochemical Centre, Amersham, Bucks., U.K.) was added to the induced culture and 75 µCi of ¹⁴C-labelled amino acid mixture (CFB.104; The Radiochemical Centre) was added to the other. Growth was allowed to proceed to 0.38 and 0.52 mg dry mass/ml (the induced culture grew more slowly), and then the bacteria were harvested, mixed and resuspended in 15 ml 0.2 M-Tris/HCl buffer, pH 8. Spheroplasts were prepared by the method of Witholt *et al.* (1976) as described by Macpherson *et al.* (1981), but the supernatant remaining when the spheroplasts were sedimented was retained, and used as the preparation of binding protein.

A second preparation was made by an identical method except that 10 mM-glucose replaced succinate as growth substrate, and 20 mM-arabinose was used as inducer.

Electrophoresis

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was performed in 12.5% acrylamide gels as described by Laemmli (1970) and modified by Blattler *et al.* (1972). Approx. 22 µg of protein of the succinate-grown preparation and 34 µg from the glucose-grown preparation were dissolved and electrophoresed in parallel with molecular-mass markers (Macpherson *et al.*, 1981).

The processed gel was fixed, stained, dried, cut into 2 mm strips and counted for radioactivity as described by Macpherson *et al.* (1981).

Materials

L-Ascorbic acid, 2,4-dinitrophenol, dicyclohexylcarbodi-imide and NaN₃ were obtained from BDH Chemicals, Poole, Dorset, U.K.; methyl β-L-arabinopyranoside was from Cambrian Chemicals, Croydon, Surrey, U.K.; LiCl was from Hopkin and Williams, Chadwell Heath, Essex, U.K.; D-talose, 2-deoxy-D-galactose and methyl β-D-galactoside were from Koch-Light Laboratories, Colnbrook, Bucks., U.K.; L-arabinose, D-arabinose, D-fucose, 2-deoxy-D-glucose, D-glucose, D-galactose (glucose-free grade), isopropyl 1-thio-β-D-galactoside, L-rhamnose, D-ribose, D-xylose, carbonyl cyanide *m*-chlorophenylhydrazine, phenazine methosulphate and valinomycin were from Sigma Chemical Co., Poole, Dorset, U.K.; [¹⁴C]methyl 1-thio-β-D-galactoside was from New England Nuclear Corp., Swindon, Wilts, U.K.; L-[U-¹⁴C]arabinose and L-[U-¹⁴C]glutamine were from The Radiochemical Centre; tetrachlorosalicylanilide was kindly given by Dr. W. A. Hamilton, Department of Microbiology, University of Aberdeen, Aberdeen, Scotland, U.K.; ionophore A217 was kindly given by Dr. R. L. Hamill, Lilly Research Laboratories, Indianapolis, IN, U.S.A. Chemicals used for growth of cultures

were of reagent grade and all others were of the highest purity available. Unless specified, the configuration of sugar refers to the D-enantiomer, except for L-arabinose.

Results

Arabinose-promoted effective proton uptake into AraE⁺ strains

The observation of an alkaline pH change during carrier-mediated diffusion of a sugar into energy-depleted cells is evidence for obligatory coupling of sugar and proton movements (Mitchell, 1970, 1973). Accordingly, AraE⁺,AraF⁻ or AraE⁻,AraF⁺ mutants were examined for sugar-H⁺ symport on

the individual transport systems AraE or AraF by measuring the ability of sugars to promote pH changes.

E. coli K12 strains were induced with arabinose during growth in batch culture. The addition of arabinose to anaerobic resting suspensions elicited an alkaline pH shift in AraE⁺,AraF⁺ and AraE⁺,AraF⁻ strains (Table 2). Strains that were AraE⁻,AraF⁺ or AraE⁻,AraF⁻ showed no arabinose-H⁺ symport (Table 2). In AraE⁻,AraF⁺,AraA⁺ strains only acid efflux was observed on addition of arabinose, indicating that it was taken up and metabolized without an obligatory H⁺ uptake. In uninduced cells no change in pH occurred in the presence of arabinose, even in AraE⁺ cells.

Table 2. pH changes elicited by the addition of arabinose or related sugars to de-energized suspensions of *E. coli*. Each strain of *E. coli* was grown on the indicated compound(s) and depleted of metabolic energy as described in the Experimental section. The alkaline pH changes occurring on addition of the listed sugars were measured as for Fig. 1 (see also Henderson *et al.*, 1977). When more than one measurement was made, usually on different batches of cells, the mean value and the standard error of the mean are given.

Strain	Relevant phenotype	Growth compound(s)	Sugar added to de-energized cells	Effective H ⁺ uptake (nmol)	H ⁺ efflux
<i>E. coli</i> K12 strains					
K10	AraE ⁺ ,AraF ⁺	Arabinose	Arabinose	28 ± 10 (8)	+
			Fucose	68 ± 10 (2)	—
			Galactose	15 ± 5 (2)	—
RS1wt	AraE ⁺ ,AraF ⁺	Arabinose	Arabinose	15 ± 2 (2)	+
			Fucose	95	—
KD2	AraE ⁺ ,AraF ⁻	Arabinose	Arabinose	15 ± 6 (5)	+
			Fucose	23 ± 8 (3)	—
KD2	AraE ⁺ ,AraF ⁻	Lactate	Arabinose	0	—
			Fucose	0	—
RS1E ⁻	AraE ⁻ ,AraF ⁺	Arabinose + lactate	Arabinose	0 (2)	+
			Fucose	0 (2)	—
RS1E ⁻ F ⁻	AraE ⁻ ,AraF ⁻	Arabinose + lactate	Arabinose	0	—
			Fucose	0	—
JM1100	AraE ⁺ ,AraF ⁺ ,GalP ⁻ , MglP ⁻	Arabinose	Arabinose	17 ± 6 (3)	+
			Fucose	9	—
JM559	LacY ^c	Glycerol	Arabinose	0	—
			Methyl thiogalactoside	93	—
			Galactose	31	—
JM559	AraE ⁺ ,AraF ⁺ ,LacY ^c	Arabinose	Arabinose	24	+
			Methyl thiogalactoside	38	—
			Galactose	18	—
<i>E. coli</i> B/r strains					
UP1000	AraE ⁺ ,AraF ⁺	Arabinose	Arabinose	17 ± 5 (2)	+
			Fucose	43 ± 5 (2)	—
			Galactose	0	—
CW2022	AraE ⁺ ,AraF ⁻	Arabinose	Arabinose	22 ± 3 (2)	+
			Fucose	32 ± 5 (2)	—
			Galactose	0	—
CW2004	AraE ⁻ ,AraF ⁺	Arabinose	Arabinose	0	+
			Fucose	2.5 ± 0.5 (2)	—
SB5314	AraE ⁺ ,AraF ⁺	Arabinose + glycerol	Arabinose	75 ± 18 (2)	—
			Fucose	60	—
SB5313	AraE ⁻ ,AraF ⁺ ,AraA ⁻	Arabinose + glycerol	Arabinose	0 (3)	—
			Fucose	0 (2)	—

