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An L-Arabinose Binding Protein and Arabinose Permeation in *Escherichia coli*

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A search was made for proteins of the arabinose system which bind arabinose, as it is expected that the permease and possibly the *C* protein could be identified by arabinose binding. One protein was found and this is most probably part of the arabinose permease. It is inducible, can be removed from cells by osmotic shock and easily purified, and consists of one polypeptide of molecular weight 35,000. In these respects it is closely similar to proteins binding sulfate, leucine, and galactose (Pardee, Prestidge, Whipple & Dreyfuss, 1966; Anraku, 1968; Penrose, Nichoalds, Piperno & Oxender, 1968).

Mutants were found with reduced ability to concentrate arabinose intracellularly but which still possessed the arabinose binding proteins. One mutant was found with a lowered level of binding protein. This strain is unable to concentrate any detectable amount of arabinose. None of the mutations affecting permeation of arabinose mapped in the threonine–leucine region where the arabinose genes *A*, *B*, *C* and *D* are located.

1. Introduction

Englesberg and his co-workers (latest reference Sheppard & Englesberg, 1967) have studied arabinose utilization in *Escherichia coli* B/r and they have shown that a product of the arabinose *C* gene is required for expression of three genes linked to it (*A*, isomerase; *B*, ribulokinase; and *D*, epimerase, see Fig. 1), and perhaps also for expression of a permease gene located elsewhere (Englesberg, Irr, Power & Lee, 1965). The *C* gene product in the presence of arabinose acts positively to turn on expression. Normal expression of arabinose genes is impossible in the absence of the *C* gene product. This control system is different from the better understood lactose operon, where absence of its regulatory protein leads to full expression of lactose genes (Jacob & Monod, 1961).

In order to examine this control mechanism, I have studied the binding of arabinose to proteins in the cell extracts. This work necessitated the isolation and characterization of mutants in the arabinose operon of strain K12. All properties of the arabinose operon and its control reported by Englesberg in his work with strain B/r were found to be true also in strain K12.

The work to be presented here reports the isolation of an arabinose binding protein which is not the *C* gene protein, but which appears to be involved in transport. Proteins are known which bind their various substrates comparatively tightly and which seem to be involved in permeation of their substrates. Anraku has studied one

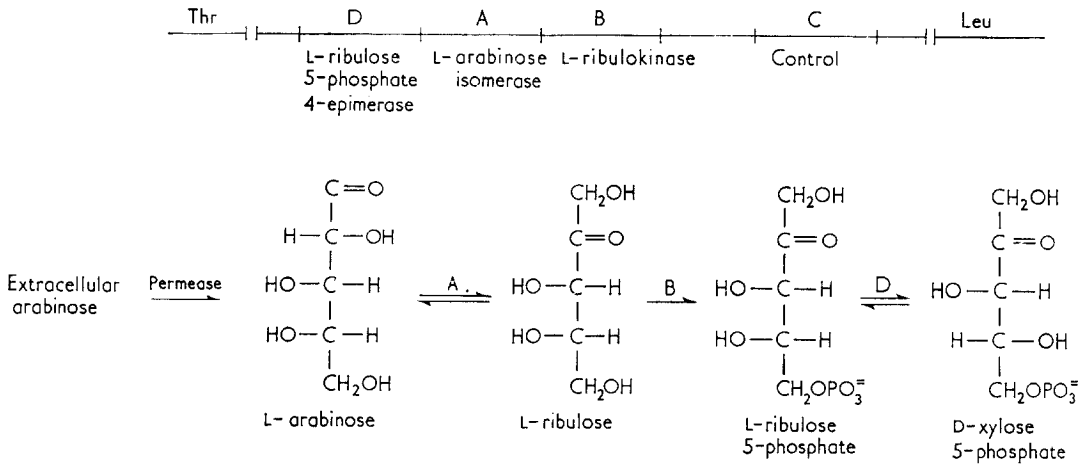

FIG. 1. The L-arabinose pathway as elucidated by Englesberg and co-workers.

binding leucine and galactose (Anraku, 1968) and Pardee has studied one binding sulfate (Pardee *et al.*, 1966).

