BBA 75781

ARABINOSE TRANSPORT IN *araC*⁻ STRAINS OF *ESCHERICHIA* B/r

JUDITH SINGER AND ELLIS ENGLESBERG

Department of Biological Sciences, University of California, Santa Barbara, Calif. 93106 (U.S.A.)

(Received June 23rd, 1971)

SUMMARY

A permease is described which transports and is inducible by L-arabinose in *araC*⁻ strains of *Escherichia coli* B/r. The cell density at which the permease begins to appear depends on several factors including the presence of added cyclic AMP, and the concentrations of the inducer and the carbon source. The kinetics of induction, the K_m, and the substrate specificity of the permease provide evidence that it is distinct from the previously described arabinose- specific permease present in *araC*⁺ strains, and is probably equivalent to the methylgalactoside permease present in *E. coli* K12.

INTRODUCTION

The product of the regulatory gene *araC* is required, together with L-arabinose, for the induction of the first three enzymes involved in the metabolism of L-arabinose[1,2] as well as for the induction of an arabinose-specific permease[3], and an arabinose-binding protein which may also play a role in transport[4]. However the control of the *araC* gene over L-arabinose transport is not complete: while the permease levels of *araC*⁻ strains are greatly reduced, such strains do show some inducible arabinose permease activity[5].

While it seemed possible that the residual permease activity of *araC*⁻ strains was due to the arabinose-specific permease previously described[3], the fact that transport in bacteria is often mediated by multiple permeases, and that the specificity of some permeases is rather broad[6] (and, in particular, the fact that the D-galactose permease has affinity for L-arabinose[7]) made it seem equally plausible that the active transport of L-arabinose by *araC*⁻ strains was due to a different permease.

The aim of the present study, then, was to characterize the permease activity of *araC*⁻ strains, in the hope that this might help clarify the mechanism of arabinose transport and the role of the gene *araC* in the control of the synthesis of arabinose transport systems.

METHODS

Strains

The strains used are shown in Table I. Transducing lysates of phage Plbt and Plkc were prepared and transductions carried out as previously described[8,11]. *Thy*⁻

strains were selected on mineral glucose plates containing thymine (50 μg/ml) and trimethoprim (50 μg/ml), or by aminopterin selection[11] as modified by A. L. TAYLOR (personal communication).

TABLE I

LIST OF STRAINS

All strains listed are F^- strains of *E. coli* B/r with the exception of T18, which is *E. coli* K12, HfrH 3000.

Strain	*Genotype*	*Origin or reference*
SB 1094	*araCΔ719 thr*	SHEPPARD AND ENGLESBERG[2]
UP 1082	*araC5 leu*	GROSS AND ENGLESBERG[8]
SB 1095	*araCΔ719*	Plbt(SB 1094) × UP 1082; selection for *leu*$^+$
SB 5045	*araCΔ719 thy*	SB 1094 by trimethoprim selection
T18	*galR*^{S} *trp*	SAEDLER *et al.*[9]
SB 5046	*araCΔ719 galR*^{S}	Plkc(T18) × SB 5045; selection for *thy*$^+$
UP 1656	*araE3*	ISAACSON AND ENGLESBERG[19]
UP 1041	*araA39*	SHEPPARD AND ENGLESBERG[2]
UP 1002	*ara*$^+$ *leu*	GROSS AND ENGLESBERG[8]
SB 5407	*araA39 thy*	UP 1041 by aminopterin selection
SB 5408	*leu thy*	Plbt (UP 1002) × SB 5407; selection for *ara*$^+$
SB 5409	*leu araE3*	Plbt (UP 1656) × SB 5408; selection for *thy*$^+$
SB 5410	*leu araE*$^+$	Plbt (UP 1656) × SB 5408; selection for *thy*$^+$
SB 5047	*araCΔ719*	Plbt(SB 1095) × SB 5410; selection for *leu*$^+$
SB 5048	*arsCΔ719 araE3*	Plbt(SB 1095) × SB 5409; selection for *leu*$^+$
UP 1012	*araC5*	GROSS AND ENGLESBERG(8)
UP 1040	*araC37*	CRIBBS AND ENGLESBERG(10)

Media

The composition of L-broth, mineral base, casein hydrolysate plus salts, and eosin methylene blue indicator agar (emb) *plus* appropriate sugar at a concentration of 1 % has been described[2,13].