Similar results were obtained with a series of mutants derived from *E. coli* B/r, as shown in Table 2 and Fig. 1. Again, only the AraE⁺ strains elicited an alkaline pH change on addition of arabinose to de-energized cells. The initial rate and extent of effective H⁺ uptake with arabinose was higher in strain SB5314 than in other strains that metabolized the sugar. This could be due to hyper-induction of the arabinose operon, which is characteristic of mutants unable to utilize the sugar (Katz & Englesberg, 1971; Beverin *et al.*, 1971). Strain SB5313, which is AraF⁺,AraE⁻,AraA⁻, showed no alkaline pH change or acidification of the external medium (Fig. 1), when grown and tested under the identical conditions used for strain SB5314. Since an alkaline pH shift was observed (Table 2) in arabinose-grown strain JM1100, which is GalP⁻,MglP⁻, arabinose-H⁺ symport is not likely to be mediated on the active-transport systems for galactose. In glycerol-grown cells of strain JM559, which is constitutive for the lactose permease, no pH shift was observed on addition of arabinose, although methyl 1-thio- β -D-galactoside and galactose, substrates of the lactose permease, elicited alkaline

pH shifts. Therefore arabinose-H⁺ symport on the lactose permease was also eliminated. Just as with the K12 strains described above, the pH increase on addition of arabinose to B/r AraE⁺ strains was followed by a subsequent H⁺ efflux in cells capable of metabolizing the sugar (Fig. 1); the absence of H⁺ efflux from strain SB5314 (Fig. 1 and Table 2), which is blocked in arabinose metabolism (*araA*), supported the conclusion that the acidification was due to the sugar being metabolized.

Proton-linked arabinose transport in membrane vesicles

The activity of the AraF system should be absent from membrane vesicles of *E. coli*, since the binding protein is lost during the preparative procedure (Kaback, 1971). Vesicles prepared from glycerol-grown arabinose-induced strain SB5314 (AraE⁺,AraF⁺) retained the ability to energize the transport of labelled arabinose (see below), and therefore their ability to effect arabinose-H⁺ symport was also tested.

An alkaline pH change was observed when

Fig. 1. Association of uncoupler-sensitive arabinose-promoted pH changes with AraE

(a) *E. coli* strain SB5314 (AraE⁺) or SB5313 (AraE⁻) was grown on 20mM-glycerol + 1mM-arabinose as inducer. The organisms were harvested and depleted as described in the Experimental section. In the three separate experiments shown 7μmol of arabinose was added to an anaerobic suspension of cells (12mg dry mass) in 4ml of 150mM-KCl/2mM-glycylglycine, pH 6.4–6.8. An upward deflexion represents an alkaline pH change. Where indicated 7μM-tetrachlorosalicylanilide (TCS) was added about 10min before the arabinose. (b) *E. coli* strain CW2022 (AraE⁺,AraF⁻) or CW2004 (AraE⁻,AraF⁺) was grown on 20mM-glycerol + 10mM-arabinose. Both strains can metabolize arabinose. The pH changes occurring on addition of 7μmol of arabinose or fucose were measured in cells prepared as for (a).

arabinose was added to an anaerobic suspension of membrane vesicles prepared from strain SB5314 grown in the presence of arabinose (Fig. 2). A

Fig. 2. Sugar-promoted pH changes in membrane vesicles of *E. coli*

Membrane vesicles (14–16 mg of protein) prepared from arabinose-induced glycerol-grown *E. coli* SB5314 were diluted into 150 mM-KCl/2 mM-glycylglycine under the conditions used for Fig. 1. Six separate experiments are shown, in which 14 μ mol of the indicated sugar was added after a period of equilibration. Where indicated tetrachlorosalicylanilide (TCS) was present at 10 μ M. In one experiment vesicles were omitted to measure the effect of arabinose alone on the pH measuring system; no significant deflexion occurred with the other compounds.

second addition of sugar gave a much smaller deflexion, presumably due to an unfavourable H^+ gradient set up by the inward H^+ movement on the first addition, which would oppose further sugar- H^+ symport into the vesicles. The small pH shift observed in the buffer control (Fig. 2) could be due to an effect of the sugar solution on the pH and/or reference electrode. After correcting for this, the mean extent of arabinose-promoted H^+ movement was 1.25 nmol of H^+ /mg of protein (compared with 6.25 nmol/mg dry mass in intact cells), from four separate experiments on different batches of vesicles.

Susceptibility of arabinose-promoted alkaline pH changes to uncoupling agents

If the alkaline pH change observed on addition of arabinose to cells or vesicles of AraE⁺ *E. coli* strains represents the movement of protons across the cell membrane, then its appearance should be abolished by compounds that render the membrane permeable to protons, i.e. uncoupling agents. Indeed, in the presence of the uncouplers, 2,4-dinitrophenol, carbonyl cyanide *m*-chlorophenylhydrazone and tetrachlorosalicylanilide, the alkaline pH shift on addition of arabinose to intact cells (Fig. 1 and Table 3) or membrane vesicles (Fig. 2) was inhibited. The inhibition by carbonyl cyanide *m*-chlorophenylhydrazone was not likely to be due to modification of thiol groups (Kaback *et al.*, 1974) in the AraE system, as the effect was not attenuated by dithiothreitol (Table 3).

Substrate specificity of the proton symport system, AraE, in intact cells and membrane vesicles

In intact cells L-arabinose and D-fucose effected H^+ symport in all AraE⁺ strains (Table 2 and Fig. 1). The addition of galactose in some strains elicited a small alkaline pH change, but no acid efflux was observed (Table 2), probably because

Table 3. Inhibition by uncoupling agents of arabinose-promoted pH changes in *E. coli* strains induced for arabinose transport

The growth conditions and method of measuring pH changes are described in the Experimental section. Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; TCS, tetrachlorosalicylanilide; DNP, 2,4-dinitrophenol.

<i>E. coli</i> strain	Uncoupling agent	Dithiothreitol (10 mM)	Effective H^+ uptake (nmol)	H^+ efflux
KD2	None	—	19.4	+
	None	+	14.4	+
	20 μ M-CCCP	—	0	+
	20 μ M-CCCP	+	0	+
	20 μ M-TCS	—	0	+
	2 mM-DNP	—	0	+
SB5314	None	—	75	—
	20 μ M-CCCP	—	0	—
	20 μ M-TCS	—	0	—
	2 mM-DNP	—	0	—

the inducible enzymes for galactose metabolism were not present. Methyl β -L-arabinoside and D-xylose gave a slow extensive alkaline pH drift. D-Arabinose, D-talose, L-rhamnose, L-fucose, D-ribose, D-glucose, 2-deoxy-D-galactose, 6-deoxy-D-glucose and methyl β -D-galactoside did not effect an alkaline pH change. The results were consistent with the findings obtained by Novotny & Englesberg (1966) and Brown & Hogg (1972) that D-fucose was a strong inhibitor, i.e. competing substrate, of the low-affinity system and that D-galactose, methyl β -L-arabinoside and D-xylose were weak inhibitors.