2. Materials and Methods

(a) *Bacterial strains and bacteriophages*

TABLE 1

Strains of bacteria used

RFS-strain number	Genotype	Comment
1	Hfr: *thi*	Strain Hfr H, requires thiamine
3	Hfr: *araA3 thi*	
6	Hfr: *araB6 thi*	
13	Hfr: *araB13 thi*	Hyperinducible
22	Hfr: *araC22 thi*	Arabinose constitutive
54	Hfr: *araD54 thi*	Arabinose sensitive
56	Hfr: *araC22 araA56 thi*	No detectable isomerase, arabinose sensitive in low phosphate medium
73	F⁻: *thr leu lac thi*	Strain C600, *lac* negative
90	Hfr: *araC22 araA56 ara90 thi*	No longer sensitive to arabinose in low phosphate medium
305	F′: *(thr-leu)/thr leu met pro thi str*	Strain KLF-1 (Low, 1968), episome covering threonine-leucine, streptomycin resistant
306	F′: *araD54 araC22/araD54 araC22 thi*	See below
381	Hfr: *ara381 araC22 met381 his381 thi*	Arabinose permease negative
496	Hfr: *araB6 ara381 thr thi*	See below
498	Hfr: *ara498 leu498 thi*	Arabinose insensitive revertant of *araD54*, deletes leucine and at least arabinose *C*, *B* and *A*
506	Hfr: *thr leu thi pur suc lac str*	Strain Hfr 312, *lac* negative, streptomycin resistant

(i) *Selection of* araA, araB, araD,

Strains 3, 6, 13, 22 and 54 were
nitrosoguanidine mutagenesis (A
(Gorini & Kaufman, 1960) was u
grow on 0·1% arabinose in the p
permease. Strain 54, defective i
plating to score arabinose-sensiti
strain inhibited by arabinose (gro
by the presence of arabinose, pre
Strain 90 was selected from strai
phosphate–arabinose–glycerol pla

(ii) *Construction of diploid D⁻ C*

Strain 306 was constructed by
and scoring for *araD54*. From a
penicillin, *araC22* was transduce
305 was introduced by selecting f
plating onto minimal plates cont
and arabinose. The arabinose-sen
growing cultures against a dried st
Males and females are distinguish

(iii) *Isolation of lowered binding p*

Strain 381 was found by screen
protein. Colonies from mutageni
tryptone. Two ml. were spun
pH 7·5, 0·2 M-KCl, 0·01 M-magn
EDTA in doubly distilled water
which they were used) containin
10^{-3} M-dinitrophenol. After 20
ml. of supernatant was counted i
of the arabinose binding protein
pellet, leaving behind about 20 ct
behind 150 cts/min. Strain 472 wa
made phenotypically female by h
The methionine and histidine non
leucine requirements and the per
then transduced to leucine plus wit
of the ribulokinase defect was chec

(iv) *Construction of arabinose delet*

Strain 54 was plated, 10^8 cells
arabinose. After 24 hr, approxim
were purified and tested for leucine
ment. Strain 498 was characterize
transduce arabinose point mutan
arabinose plus of less than 10^{-12}
than 1/20 the normal wild-type ba

Bacteriophages R17 (male speci
duction with phage P1 was carri
Adams & Ting (1960). The recipien
used while in exponential growth.
few lysogenic transductants.

(i) *Minimal medium for plates*

In 500 ml. water: 7 g Na_2HPO_4,
water: 5 g sugar, 15 g agar: comb
acids at 100 μg/ml. and thiamine a

(i) *Selection of* araA, araB, araD, *constitutive, and defective permease mutants*

Strains 3, 6, 13, 22 and 54 were derived from strain 1 and strains 56 and 381 from 22 by nitrosoguanidine mutagenesis (Adelberg, Mandel & Chen, 1965). Penicillin enrichment (Gorini & Kaufman, 1960) was used in selection of 54 and 306. Strain 22 was selected to grow on 0·1% arabinose in the presence of 0·2% D-fucose, an inhibitor of the arabinose permease. Strain 54, defective in ribulose phosphate epimerase, was found by replica plating to score arabinose-sensitive colonies after 3 cycles of penicillin enrichment for a strain inhibited by arabinose (growth of a strain lacking the epimerase is greatly inhibited by the presence of arabinose, presumably due to the accumulation of ribulose phosphate). Strain 90 was selected from strain 56 to be resistant to the inhibition by arabinose on low phosphate–arabinose–glycerol plates.