Preparation of cells and assay of permease activity

Cells were grown at 37°. In a typical experiment a 5–7-h L-broth culture was diluted 50-fold into 1 % casein hydrolysate *plus* salts and incubated overnight for 12 h. The overnight culture was then used to inoculate the same medium to a turbidity of 20–30 Klett units (blue filter). Cells in late-log phase were harvested by centrifugation (10000 rev./min for 10 min), washed in 0.5 vol. of casein hydrolysate *plus* salts at 4°, and resuspended in the same medium to a turbidity of 0.46 units measured with a Fisher electrophotometer[3]. Aliquots containing 1.5 ml of cells were then placed in a shaking water bath at 25° for 5 min, after which 1 ml was incubated with L-[1-^{14}C] arabinose (final concn. 2 mM) and chloramphenicol (final concn. 25 μg/ml), in a final volume of 1.25 ml. After 15 min of incubation at 25° (or 30 sec at 20° where initial velocity of uptake was measured), 1 ml of the reaction mixture was filtered on a Millipore filter (0.45 μm pore size), then washed twice, each time by the rapid (3 sec) filtration of 1 ml of casein hydrolysate *plus* salts at room temperature. The filters were dried, and radioactivity was determined in a Beckman LS 200 B liquid-scintillation counter. In calculating the specific activity (μmoles of L-[1-^{14}C]arabinose per g dry wt.), a background of 40 counts/min was substracted from all values, and

where accumulation was measured, correction was made for the counts/min expected on the basis of diffusion alone. The values shown are the averages of duplicates.

Identification of accumulated radioactive compound

Cells were incubated with L-[1-^{14}C]arabinose for 10 min, then filtered and washed, as described in the previous section. The filters were then placed in 5 ml of boiling 70 % ethanol, and boiled for 5 min. The resulting extract was centrifuged (15000 rev./min, 15 min), and the supernatant was collected, then evaporated to dryness; the residue was reconstituted in approx. 20 μl of water, and duplicate samples were spotted, together with cold carrier L-arabinose, on Whatman No. 1 filter paper for pie plate chromatography. A control consisting of chloramphenicol, L-[1-^{14}C]arabinose, and carrier was spotted at the same time. The chromatograph was developed in water-saturated *n*-butanol for 7.5 h at 37°, then dried and exposed to medical X-ray film (Kodak) for 30 days. The X-ray film was then developed. For one of the duplicate samples, the distance of the radioactive spots from the origin was compared with that of the L-[1-^{14}C]arabinose standard, and regions on the chromatogram corresponding to the radioactive spots were cut out and counted in the scintillation counter. The chromatogram of the second duplicate sample was developed with triphenyltetrazolium chloride spray, and the position of the carrier L-arabinose was compared with the position of the radioactive spots.

Chemicals

L-[1-^{14}C]Arabinose from Calbiochem was further purified by descending paper chromatography in water-saturated *n*-butanol. In some shipments, about 50 % of the radioactivity was due to impurities. Trimethoprim was purchased from Calbiochem, D-galactose (glucose-free) from Sigma, and β-methyl-D-galactopyranoside from Mann Research Laboratories.

RESULTS

Induction

One striking characteristic of the permease is its pattern of induction. If L-arabinose (final concn. 0.2 %) is added to a culture of cells in the exponential phase of growth, induction occurs only when the cell density corresponds to about 400 μg dry wt./ml (Fig. 1).

The cell density at which the permease begins to appear can be lowered by;

(1) *The addition of cyclic AMP.* If cyclic AMP is added to the culture (final concn. 5 mM) at the same time as L-arabinose, induction is immediate (Fig. 1). The slope representing the differential rate of synthesis of the permease (300 μmoles of L-arabinose accumulated per g dry wt. of cells) is the same in induced cultures with and without cyclic AMP, indicating that the permease is being synthesized at the same rate in both cases. Cyclic AMP added to cells in the absence of L-arabinose increases the differential rate of synthesis of the permease to 50 μmoles of L-arabinose accumulated per g dry wt. of cells (results not shown).

(2) *An increase in inducer concentration.* If L-arabinose is added to the culture at a final concentration of 1 %, synthesis of the permease begins at a lower turbidity (Fig. 2); however, the rate of permease synthesis is not constant in this case, but rather increases gradually until the maximum rate is attained.