In membrane vesicles the ability of D-fucose, D-galactose and methyl 1-thio- β -D-galactoside to elicit H^+ symport was examined. Fucose or galactose, but not methyl 1-thio- β -D-galactoside, were effective (Fig. 2). The H^+ uptake was considerably inhibited when arabinose was added after fucose or galactose, but it was not after methyl 1-thio- β -D-galactoside addition. Probably an unfavourable H^+ gradient set up by the inward movement of protons with fucose or galactose prevented H^+ symport with arabinose.

The results imply that translocation on the arabinose carrier in the vesicles, presumably AraE, occurs by a sugar- H^+ symport mechanism. The sugar and inducer specificities indicate that transport is not mediated by the LacY, GalP or XylP system.

Arabinose- H^+ symport in spheroplasts

Spheroplasts prepared from arabinose-induced cells of strain SB5314 were tested for their ability to effect sugar- H^+ symport. Arabinose or fucose promoted alkaline pH shifts (Table 4). Arabinose- H^+ symport was abolished by the uncoupler tetrachlorosalicylanilide. The presence of galactose diminished the alkaline pH change observed on adding arabinose, whereas methyl 1-thio- β -D-galactoside had no significant effect. No pH change occurred when methyl β -L-arabinoside, 2-deoxy-D-galactose, xylose, ribose or rhamnose was added. These results essentially corroborated the observations with intact cells and membrane vesicles.

Temperature-dependence of the sugar-promoted pH changes on AraE

The effect of temperatures between 5°C and 35°C on the velocity of H^+ symport with arabinose or fucose was measured in energy-depleted suspensions of cells after growth on arabinose. Arrhenius plots of the results showed a reasonably linear relationship between the logarithm of the H^+ uptake velocity and the reciprocal of absolute temperature (Fig. 3). The apparent energy of activation was 78.5 kJ/mol for fucose- H^+ symport and 68.9 kJ/mol for arabinose- H^+ symport. These values for H^+ transport on AraE are similar to the activation energy of 63.5 kJ/mol observed for galactose- H^+ symport on GalP (P. J. F. Henderson, unpublished work), and the activation energy for the LacY transport system of 75.3 kJ/mol (Thilo *et al.*, 1977). The temperature coefficients, Q_{10} , were calculated to be 2.4 and 2.6 for H^+ transport elicited by fucose and arabinose respectively on AraE. A high temperature coefficient (Q_{10} 2–3) is expected for carrier-mediated solute transport, whereas simple diffusion would yield a Q_{10} of about 1.03 in the 'generally used temperature range' (Kotyk & Janacek, 1970).

Uptake of radioactive sugar on the arabinose-transport systems AraE and AraF

The accumulation of arabinose into various induced strains of *E. coli* is shown in Table 5. From an external concentration of 0.05 mM sugar, assuming that there is 2.7 μ l of intracellular water/mg dry mass of cells (Winkler & Wilson, 1966), if uptake was by passive diffusion and isolated from further metabolism, then the concentration of sugar reached inside would approach 0.14 nmol/mg dry mass. The initial rates of uptake showed that arabinose transport on the AraE and AraF systems established a gradient of 150–200-fold higher inside than outside within 0.25 min in strain SB5314, which cannot metabolize the sugar because it is arabinose isomerase-negative. In strains where the initial uptake of arabinose is rapidly followed by metabolism, an

Table 4. Sugar-promoted alkaline pH changes with spheroplasts

Spheroplasts were prepared from arabinose-induced *E. coli* SB5314 grown on glycerol as described in the Experimental section. The pH of a suspension (5.6 mg of protein) in 150 mM-KCl/2 mM-glycylglycine was recorded continuously as 7 μ mol of sugar was added (see the Experimental section).

Sugar added	Effective H^+ uptake
Arabinose	32.1 (3)
Arabinose (+ 20 μ M-tetrachlorosalicylanilide)	0
Fucose	19.3
Galactose	0
(followed by arabinose)	11.8
Methyl thiogalactoside	0
(followed by arabinose)	28.2

Fig. 3. Arrhenius plots of H^+ movement linked to sugar transport on AraE

Arabinose- H^+ symport was measured in *E. coli* K10 (Δ) and fucose- H^+ symport in *E. coli* RS1 ($\circ$) as described in Fig. 1. Both strains were grown on 20 mM-glycerol + 10 mM-arabinose. The initial rates of sugar-promoted alkaline pH changes (nmol/min) were measured with cells equilibrated at temperatures in the range 5–35°C maintained by circulation from a refrigerated water bath (Grant Instruments LB8).

apparent 23–70-fold higher accumulation inside than outside was observed. The initial rates of arabinose uptake at 0.25 min were lower in strains metabolizing the sugar than in the arabinose isomerase-negative mutants, which probably reflects the hyper-induction of the arabinose operon previously reported in such mutants, as mentioned above (Katz & Englesberg, 1971; Beverin *et al.*, 1971).

Strains RS1wt and K10 failed to accumulate an appreciable amount of arabinose when grown in the absence of the sugar (Table 5). Also, there was no significant arabinose uptake when strains RS1wt, KD2 and RS1E⁻ were grown on lactate with methyl β -L-arabinoside or fucose. The results clearly showed that the active-transport systems for arabinose are induced by arabinose, and not by fucose or methyl β -L-arabinoside.

Effect of arsenate on the AraE and AraF transport systems

The phosphate analogue arsenate substantially lowers ATP concentrations in *E. coli* (Klein & Boyer, 1972). Therefore, if energization of arabinose transport involves ATP directly, uptake would be inhibited by arsenate. By contrast, transport energized by a transmembrane protonmotive force generated by respiration, without the intermediate formation of ATP, would not be susceptible to arsenate.

Arsenate severely inhibited the initial rates of

Table 5. Uptake of arabinose by *E. coli* strains

The number of measurements, where made on more than one batch of cells, is indicated in parenthesis. Otherwise the values obtained are means of duplicates. Cell suspensions were incubated with the energy source for 3 min at 25°C. Radioactive arabinose (50 μ M) was then added and the accumulation of the sugar was measured, as described in the Experimental section.