(ii) *Construction of diploid* $D^-\ C^c/D^-\ C^c$

Strain 306 was constructed by transducing *araD*54 into 73, selecting for leucine plus and scoring for *araD*54. From a spontaneous leucine-requiring derivative enriched by penicillin, *araC*22 was transduced in, again selecting for leucine plus. The episome from 305 was introduced by selecting for threonine plus. The homozygote was found by replica plating onto minimal plates containing glycerol and minimal plates containing glycerol and arabinose. The arabinose-sensitive clones were tested for maleness by cross-steaking growing cultures against a dried streak of R17 at 10^{10}/ml. on a yeast–tryptone R17 plate. Males and females are distinguished after 4 hr.

(iii) *Isolation of lowered binding protein mutant*

Strain 381 was found by screening 1200 clones for those lacking the arabinose binding protein. Colonies from mutagenized 22 were grown to stationary phase in 2 × yeast–tryptone. Two ml. were spun down and resuspended in buffer A (0·01 M-Tris–HCl, pH 7·5, 0·2 M-KCl, 0·01 M-magnesium acetate, 0·0005 M-β-mercaptoethanol, 10^{-4} M-EDTA in doubly distilled water at 0°C. The pH of buffers refers to the temperature at which they were used) containing $3{\cdot}5 \times 10^{-7}$ M-L-[^{14}C] in arabinose 3·5 mc/m-mole and 10^{-3} M-dinitrophenol. After 20 min in the cold, the cells were spun down and 0·100 ml. of supernatant was counted in 10 ml. Bray's solution. A strain producing high levels of the arabinose binding protein carries most of the radioactive arabinose into the cell pellet, leaving behind about 20 cts/min, where as a strain without binding protein leaves behind 150 cts/min. Strain 472 was made by crossing strain 506 with 381 which had been made phenotypically female by holding cells in stationary phase for 30 hr before mating. The methionine and histidine non-requiring recombinants were scored for threonine and leucine requirements and the permease defect of strain 381. One such recombinant was then transduced to leucine plus with P1 grown on strain 6, yielding strain 496. The presence of the ribulokinase defect was checked enzymically.

(iv) *Construction of arabinose deletion*

Strain 54 was plated, 10^8 cells per plate on yeast–tryptone plates containing 0·2% arabinose. After 24 hr, approximately 200 arabinose-resistant colonies grew up. These were purified and tested for leucine requirement. Approximately 0·5% had such a requirement. Strain 498 was characterized as a deletion by the inability of P1 grown on it to transduce arabinose point mutants to arabinose plus, by a reversion frequency to arabinose plus of less than 10^{-12}, and by a basal level of arabinose isomerase of less than 1/20 the normal wild-type basal level.

Bacteriophages R17 (male specific) and P1 are indigenous in this laboratory. Transduction with phage P1 was carried out by a modification of the procedure of Luria, Adams & Ting (1960). The recipient cells were grown to stationary phase instead of being used while in exponential growth. A low (0·01) multiplicity of infection resulted in very few lysogenic transductants.

(b) *Media*

(i) *Minimal medium for plates*

In 500 ml. water: 7 g Na_2HP0_4, 3 g KH_2P0_4, 1 g NH_4Cl, 0·8 g Na_2S0_4; and in 500 ml. water: 5 g sugar, 15 g agar: combined after autoclaving and supplemented with amino acids at 100 μg/ml. and thiamine at 10 μg/ml.

(ii) *M9 (Anderson, 1946)*

Minimal salts medium, 0·2% sugar, 10 μg thiamine/ml. supplemented where noted with 1% Casamino acids.

(iii) *Low-phosphate medium (Englesberg, 1966)*

In 1 liter water: 0·3 g KH_2P0_4, 0·7 g K_2HPO_4, 0·1 g $MgSO_4$, $7H_2O$, 1 g $(NH_4)_2SO_4$, after autoclaving 10 mg thiamine, 0·5 g arabinose, 1·0 g glycerol were added.