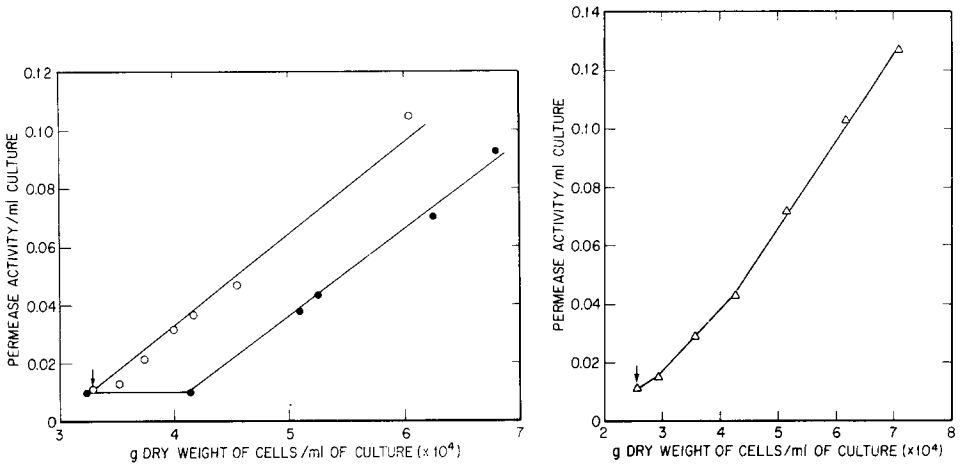

Fig. 1. The kinetics of permease induction of SB 1094 in the presence and absence of cyclic AMP. A log-phase culture of SB 1094, grown in 1% casein hydrolysate as described in METHODS, was induced with L-arabinose (final concn. 0.2%) at a Klett unit value of 150 (indicated by arrow); to 0.5 of the culture cyclic AMP was added as well. At various times after the addition of L-arabinose, samples of culture were removed, chloramphenicol was added (final concn. 40 μg/ml), and the sample was rapidly chilled to 0°. The cells were then harvested and assayed for their ability to accumulate L-arabinose as described in METHODS. Permease activity is expressed as μmoles of L-arabinose accumulated per ml of culture. L-[1-^{14}C]Arabinose, 2 mM; specific activity $9 \cdot 10^4$ counts/min per μmole. ○, + ara, + cyclic AMP; ●, + ara, − cyclic AMP.

Fig. 2. The kinetics of permease induction of SB 1094 induced with 1% L-arabinose. Cells were grown, sampled and assayed for permease activity as described in the legend to Fig. 1. Klett unit value at time of addition of L-arabinose (final concn. 1%), 120. L-[1-^{14}C]Arabinose, specific activity $6 \cdot 10^4$ counts/min per μmole.

(3) *A decrease in the concentration of the carbon source.* If the concentration of casein hydrolysate is reduced from 1 to 0.5 %, induction occurs at a lower turbidity. Similarly for cells grown in mineral glycerol, induction occurs sooner if the concentration of glycerol is reduced from 0.2 to 0.1 %.

The strain used in most of these experiments, SB 1094, has a deletion of the *araC* gene extending into the controlling sites of the *ara* operon[2]. Strains UP 1012, *araC*5, a nonsense mutant, and UP 1040, *araC*37, a missense mutant, were found to display the same kinetics of induction as SB 1094, indicating that neither the permease nor its pattern of induction are unique to SB 1094.

K_m

The value of the K_m of the permease for L-arabinose was found to be 0.6 mM, by measurement of the initial velocity of uptake of L-arabinose as a function of the external L-arabinose concentration (Fig. 3). An identical value for the K_m was obtained when steady-state accumulation rather than initial velocity was measured (results not shown).

Substrate specificity

As can be seen from Table II, D-galactose, D-glucose, D-fucose, β-methyl-D-galactopyranoside, and D-xylose each inhibit the accumulation of L-arabinose by an

arabinose-induced culture of SB 1094. In other experiments the uptake of β-methyl-D-galactopyranoside, D-xylose, D-fucose, and D-galactose by arabinose-induced cells was demonstrated directly by use of the appropriate radioactively labelled sugar (results shown only for β-methyl-D-galactopyranoside; see Table III).

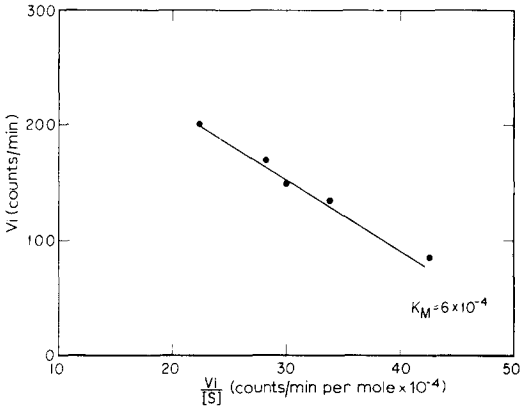

Fig. 3. The initial velocity of entry of L-arabinose as a function of its external concentration. Cells of strain SB 1094 were induced with L-arabinose, harvested at a turbidity of 345 Klett units, and assayed for initial velocity of uptake of L-arabinose as described in METHODS. V_i represents the counts/min of L-[1-^{14}C]Arabinose per filter, and [S] the external L-[1-^{14}C]arabinose concn. L-[1-^{14}C]Arabinose, concn. range 0.2–0.9 mM; specific activity $7 \cdot 10^6$ counts/min per μmole.