Strain	Arabinose-transport phenotype	Carbon compounds in growth medium	Energy source for uptakes	Arabinose-uptake rate (nmol/min per mg)
RS1wt	AraE ⁺ , AraF ⁺	Arabinose, glycerol	Glycerol	7.3 (10)
		Lactate	Lactate	0.8
		Fucose + lactate	Lactate	0.37
		Methyl β -L-arabinose + lactate	Lactate	0.27
KD2	AraE ⁺ , AraF ⁻	Arabinose, glycerol	Glycerol	9.6 (7)
		Fucose + lactate	Lactate	0.33
		Methyl β -L-arabinose + lactate	Lactate	0.15
RS1E ⁻ thi ⁻	AraE ⁻ , AraF ⁺	Arabinose, glycerol	Glycerol	5.9 (11)
		Fucose + lactate	Lactate	0.45
		Methyl β -L-arabinose + lactate	Lactate	0.5
UP1000	AraE ⁺ , AraF ⁺	Arabinose	Glycerol	5.5 (6)
CW2022	AraE ⁺ , AraF ⁻	Arabinose	Glycerol	5.3 (4)
CW2004	AraE ⁻ , AraF ⁺	Arabinose	Glycerol	5.8 (5)
SB5314	AraE ⁺ , AraF ⁺ , AraA ⁻	Arabinose, glycerol	Glycerol	29.8 (2)
K10	AraE ⁺ , AraF ⁺	Arabinose	Glycerol	6.4 (7)
		Glycerol	Glycerol	0.2

arabinose uptake into the AraE⁻,AraF⁺ strain, whereas the inhibition occurred to a smaller degree in the AraE⁺,AraF⁻ strain (Fig. 4). In the wild-type AraE⁺,AraF⁺ strain, transport was resistant to arsenate, similarly to the AraE⁺,AraF⁻ strain. Control measurements were made of transport systems whose mechanisms of energization are considered to be established in *E. coli*; the glutamine-uptake system was selected as representative of phosphate-bond-energized transport (Berger, 1973), and proline and methyl 1-thio- β -D-galactoside uptake as representative of protonmotive-force-energized transport (Hirata *et al.*, 1973). The effect of arsenate on glutamine, proline and methyl 1-thio- β -D-galactoside uptake is illustrated in Fig. 5. Glutamine uptake was strongly inhibited by arsenate, whereas protonmotive-force-driven methyl 1-thio- β -D-galactoside and proline uptakes were more resistant. The partial inhibition of these last two uptakes may occur because the lowering of ATP concentrations

by arsenate would moderately affect the formation of the protonmotive force via ATP hydrolysis by the Ca²⁺/Mg²⁺-dependent ATPase. Other factors, such as the carbon source for growth, the non-specific effects of arsenate at high concentrations, and the compound used to energize uptakes, were shown by Rhoads & Epstein (1977) to moderate the susceptibility of transport to arsenate. Such considerations should not affect the experiment shown in Fig. 4, because the isogenic strains were grown under identical conditions and the transport measurements were performed with the same energy source and substrate. However, in the results shown in Fig. 5 the necessary inclusion of isopropyl β -D-galactoside or proline or glutamine in the growth medium would prevent the metabolic conditions being precisely the same in the three experiments.

With this reservation, it can still be concluded that transport on AraE is no more susceptible to arsenate than is methyl 1-thio- β -D-galactoside or

Fig. 4. Inhibition by arsenate of arabinose uptake into *E. coli*

E. coli strain KD2 (AraE⁺,AraF⁻; ○) or RS1E⁻ (AraE⁻,AraF⁺, △) was grown on 20mM-glycerol + 10mM-arabinose. After depletion and a washing in 150mM-KCl/5 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/KOH buffer, pH 6.5, the glycerol-energized uptake of 182 μ M-[¹⁴C]arabinose was measured as described by Henderson *et al.* (1977). The cells were exposed to the indicated concentrations of potassium arsenate 3 min before the addition of arabinose; the concentration of KCl was adjusted to maintain constant osmolality in the presence of arsenate. Values are the means of duplicate measurements on two batches of cells. Control values were 5.6 nmol/min per mg (○) and 8.1 nmol/min per mg (△).

Fig. 5. Inhibition by arsenate of methyl thiogalactoside, proline or glutamine uptakes into *E. coli*

E. coli strain KD2, RS1E⁻ or RS1wt was grown on 20mM-glycerol with 1mM-isopropyl 1-thio- β -D-galactoside, 10mM-proline or 10mM-glutamine. The glycerol-energized uptakes of 45 μ M-[¹⁴C]methyl 1-thio- β -D-galactoside (○), 45 μ M-[¹⁴C]proline (□) or 45 μ M-glutamine (△) were measured at the indicated arsenate concentrations under the conditions used for Fig. 4. Each strain gave very similar results for a particular substrate. Therefore duplicate measurements on one batch of each of the three strains, i.e. six in all, were averaged to give each value shown. Mean control values were 9.8 nmol/min per mg (○), 11.6 nmol/min per mg (□) and 3.5 nmol/min per mg (△).

proline uptake, and transport on AraF is nearly as susceptible to arsenate as is glutamine uptake. These results indicate that the AraF transport system is directly energized by ATP or another high-energy phosphate compound and that the AraE transport system is not. When both transport systems were present in wild-type organisms, transport was not susceptible to arsenate, indicating that the AraE system functioned independently of any inactivation of AraF.

Effect of arsenate on the GalP and MglP transport systems

The susceptibility of the binding-protein-containing galactose transport system, MglP, to arsenate seems to be well established (Vorisek & Kepes, 1972; Parnes & Boos, 1973; Boos, 1974; Wilson, 1974b). However, the report that GalP activity was also apparently susceptible to arsenate (Wilson, 1974b) is inconsistent with the results that arsenate-insensitive galactose transport (presumably GalP) was energized by respiration in vesicles (Kerwar *et al.*, 1972) and that GalP activity was proton-linked in intact cells or vesicles (Henderson *et al.*,

1977; Horne, 1980). We sought to clarify this question by using *E. coli* mutants that are GalP⁺, MglP⁻, or GalP⁻, MglP⁺, but otherwise virtually isogenic, JM1097 and JM1104 (Henderson *et al.*, 1977). The strains were grown under identical conditions and the transport measurements were performed with the same energy source and substrate.

The results are shown in Fig. 6. Galactose transport on MglP was much more susceptible to inhibition by arsenate than was galactose transport on GalP. The ability of arsenate to decrease GalP activity by 20–30% is not considered significant, because uptake of methyl 1-thio- β -D-galactoside into the isopropyl 1-thio- β -D-galactoside-induced strains, i.e. proton-linked lactose permease activity, was also decreased by 20–25% (Daruwalla, 1979). Galactose transport into vesicles where MglP activity is absent was unaffected by 10 mM-arsenate, and 50 mM-arsenate inhibited by only 29% (Kerwar *et al.*, 1972).

The arsenate-resistance of GalP activity is consistent with its energization by a chemiosmotic mechanism, whereas the arsenate-sensitivity of MglP indicates involvement of phosphate bonds in energization. In this respect, the similarity of GalP to AraE, and of MglP to AraF, is readily apparent from Figs. 4 and 6.