(iv) *Yeast–tryptone*

In 1 liter water: 5 g NaCl, 8 g tryptone (Difco), 5 g yeast extract (Difco). For 2 × solution, tryptone and yeast extract were doubled, for solid media 15 g agar (Difco)/l. was used. Media for R17 and P1 were made 2×10^{-3} M in $CaCl_2$ after autoclaving.

(v) *Tetrazolium plates*

In 1 liter water: 25·5 g Bacto antibiotic medium 2, 50 mg 2, 3, 5, triphenyltetrazolium chloride. After autoclaving, 10 g sugar was added. Carbohydrate-utilizing colonies are white, carbohydrate non-utilizing are red.

(c) *Assays*

(i) *Arabinose isomerase*

The assay is essentially that described by Cribs & Englesberg (1964). Up to 0·050 ml. of extract was incubated with 0·1 ml. of assay mix (0·1 M-Tris–HCl (pH 7·5), 0·1 M-L-arabinose, $1{\cdot}5 \times 10^{-4}$ M-$MnCl_2$) for 2 to 60 min. The reaction was terminated by adding 0·9 ml. 0·1 M-HCl, and the ribulose produced was assayed colorimetrically by the cysteine–carbazole test by adding 0·1 ml. of 1·5% cysteine, 0·1 ml. of carbazole in 95% ethanol, and immediately 3 ml. of 70% H_2SO_4. After 20 min the absorbance at 550 mμ was read on a Bausch & Lomb Spectronic 20 against a sample not incubated. 10^{-7} mole of ribulose produces an absorbance of 0·3 which is an O.D.$_{550}$ of 0·50.

Whole cells were assayed by vortexing 1 ml. with a drop of toluene. In assays of fully induced strains grown to stationary phase, 0·010 ml. of cells incubated 30 min. in 0·1 ml. of assay mix produces 2×10^{-7} mole ribulose. For assays of low enzyme levels, up to 100 ml. of cells were spun down and resuspended in 1 ml. of chilled assay mix and immediately vortexed with toluene. 0·2 ml. of the heavy suspension was incubated with shaking to prevent clumping. Incubations were stopped by adding 0·8 ml. of 0·5 M-HCl. The assay is very specific for arabinose isomerase. Strain 56, constitutive but lacking the isomerase, has less than 5% as much isomerase activity as the basal level in its parent Hfr H or strain C600.

(ii) *Ribulokinase*

The ribulokinase assay of Lee & Englesberg (1966) was used. Ten ml. of cells that have grown to stationary phase were spun down, resuspended in 1 ml. buffer B with 10^{-2} M-glutathione (0·05 M-Tris–HCl (pH 7·5), 0·2 M-KCl, 0·01 M-magnesium acetate, 0·0005 M-β-mercaptoethanol, 5% glycerol) and sonicated. The cell debris was spun out at 10,000 *g* for 10 min. [^{14}C]Ribulose was prepared for the assay essentially as described by Englesberg (1960) with the omission of the final chromatographic purification. In this preparation, strain 13 is used to convert arabinose to ribulose.

(iii) *Assay of arabinose permease*

0·5 ml. of cells growing exponentially at 2×10^8/ml. in M9, 0·2% glycerol, 1% casein hydrolysate was added to 0·5 ml. of medium at 37°C with [^{14}C]arabinose at 7×10^{-6}M. After incubation for 2 min the cells were filtered on Millipore 0·45 μ papers and rinsed with 10 ml. M9 at 22°C, dried and counted. As noted by Novotny & Englesberg (1966), changing the temperature of the rinse to 0°C releases more than 80% of accumulated arabinose. Under these conditions an uninduced isomerase minus strain takes up 50 to 100 cts/min above a background of 100, while a constitutive isomerase minus mutant takes up 1500 cts/min (permease begins to appear 2·5 min after induction). The contribution of counts provided by the arabinose binding protein is calculated to be less than 20 cts/min. The uptake is energy dependent, for it is inhibited more than 90% by dinitrophenol at

concentrations greater than 3×10
cm^3 for calculation of the amoun
without cells gives the background
The data presented here have all b