TABLE II

EFFECT OF VARIOUS SUGARS ON THE ACCUMULATION OF L-ARABINOSE IN SB 1094

Cells of SB 1094 were grown at 37° in 1% casein hydrolysate *plus* 0.2% L-arabinose and harvested as described in METHODS, then assayed for accumulation of L-[1-^{14}C]arabinose after 15 min at 25° in the presence of other non-radioactive sugars. The external concentration of L-[1-^{14}C]arabinose was 3 mM, that of the added sugar, 8 mM. The percentage inhibition was calculated with respect to the value obtained without additions. Specific activity of L-[1-^{14}C]arabinose, $1.29 \cdot 10^5$ counts/min per μmole.

Additions	*Amount of L-[1-^{14}C]arabinose accumulated (μmoles/g dry wt.)*	*Inhibition (%)*
None	310	–
D-Galactose	45	85
D-Glucose	59	81
D-Fucose	61	80
β-Methyl-D-galactopyranoside	68	78
D-Xylose	115	63
L-Arabinose	152	51
D-Arabinose	293	5
L-Arabitol	330	0

Inducer specificity

Table III shows the inducer specificity of the permease. L-Arabinose induces a permease which transports L-arabinose and β-methyl-D-galactopyranoside. Induction is due to L-arabinose itself rather than to an impurity, as shown by the fact that the

TABLE III

INDUCER SPECIFICITY OF THE PERMEASE OF SB 1094

Cells of strain SB 1094 were grown in the presence of various inducers (inducer concn. 0.2%) for 5 h, then assayed for the ability to accumulate L-[1-^{14}C]arabinose, β-methyl-D-[^{14}C]galactopyranoside ([^{14}C]MG), and L-[1-^{14}C]arabinose in the presence of an equimolar amount of non-radioactive β-methyl-D-galactopyranoside. External concentration of L-[1-^{14}C]arabinose, β-methyl-D-[^{14}C] galactopyranoside, and β-methyl-D-galactopyranoside, 2 mM. Specific activity of L-[1-^{14}C] arabinose, $6 \cdot 10^4$ counts/min per μmole; specific activity of β-methyl-D-[^{14}C]galactopyranoside, $1 \cdot 10^5$ count/min per μmole.

Inducer	*Substrate (μmoles sugar/g dry wt.)*		
	L-[1-^{14}C] *Arabinose*	[^{14}C]*MG*	L-[1-^{14}C] *Arabinose + MG*
None	31	53	15
L-Arabinose	376	564	30
L-Arabinose*	364	570	30
D-Fucose	196	5	17
MG	30	2	12
D-Galactose	16	8	20

* L-Arabinose recrystallized from ethanol

permease is induced equally well whether L-arabinose is used directly or recrystallized from ethanol. Neither D-fucose, β-methyl-D-galactopyranoside, nor D-galactose causes induction, although D-fucose seems to induce another permease whose ability to transport L-arabinose is inhibited by β-methyl-D-galactopyranoside.

Accumulation of L-*arabinose*

The compound accumulating as a result of the permease activity of SB 1094 was found to be at least 97% L-arabinose by chromatography and radioautography (see METHODS).

Effect of galR^S

The specificity of the permease suggested that it might be the equivalent of one of the permeases known to transport D-galactose in *E. coli* K-12 (ref. 7). In order to see whether the permease was under the control of the *galR* gene, the *galR*^{S} marker was transduced into SB 5045, a *thy*$^-$ derivative of SB 1094, as described in Table I. One of the purified *gal*$^-$ transductants, SB 5046, *thr araCΔ719 galR*^{S}, was found to have the same inducible permease activity as the parent strain, SB 1094, *thr araCΔ719 galR*$^+$, thus providing evidence that the permease of *araC*$^-$ strains is not under the control of the *galR* gene.

Effect of araE

The product of the *araE* gene is thought to be required for full expression of the permease present in *araC*$^+$ strains, as shown by the fact that strains containing mutant alleles of *araE* have reduced permease levels. If the *araE* gene plays a role in the permease activity of SB 1094, introduction of an *araE*$^-$ allele should alter the activity. However, the kinetics of permease induction of strains SB 5047 and SB 5048, two isogenic *araC*$^-$ strains differing only in the presence or absence of a functional *araE*

gene, were found to be the same within experimental error (Fig. 4), demonstrating that this permease does not require the product of the *araE* gene.