Fig. 6. Inhibition by arsenate of galactose uptake into *E. coli*

E. coli strain JM1097 (GalP⁺, MglP⁻) or JM1104 (GalP⁻, MglP⁺) was grown on 20 mM-glycerol + 1 mM-D-fucose as inducer of both transport systems. The glycerol-energized uptake of 182 μ M-[1-³H]-galactose was measured at the indicated arsenate concentrations as described for Fig. 4. For *E. coli* JM1097 (○) each measurement is the mean of duplicates from three batches of cells; for *E. coli* JM1104 (△) each value is the mean of duplicates from one batch. Control values were 5.8 nmol/min per mg (○) and 7.5 nmol/min per mg (△).

Arabinose transport in membrane vesicles

Kaback (1972) reported briefly that vesicles prepared from *E. coli* ML30 induced with arabinose retained D-lactate-energized arabinose transport. For further evidence that this activity was of the sugar-H⁺ symport type, we have prepared vesicles from *E. coli* SB5314 by the method of Kaback (1971) and measured the susceptibility of arabinose transport to uncouplers, inhibitors and ionophores. Such vesicles retain the active AraE transport protein (Macpherson *et al.*, 1981), and have the advantage over intact cells of accessibility to ionophore antibiotics, which can be used to investigate the transport mechanism, and also secondary effects of uncoupling agents (see the Discussion section) are eliminated by removal of metabolic enzymes and intermediates.

Table 6 shows the uptake of arabinose energized by different respiratory substrates at pH 6.5. Ascorbate in the presence of phenazine methosulphate was the most efficient energy source, whereas D-lactate, succinate and DL- α -glycerophosphate were only marginally effective. This agrees with previous observations on the relative efficiency of these respiratory substrates in energizing transport. Energized transport of arabinose was not observed in vesicles prepared from uninduced cells. Uptake was inhibited by azide and cyanide (Table 7). All these observations indicate that the energy for active

Table 6. *Effect of different energy sources on arabinose transport into membrane vesicles of E. coli SB5314*

Membrane vesicles were prepared from *E. coli* SB5314 grown with 20 mM-glycerol + 1 mM-arabinose (induced) or 20 mM-glycerol (uninduced). Ascorbate and phenazine methosulphate were present at concentrations of 20 mM and 0.1 mM, respectively. D-Lactate, succinate and DL- α -glycerophosphate were present at 10 mM concentrations. Uptake was measured after addition of 0.2 mM-[14 C]arabinose as described in the Experimental section.

Energy source	Initial rate (nmol/min per mg)	Extent (nmol/2 min per mg)
Induced		
None	3.1	1.0
Ascorbate + phenazine methosulphate	12.8	5.7
DL-Lactate	5.0	2.3
Succinate	3.4	2.0
DL- α -Glycerophosphate	3.7	1.7
Uninduced		
None	0.3	0.08
Ascorbate + phenazine methosulphate	0.2	0.08

Table 7. *Effect of metabolic inhibitors and ionophores on energized arabinose transport into membrane vesicles of E. coli SB5314*

Membrane vesicles were prepared from *E. coli* SB5314 grown on 20 mM-glycerol + 1 mM-arabinose. Uptake of 0.2 mM-[14 C]arabinose was energized by ascorbate + phenazine methosulphate and measured as described in the Experimental section. Values are means of duplicate measurements. Where the compounds used were prepared as ethanolic solutions, the uptakes were measured with ethanol at the same final concentration of 2% (v/v).

Inhibitor or ionophore	Concn.	Uptake activity (% of control initial rate)
Carbonyl cyanide <i>m</i> -chlorophenylhydrazine	20 μ M	28
Tetrachlorosalicylanilide	20 μ M	18
2,4-Dinitrophenol	1 mM	18
Azide	10 mM	35
Cyanide	1 mM	62
	5 mM	12
Arsenate	5 mM	74
	20 mM	119
Ionophore A217	1 μ g/ml	59
	2 μ g/ml	82
	4 μ g/ml	54
Valinomycin	1 μ M	93
	2 μ M	81
	4 μ M	62
Ionophore A217 + valinomycin	1 μ g/ml + 1 μ M	50
	2 μ g/ml + 2 μ M	31
	4 μ g/ml + 4 μ M	26
No energy source		17–24

transport of arabinose by the membrane-bound system is supplied by respiration.

Arsenate, which uncouples ATP synthesis without affecting the protonmotive force, did not prevent the active uptake of arabinose (Table 7). This substantiates the conclusion from experiments with intact cells that phosphate-bond energy is not directly coupled to AraE activity.

Arabinose accumulation on AraE was abolished in the presence of the uncoupling agents carbonyl cyanide *m*-chlorophenylhydrazine, tetrachlorosalicylanilide and 2,4-nitrophenol. This indicated that active transport on AraE was linked to the trans-

membrane protonmotive force. To determine the extent of involvement of the electrical-potential gradient ($\Delta\psi$) and the pH-gradient (Δ pH) components of the protonmotive force, the effects of the ionophores A217 and valinomycin were examined. Ionophore A217, a nigericin-like compound, exchanges K^+ for H^+ , thus decreasing the transmembrane pH. Valinomycin in the presence of K^+ causes a decrease of the transmembrane $\Delta\psi$, which is partly compensated by an increase in Δ pH (Ramos & Kaback, 1977a,b). In the presence of ionophore A217 or valinomycin, energized arabinose uptake was partially inhibited (Table 7), indicat-

ing that at pH 6.5 both pH and $\Delta\psi$ contributed to the total protonmotive force. This is in agreement with the findings obtained by Ramos & Kaback (1977a) that at pH 6.5 the protonmotive force generated through respiration on ascorbate + phenazine methosulphate has a ΔpH component of -75 mV and a $\Delta\psi$ component of -60 mV . The ionophores when present together effectively uncoupled energized transport, presumably by disrupting both ΔpH and $\Delta\psi$ simultaneously (Table 7).

These results strongly implicate the protonmotive force as the driving force for active transport of arabinose on AraE. The observations point to the involvement of both the pH-gradient and electrical-gradient components of the protonmotive force in energizing arabinose uptake at pH 6.5.

Kinetic parameters of transport on AraE or AraF

The apparent K_m and V_{max} values obtained for arabinose transport are listed in Table 8. In AraE⁺, AraF⁻ strains a low-affinity arabinose-transport system was present with an apparent K_m of 145–168 μM . These values are close to those of 130 μM and 100 μM obtained by Novotny & Englesberg (1966) and Hogg (1977) respectively, but somewhat higher than the apparent K_m of 63 μM in an AraE⁺, AraF⁻ B/r strain (Brown & Hogg, 1972).