(iv) *Assay of arabinose binding pro*

In the equilibrium dialysis assay
at concentrations up to 100 mg/m
dialysis tubing (Union Carbide) ag
cts/min/0·1 ml.) in 10 ml. of buffe
free arabinose is greater than 2, it
and experimental measurements b
creased by the ratio of internal to
is 7 min when there is no arabinos
of solution from outside and insid
fluid. Samples with a 5% excess o
the affinity, K, may be determine
against several concentrations of
arabinose is not chemically mod
"isotopically pure" L-arabinose ac
but one which can be used only wh
filter paper assay. The sample co
20°C with 1 ml. of buffer A contai
the sample was filtered on to a 0·4
20°C and counted.

(d) *Purifica*

The cells of strain 56 were grown
and reached an O.D.$_{550\ m\mu}$ of 15.
10 hr of growth. 60 l. were harves
400 to 600 g of cells.

The freshly grown cells were wash
NaCl at 4°C. After resuspension in
sucrose at 22°C for 20 min, they
resuspending in 6 l. of 5×10^{-4} M-
The supernatant solution from
(pH 7·5) to 0·01 M and β-mercapto
small portions, 60 g/100 ml., while

Summary of puri

	Vol. (ml.)	Protein concn (mg/ml.
Whole cells	550	240
Osmotic shock fluid	6000	0·5
DEAE-cellulose chromatography	180	1·8
Sephadex G100 filtration	15	9·5

†Activity is defined as the ratio of
arabinose at 4°C.

concentrations greater than 3×10^{-4} M. The intracellular volume is assumed to be 10^{-12} cm^3 for calculation of the amount by which arabinose is concentrated. A blank sample without cells gives the background from non-specific adsorption of arabinose to the filters. The data presented here have all been corrected for such a background.

(iv) *Assay of arabinose binding protein*

In the equilibrium dialysis assay, approximately 0·15 ml. of solution containing protein at concentrations up to 100 mg/ml. was normally dialyzed 1 hr in a small sack of no. 20 dialysis tubing (Union Carbide) against [^{14}C]arabinose at 7×10^{-7} M, 3·5 mC/m-mole (350 cts/min/0·1 ml.) in 10 ml. of buffer A. In assays where the equilibrium ratio of bound to free arabinose is greater than 2, it is necessary to dialyze longer. A detailed calculation and experimental measurements both show that the half-time of arabinose entry is increased by the ratio of internal to external arabinose found at equilibrium. The half-time is 7 min when there is no arabinose binding protein in the sack. After dialysis, 0·100 ml. of solution from outside and inside the sack is counted in 10 ml. of Bray's scintillation fluid. Samples with a 5% excess of counts in the sack can be detected reproducibly, and the affinity, K, may be determined with a precision of $\pm$ 0·3 K by dialyzing samples against several concentrations of arabinose. Chromatography verified that the bound arabinose is not chemically modified. Such chromatography found one shipment of "isotopically pure" L-arabinose actually was 80% L-arabitol. A more convenient assay, but one which can be used only when the protein is greater than 20% pure, is the Millipore filter paper assay. The sample containing from 1 to 30 μg of protein was incubated at 20°C with 1 ml. of buffer A containing radioactive arabinose at 7×10^{-7}M. After 10 min the sample was filtered on to a 0·45 μ Millipore paper, rinsed with 10 ml. of buffer A at 20°C and counted.

(d) *Purification of arabinose binding protein*

The cells of strain 56 were grown with vigorous aeration at 22°C in 2 × yeast-tryptone and reached an O.D.$_{550\ m\mu}$ of 15. There is little, if any, mass increase during the last 10 hr of growth. 60 l. were harvested in a Lourdes continuous flow centrifuge, yielding 400 to 600 g of cells.

The freshly grown cells were washed 3 times in 3 l. of 0·01 M-Tris–HCl, pH 7·3, 0·03 M-NaCl at 4°C. After resuspension in 3 l. of 0·03 M-Tris–HCl, pH 7·3, 10^{-4} M-EDTA, 20% sucrose at 22°C for 20 min, they were spun down and osmotically shocked by quickly resuspending in 6 l. of 5×10^{-4} M-$MgCl_2$ at 4°C. The cells were spun out and discarded.