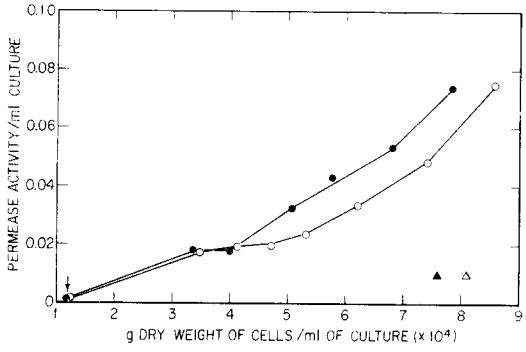

Fig. 4. The effect of the *araE* allele on the kinetics of permease induction. Cells of strain SB 5047 (*araCΔ*719) and SB 5048 (*araCΔ*719 *araE*3) were grown, sampled and assayed for permease activity as described in the legend to Fig. 1. L-Arabinose (final concn. 0.4%) was added when the cells reached a turbidity of 148 Klett units, as shown by the arrow. L-[1-^{14}C]Arabinose, specific activity $4.4 \cdot 10^4$ counts/min per μmole. ●, SB 5047, + ara; ○, SB 5048, + ara; ▲ SB 5047, − ara; △, SB 5048, − ara.

DISCUSSION

Evidence has been presented demonstrating the presence in *araC*$^-$ strains of a permease which transports and is inducible by L-arabinose. The cell density at which inducible synthesis of the permease begins, and the kinetics of induction are variable, depending upon the presence of added cyclic AMP, and upon the concentration of the inducer and of the carbon source.

One possible explanation for the unusual induction pattern is that L-arabinose is a poor inducer, and that the permease is extremely sensitive to catabolite repression. If this is the case, then the kinetics of induction seen when L-arabinose is added to a final concentration of 0.2% can be explained on the grounds that not enough L-arabinose enters the cells to cause induction until late in exponential growth, when the intracellular cyclic AMP concentration rises[14]. The increase in intracellular cyclic AMP concentration — which can also be brought about by adding cyclic AMP directly (Fig. 1), and made to occur at a lower cell density by decreasing the concentration of a limiting carbon source — would have a 2-fold effect: first, it would increase the basal level of the permease so that L-arabinose would be present in sufficient amounts to cause induction, and, secondly, it would allow the synthesis of the permease to occur at its maximum rate. The kinetics of induction seen when L-arabinose is added to a final concentration of 1% might then be explained by postulating that at that concentration, enough L-arabinose enters the cells by diffusion to cause almost immediate induction of the permease, but that its rate of expression increases only gradually as the intracellular cyclic AMP concentration increases.

The permease is distinct from the one previously characterized in *araC*$^+$ strains; its K_m is different (0.6 *vs* 0.125 mM for the permease in *araC*$^+$ strains). and it has a different substrate specificity as measured by the inhibition of L-arabinose accumulation by other sugars (see Table II); although D-fucose greatly inhibits the activity

of both permeases, D-galactose and D-xylose are much more potent inhibitors of this permease than of the permease in *araC*$^+$ strains[3]. This permease is also more severely inhibited by D-glucose, which presumably prevents L-arabinose accumulation *via* non-competitive inhibition[15]. The permease is not coded for by the *araE* gene as shown by the fact that the *araE* allele does not affect its induction. It also appears to be distinct from the arabinose binding protein, since no binding protein is detectable in *araC*$^-$ strains[4].

Although of the four permeases which transport D-galactose in *E. coli* K-12, only the galactose permease has been reported to be inducible by L-arabinose[7], the permease described here has the substrate specificity and sensitivity to physiological conditions of the methylgalactoside permease[7,16-18]. If it is the methylgalactoside permease, it differs from that of *E. coli* K-12 in that it is not inducible by D-fucose (Table III).

While the permease described here is different by several criteria from the one previously found in *araC*$^+$ strains, preliminary observations suggest that the *araC* allele may affect its synthesis. This possibility is currently under investigation.

ACKNOWLEDGMENTS

We thank Linda Gielow for the construction of strains SB 5047 and SB 5048, and H. J. Besemer for kindly sending us strain T18. This research was supported by National Science Foundation Grant No. GB 24093 to E.E., and J. S. was supported by a National Institutes of Health Predoctoral Fellowship (No. 5 F01 GM 41692-03).

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