In membrane vesicles the apparent K_m for arabinose uptake was 317 μM , which compares with a value of 140 μM in membrane vesicles reported by Kaback (1972). This indicated that the low-affinity transport system for arabinose is the membrane-bound one. Its higher K_m in vesicles may reflect some loss of efficient energization, as described for the lactose permease (Wright & Overath, 1980).

In AraE⁻, AraF⁺ cells, the apparent K_m value was 4–6 μM (Table 8), indicating the presence of a high-affinity transport system. The results were consistent

with K_m values of 2–6 μM and 1 μM reported by Brown & Hogg (1972) and Hogg (1977) respectively in AraE⁻, AraF⁺ strains.

The velocities of arabinose uptake in AraE⁺, AraF⁺ strains gave reasonably hyperbolic relationships to substrate concentration and did not fit the sum of two hyperbolas, indicating that the two distinct transport systems were not functioning independently in an additive manner. The K_m value obtained was intermediate between and distinct from the K_m of the high-affinity system in AraE⁻, AraF⁺ strains or the low-affinity system in the AraE⁺, AraF⁻ strains. The suggestion by Schleif (1969), that the intracellular arabinose after growth on the sugar obscures completely the presence of the high-affinity system, does not satisfactorily explain the difference in the K_m values. The failure to observe clear biphasic kinetics could be due to the two transport systems competing for the second substrate, the energy source, which may not be at a saturating concentration. Hogg (1977) also obtained kinetic data that suggested that transport in AraE⁺, AraF⁺ strains is not an additive function of the individual systems, but that they operate in a synergistic way.

The V_{max} values for arabinose transport were similar in the different strains, except for the higher value obtained with intact cells of strain SB5314; once again, this is consistent with hyper-inducibility of the arabinose operon in this strain.

The results of the present study supplement previous kinetic evidence in support of two different active-transport systems for arabinose, the membrane-bound AraE system with a low affinity for arabinose uptake and the AraF system with a high affinity for uptake of the sugar. It appears that in AraE⁺, AraF⁺ strains the two systems interact to function co-operatively in the uptake of arabinose. A similar phenomenon was reported for the two

Table 8. *Kinetic parameters of the arabinose-transport systems in E. coli.*

Cultures were grown on 20 mM-glycerol + 10 mM-arabinose, except for strain SB5314, which was grown on 20 mM-glycerol + 1 mM-arabinose. Cell suspensions were prepared for uptakes as described in the Experimental section. Uptakes were energized by 10 mM-glycerol for 3 min before the addition of radioactive arabinose. The K_m , V_{max} and standard deviations were calculated by the computerized method of Cleland (1967). For some strains the experiment was repeated on the indicated number of batches, and the average of the K_m and V_{max} determinations is shown.

<i>E. coli</i> strain	Arabinose-transport phenotype	K_m (μM)	V_{max} (nmol/min per mg)
K10	AraE ⁺ , AraF ⁺	44.8 ± 5.7 (4)	9.2 ± 0.7 (4)
RS1wt	AraE ⁺ , AraF ⁺	82.3 ± 11.3	13.9 ± 0.6
UP1000	AraE ⁺ , AraF ⁺	75.1 ± 10.2 (3)	12.8 ± 0.9 (3)
SB5314	AraE ⁺ , AraF ⁺	22.3 ± 5.4 (2)	105.2 ± 5.4 (2)
SB5314 membrane vesicles	AraE ⁺ , AraF ⁻	316.6 ± 134.6	24.8 ± 6.1
KD2	AraE ⁺ , AraF ⁻	168.0 ± 29.5	17.7 ± 1.3
CW2022	AraE ⁺ , AraF ⁻	145.9 ± 27.6	15.2 ± 2.0
RS1E ⁻ thi ⁻	AraE ⁻ , AraF ⁺	4.1 ± 0.7	7.6 ± 0.2
CW2004	AraE ⁻ , AraF ⁺	6.1 ± 2.0	11.4 ± 0.6

galactose-transport systems, GalP and MglP by Wilson (1974a).

AraF is more susceptible to catabolite repression than is AraE

Strain SB5314 was grown on succinate as growth substrate, conditions under which catabolite repression is minimal. The proteins released when spheroplasts were formed from a mixture of arabinose-induced cells supplied with ^3H -labelled amino acids and uninduced cells supplied with ^{14}C -labelled amino acids were solubilized in sodium dodecyl sulphate and separated by polyacrylamide-gel electrophoresis (Macpherson *et al.*, 1981). The $^3\text{H}/^{14}\text{C}$ ratios in sequential 2 mm slices of the gel were then measured. A high ratio was found in a protein of apparent M_r 34000, which was taken to be the arabinose-binding protein (reported M_r 33100; Hogg, 1977). The appearance of this arabinose-inducible protein was completely abolished by growth on glucose instead of succinate, even with 20 mM-arabinose present as inducer (Fig. 7). Nevertheless, such cells still transported ^{14}C arabinose (results not shown), albeit at a rate about 20% of that found in glycerol-grown cells. The addition of arabinose or fucose to anaerobic suspensions of glucose-grown cells induced with 1 mM- or 20 mM-arabinose evoked an alkaline pH change, with initial rates 20–40% of those obtained with glycerol-grown bacteria.

These results imply that the expression of the *araF* gene(s) is completely repressed by growth on glucose, whereas expression of the *araE* gene(s) is only partially repressed.

MglP is more susceptible to catabolite repression than is GalP

Wilson (1974a) measured GalP activity and galactose-binding protein (*mglB* gene product; Ordal & Adler, 1974a,b; Robbins *et al.*, 1976) in wild-type and GalP-constitutive *E. coli* strains, growing on glucose, glycerol or galactose. He concluded that the MglP galactose-transport system is much more susceptible to catabolite repression than is the GalP galactose-transport system. We have substantiated this conclusion by using the non-metabolizable 6-deoxy-D-galactose, D-fucose, to induce both the MglP and GalP systems on various growth substrates, and by exploiting the availability of radioisotope-labelled galactose analogues that are substrates of either MglP or GalP. They are methyl β -D-galactoside, which is specific for the MglP system (Rotman, 1959; Rotman *et al.*, 1968), and 2-deoxy-D-galactose, which is specific for GalP (Henderson & Giddens, 1977).