The supernatant solution from the osmotic shock was buffered by adding Tris–HCl (pH 7·5) to 0·01 M and β-mercaptoethanol to 0·005 M. Ammonium sulfate was added in small portions, 60 g/100 ml., while the pH was held constant with KOH. After 12 hr the

TABLE 2

Summary of purification of arabinose binding protein

	Vol. (ml.)	Protein concn (mg/ml.)	Activity† /ml.	Specific activity	Yield (%)	Fold purification
Whole cells	550	240	55	0·23	100	1
Osmotic shock fluid	6000	0·5	2·6	5·2	53	22
DEAE-cellulose chromatography	180	1·8	45·0	25	27	110
Sephadex G100 filtration	15	9·5	330	35	17	150

†Activity is defined as the ratio of bound to free arabinose after dialysis against 7×10^{-7} M-arabinose at 4°C.

precipitated protein was spun out. The pellet, resuspended in 200 ml. of buffer A, was dialyzed against three changes of 4 l. each of buffer C (0·01 M-Tris–HCl, pH 8·0, 0·001 M-β-mercaptoethanol, 0·005 M-KCl). Precipitated protein was spun out and the supernatant solution applied to a 2·8 cm × 70 cm DEAE-cellulose column equilibrated with buffer C. If the ammonium sulfate step was omitted, the osmotic shock fluid was adjusted to pH 8·0 with KOH, which gave a conductivity corresponding to 0·01 M-KCl. Then the 6 l. were applied to the column in 48 hr. After application of the sample, the binding protein was eluted in buffer C at 0·025 M-KCl with a linear 1800-ml. gradient of KCl increasing from 0·005 to 0·08 M.

The pooled fractions, containing 80% of the binding protein as assayed by the Millipore filter paper method, were concentrated by precipitation in which ammonium sulfate is added to 85% of saturation, then dialyzed against buffer A, and further concentrated to 2 ml. by immersing a dialysis sack containing the solution in dry Sephadex G200. The sample was passed through a 1·5 cm × 60 cm Sephadex G100 column in buffer A, yielding 50 to 100 mg of protein free of detectable contamination on polyacrilamide gel electrophoresis. The optical density at 1 mg/ml. determined by the biuret method calibrated against bovine serum albumin was: O.D.$_{260}$=0·65, O.D.$_{280}$=1·18.

3. Properties of the Arabinose Binding Protein

(a) *Detection, purification and partial genetic mapping of the protein*

Arabinose binding is detected by dialyzing a small protein sample against radioactive arabinose. At equilibrium, the concentrations of free arabinose inside and outside the dialysis sack are equal, but the arabinose bound to protein increases the total arabinose concentration inside the sack. In crude extracts of *E. coli*, a 10% excess of arabinose in the dialysis sack can be seen with this equilibrium dialysis assay. The magnitude of binding depends on the strain, but in all cases it can be seen in the protein fraction precipitated between 60 and 85% saturation in ammonium sulfate. The protein can be removed from cells by mild osmotic shock. As described in Materials and Methods, it can be purified further by elution from a DEAE-cellulose column and then by gel filtration on Sephadex G100 to yield a protein without detectable impurities on polyacrylamide gel electrophoresis.

This binding protein was shown not to be the product of arabinose genes *A*,*B*,*C* or *D* since it is present in strain 498 deleting these genes. The binding protein is inducible by growth of cells in the presence of arabinose, but still greater quantities of the protein are found in some constitutive strains. One such constitutive strain, 56, was the source of the binding protein used in these studies.

(b) *Binding properties*

The affinity of binding protein can be calculated from equilibrium dialysis data. For binding characterized by a single binding constant, the relation $Bd = P_o - K(Bd/F)$ holds, where Bd is the concentration of bound arabinose, P_o is the concentration of binding sites, K is the dissociation constant and F is the concentration of free arabinose. Figure 2 shows typical binding at various arabinose concentrations at 37°C with the data plotted as the amount bound as a function of the ratio of bound to free. The binding closely fits a straight line, as expected for a single dissociation constant, and the slope represents $K(37°C) = 2\times10^{-6}$M. The arabinose affinity varies markedly with temperature $K(4°C) = 2\times10^{-7}$ M; $K(24°C) = 7\times10^{-7}$ M; $K(37°C) = 2\times10^{-6}$ M. The enthalpy and free energy of the binding reaction are calculated to be $1{\cdot}2\times10^4$ and $8{\cdot}1\times10^3$ cal./mole, respectively, yielding the entropy change for binding of 20 cal./mole degree.