When *E. coli* K10 was grown on glucose plus fucose, MglP activity failed to appear, although GalP activity was evident (Table 9). It was necessary to raise the fucose concentration to 20 mM to

Fig. 7. Catabolite repression of an arabinose-inducible periplasmic protein

Mixed $^3\text{H}/^{14}\text{C}$ -labelled periplasmic proteins from arabinose-induced (^3H) or uninduced (^{14}C) *E. coli* SB5314 grown on succinate were prepared (see the Experimental section). After separation of the proteins by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (12.5%), the gel track was cut into 2 mm slices and the $^3\text{H}/^{14}\text{C}$ (induced/uninduced) ratio in each slice was measured and normalized (Macpherson *et al.*, 1981). The procedure was repeated with arabinose-induced (^3H) or uninduced (^{14}C) cells grown on glucose. The expected position of the arabinose-binding protein is indicated by an arrow.

decrease competitive inhibition of fucose entry by glucose (see Henderson *et al.*, 1977). The presence of the GalP system was further confirmed by the detection of galactose- H^+ pH changes (results not shown). When the growth substrate was glycerol with 1 mM-fucose as inducer, the methyl β -D-galactoside uptake (MglP activity) was substantial, 5 nmol/2 min per mg (Henderson & Giddens, 1977), and with succinate it increased to 13 nmol/2 min per mg (Table 9). By contrast, the 2-deoxy-D-galactose uptake was similar, 0.6–1.7 nmol/2 min per mg, whatever the substrate for growth (Table 9; Henderson & Giddens, 1977).

These results confirm that the MglP galactose-transport system, which is associated with a periplasmic binding protein, is much more susceptible to catabolite repression than is the GalP system.

Table 9. *Susceptibility of GalP and MglP to catabolite repression*

E. coli K10 was grown in 50 ml of minimal medium in a 250 ml conical flask with either 20 mM-sodium succinate or 10 mM-glucose. Fucose, the inducer of both GalP and MglP, was present as indicated. The organism was harvested during exponential growth, and transport was measured with 0.05 mM-2-deoxy-D-[1-³H]galactose or 0.05 mM-[¹⁴C]methyl β -D-galactoside as described in the Experimental section. Values are means of duplicate measurements on one batch of cells.

Growth substrate	Inducer	GalP activity (nmol of 2-deoxygalactose/ 2 min per mg)	MglP activity (nmol of methyl galactoside/ 2 min per mg)
Succinate	None	0.12	3.48
Succinate	1 mM-Fucose	0.91	13.07
Glucose	None	0.05	0.05
Glucose	1 mM-Fucose	0.09	0.05
Glucose	20 mM-Fucose	0.60	0.05

Table 10. *Doubling time and cell yield of E. coli strains grown on arabinose in batch cultures*

The cultures were grown on 10 mM-arabinose. Growth parameters were determined as described in the Experimental section.

<i>E. coli</i> strain	Arabinose-transport phenotype	Doubling time (min)	Carbon cell yield (g/mol)
RS1wt	AraE ⁺ ,AraF ⁺	84	88
KD2	AraE ⁺ ,AraF ⁻	96	115
RS1E ⁻ thi ⁻	AraE ⁻ ,AraF ⁺	180	58
UP1000	AraE ⁺ ,AraF ⁺	60	68
CW2022	AraE ⁺ ,AraF ⁻	76	81
CW2004	AraE ⁻ ,AraF ⁺	150	47
JM1100	AraE ⁺ ,AraF ⁺ ,GalP ⁻ ,MglP ⁻	114	83

Growth study of arabinose-transport mutants in batch culture

The exponential growth rates of AraE⁺,AraF⁺, AraE⁺,AraF⁻ and AraE⁻,AraF⁺ strains of *E. coli* cultured in batch at 37°C in 10 mM-arabinose were measured. The organisms grew exponentially for a few generations, followed by a decline in the growth rate.

The doubling times and cell yields are presented in Table 10. The AraE⁻,AraF⁺ strains had a doubling time nearly twice as long as strains in which the AraE system is present. The cell yields in AraE⁻,AraF⁺ mutants were 40–50% lower than in the AraE⁺ strains. Cell yields were highest in the AraE⁺,AraF⁻ strains. The ability of AraE⁺ strains to grow at about twice the rate of an AraE⁻ strain was confirmed at arabinose concentrations in the range 0.5–40 mM by using strains RS1wt (AraE⁺,AraF⁺), KD2 (AraE⁺,AraF⁻) and RS1E⁻ (AraE⁻,AraF⁺).

The results suggest that energization of AraE may be more efficient than energization of AraF. The relatively short doubling times and high cell yields of the AraE⁺ strains compared with the AraE⁻,AraF⁺ strains could imply that protonmotive-force-driven transport via AraE is more efficient in arabinose utilization than is high-energy-phosphate-energized transport on AraF. The doubling time was

shortest in the AraE⁺,AraF⁺ strains, indicating that the AraE transport system's characteristics predominate in an AraE⁺,AraF⁺ strain.

Discussion

The present study of arabinose transport into AraE⁺,AraF⁻ and AraE⁻,AraF⁺ mutants of *E. coli* has characterized two mechanisms for energizing the transport process. For the AraE system a chemiosmotic mechanism operates, and the evidence can be summarized as follows. Addition of substrate, arabinose or fucose, to intact cells, spheroplasts or membrane vesicles elicited an alkaline pH change indicative of sugar-H⁺ symport; the accumulation of arabinose into vesicles was dependent on respiration; both the alkaline pH changes and the sugar accumulation were prevented by protonophores; the accumulation into vesicles at pH 6.5 was partially inhibited by valinomycin or ionophore A217, and both ionophores together caused complete inhibition. The specificity of the transport for substrates and inducer, and the separate location of the *araE* gene on the *E. coli* chromosome, showed that the arabinose transport was not mediated by the LacY, GalP or XylP systems, which also exhibit the characteristics of a chemiosmotic energization mechanism (West & Mitchell, 1973; Henderson *et al.*, 1977; Lam *et al.*, 1980).

We cannot be certain of the nature of energization of the AraF transport system. Two observations provide some information. Firstly, no alkaline pH change was observed when arabinose was added to AraF⁺ strains. This indicated that the system does not operate by a chemiosmotic mechanism. However, such a conclusion might be invalid for the following reasons. The sugar movement on AraF could be linked to the transmembrane electrical potential and not to ΔpH . The AraF system might be inoperative under the de-energized conditions necessary to observe H⁺ symport; however, this is probably not the case, since an acid efflux indicative of arabinose metabolism (and therefore entry) was observed (non-carrier-mediated 'leakage' of arabinose was unlikely, since strain RS1E⁻ showed no growth after 2–3 days on arabinose minimal-medium plates, except as a result of mutation). The acid efflux might have obscured an alkaline pH change with arabinose, but not with fucose, which is a non-metabolizable substrate of the AraF transport system. Also, the movement of the sugar on AraF could be linked to the movement of ions other than protons. The possibility that the AraF⁺ strains were permeable to protons was eliminated by the demonstration of methyl 1-thio- β -D-galactoside-H⁺ symport when the LacY system was induced by growth in the presence of isopropyl 1-thio- β -D-galactoside (results not shown).

The second pertinent observation was that transport of arabinose on AraF was inhibited by arsenate. This implies that a phosphorylated compound is the source of energy. Berger (1973) and Berger & Heppel (1974) first described a class of transport systems with the following characteristics: susceptible to 'cold-shock', which removes periplasmic binding proteins; inhibited by arsenate; energized by a product of glycolysis, e.g. ATP, under conditions where respiration was prevented; having a high affinity for substrates. The properties of AraF identified so far would justify its inclusion in this class, although it is noteworthy that some transport systems in *E. coli* may exhibit characteristics of both this type and the chemiosmotic mechanism (Rhoads & Epstein, 1977; reviewed by Silhavy *et al.*, 1978). Subsequently, Hong *et al.* (1979) proposed that acetyl phosphate was the energy source, rather than ATP. Our results do not illuminate this possibility.

Uncouplers of oxidative phosphorylation dissipate the transmembrane protonmotive force generated by respiration and so prevent ATP synthesis (Mitchell, 1970). Hence transport systems that are protonmotive-force-energized should be inhibited by uncoupling agents, whereas systems that require phosphate-bond energy should be insensitive, provided that a high concentration of ATP is maintained in the presence of the uncoupler.

Daruwalla (1979) undertook an extensive survey of the influence of 2,4-dinitrophenol, carbonyl cyanide *m*-chlorophenylhydrazone and tetrachlorosalicylanilide on energized arabinose transport into intact cells of the AraE⁺,AraF⁻ or AraE⁻,AraF⁺ *E. coli* strains described in the present paper. She concluded that sensitivity to these uncouplers is not a satisfactory way of distinguishing the mechanisms of energization of the AraE and AraF transport systems. The most probable reason is that the primary effect of discharging the protonmotive force is followed by changes in cytoplasmic adenine nucleotide concentrations that impair metabolism of the energizing substrate(s) and/or the transported compound. This concurs with the results obtained by Rhoads & Epstein (1977), who found, for example, that glutamine uptake was more sensitive to 2,4-dinitrophenol in glycerol-grown *E. coli* than in glucose-grown cells. Also, individual uncoupling agents may exert specific effects additional to their influence on the membrane. Kaback *et al.* (1974) reported that carbonyl cyanide *m*-chlorophenylhydrazone apparently reacts with thiol groups in proteins, a facility reversed by thiol reagents such as dithiothreitol, and Daruwalla (1979) found that inhibition of AraE or AraF by high concentrations of carbonyl cyanide *m*-chlorophenylhydrazone was moderated by dithiothreitol; both the AraE and the AraF systems have a thiol group in the substrate-binding site (Hogg & Hermodson, 1977; Miller *et al.*, 1979; Macpherson *et al.*, 1981). Brummet & Ordal (1977) reported that tetrachlorosalicylanilide inhibited glycine or proline transport in *Bacillus subtilis* by interacting at specific sites in the carrier proteins rather than by de-energizing the membrane. Thus the use of any of the three uncouplers on intact bacterial cells involves some ambiguity in interpretation of results.

Arabinose transport into the AraE⁺,AraF⁺ strain was not susceptible to arsenate, nor did the kinetic analysis indicate that two systems were operating. Possibly the AraE system predominated when both were present, or more probably the induction of the AraF system was substantially repressed. The following observations support the latter possibility. Hogg & Englesberg (1969) reported that only a small amount of binding protein (product of the *araF* gene) was produced during batch culture of wild-type strain SB5001 on arabinose. They suggested that the low concentrations of binding protein resulted from catabolite repression by arabinose (see also Katz & Englesberg, 1971; Beverin *et al.*, 1971). We have found that growth of strain SB5314 on glucose with 1 mM- or even 20 mM-arabinose as inducer did not produce detectable binding protein, although the succinate-grown organisms did so (see also Macpherson *et al.*, 1981). By using the arabinose-promoted pH change as a specific assay

Table 11. *Characteristics of the arabinose and galactose active-transport systems in E. coli*

System	Known transport components		Approximate location of gene(s) on chromosome	Response to osmotic shock	K_m (μ M) for arabinose or galactose	Inhibition by 5 mM-arsenate (%)	Sugar-H ⁺ symport	Mechanism of energization	Relative sensitivity to catabolite repression
	Membrane-bound	Periplasmic							
AraE	AraE	—	61.0 min	Resistant	146–168	8	Yes	Chemiosmotic	Resistant
GalP	GalP	—	61.0 min	Resistant	70–170	28	Yes	Chemiosmotic	Resistant
AraF	—	AraF binding protein	44.5 min	Sensitive	4–6	55	No	Phosphate-bond energization?	Sensitive
MglP	MglAC?	MglB binding protein	45.5 min	Sensitive	0.4–0.7	81	No	Phosphate-bond energization?	Sensitive

of AraE in the glucose- or succinate-grown cells, it was evident that AraE is much less susceptible to catabolite repression than is AraF.

It may be concluded that the AraF system operates only when the bacteria are growing under relatively 'starved' conditions, whereas the AraE system is operative when higher concentrations of substrate are available. The advantages of the chemiosmotic mechanism may be that, for a reason that is not yet clear, it enables higher growth rates and cell yields to be achieved.

The analogy between the two types of arabinose-transport system, and the two genetically and biochemically distinct galactose-transport systems, is striking. Their properties are summarized in Table 11. AraE and GalP are both membrane-bound shock-resistant systems on which transport is energized by a chemiosmotic mechanism, and they have a relatively low affinity for their substrates. It may be of evolutionary significance (Hartley, 1974) that the genes coding for each map close together at about 61 min on the *E. coli* chromosome (Bachmann & Low, 1980), although fine structure mapping reveals that they are separated by other genes (Macpherson *et al.*, 1981).

Like the AraF system, the MglP system has properties typical of the binding protein class of transport systems (reviewed by Boos, 1974; Silhavy *et al.*, 1978), including a very high affinity for substrate and susceptibility to catabolite repression (see also Rotman & Radojkovic, 1964; Boos, 1969; Ordal & Adler, 1974*a,b*; Wilson, 1974*a*; Henderson *et al.*, 1977). Sequence and X-ray-crystallographic analysis of the arabinose-binding or galactose-binding proteins have revealed a degree of homology between them (Hogg, 1977; Hogg & Hermodson, 1977; Quijcho & Pflugrath, 1980; Gilliland & Quijcho, 1981), and Hogg (1977) has suggested that they evolved through gene duplication (Hartley, 1974) because their gene loci are close: *araF* 44.5 min and *mglP* 45.5 min (Heffernan *et al.*, 1976; Bachmann & Low, 1980). No information is available on membrane-bound components of these systems, although it is postulated that the products of genes *mglA* and *mglC* are such components in the case of MglP (Ordal & Adler, 1974*b*; Robbins *et al.*, 1976).

We are grateful to the Science Research Council for support of this research through Grants B/RG/6761.9 and G/RA/4472.5. K. R. D. indebted to The Cambridge Society of Bombay for a scholarship. Dr. R. W. Hogg and Dr. E. Englesberg kindly donated some of the bacterial strains, and we thank Dr. M. C. Jones-Mortimer for advice on genetic manipulation of *E. coli*.

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