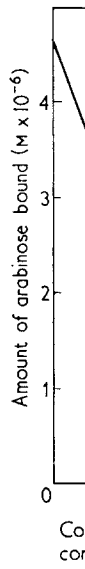

FIG. 2. Bindin
Purified arabinose binding protein at
at 3·5 mc/m-mole in 10 ml. of buffer A
concentrations were $3{\cdot}4\times10^{-7}$, 7×10^{-7},
the form of cts/min/0·100 ml. from outs
centrations have been converted to the
of bound arabinose.

At the higher temperatures ther
which could arise from irreversible
ever, for the protein is very heat s
considerably higher temperatures,
While at the elevated temperature
24°C full binding is restored. No b
phosphoenol pyruvate was seen.
requires that the dissociation be gr
2×10^{-4} M for phosphoenol pyruva
petitively inhibits arabinose uptake
tein with a dissociation constant of 1
constants of 2×10^{-6} and 4×10^{-7}
fucose, xylose and galactose were

(c)

The molecular weight of dissoci
presence of sodium dodecyl sulfate
band corresponding to a molecular
per molecular weight of 35,000 is
calculated from equilibrium dialysis
show that each polypeptide of m
molecule.

The molecular weight of the na

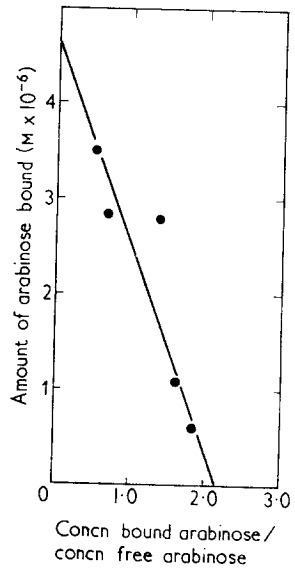

FIG. 2. Binding of arabinose to the protein.
Purified arabinose binding protein at 0·16 mg/ml. was dialyzed 12 hr against L-[^{14}C]arabinose at 3·5 mc/m-mole in 10 ml. of buffer A at 37°C with phosphate replacing Tris. The arabinose concentrations were $3{\cdot}4\times10^{-7}$, 7×10^{-7}, $2{\cdot}1\times10^{-6}$, $4{\cdot}3\times10^{-6}$ and $7{\cdot}1\times10^{-6}$ M. The raw data in the form of cts/min/0·100 ml. from outside and inside the dialysis sack at these arabinose concentrations have been converted to the ratio of bound to free arabinose and the concentration of bound arabinose.

At the higher temperatures there was no discernible reduction in binding sites, which could arise from irreversible denaturation. Denaturation is not expected, however, for the protein is very heat stable. When it is 10% pure, it may be heated to considerably higher temperatures, e.g. 70°C, without being irreversibly denatured. While at the elevated temperature, no binding is detectable, but upon cooling to 24°C full binding is restored. No binding of radioactive ATP, GTP, CTP, UTP or phosphoenol pyruvate was seen. Under the experimental conditions used, this requires that the dissociation be greater than 5×10^{-4} M for the triphosphates and 2×10^{-4} M for phosphoenol pyruvate. D-Fucose (5-methyl-L-arabinose) which competitively inhibits arabinose uptake (Novotny & Englesberg, 1966) binds to the protein with a dissociation constant of 10^{-5} M. D-Xylose and D-galactose have dissociation constants of 2×10^{-6} and 4×10^{-7} M, respectively. These dissociation constants for fucose, xylose and galactose were measured by competition for arabinose binding.

(c) *Molecular weight*

The molecular weight of dissociated denatured protein electrophoresed in the presence of sodium dodecyl sulfate (Shapiro, Viñuela & Maizel, 1967) shows only one band corresponding to a molecular weight of approximately 35,000. One binding site per molecular weight of 35,000 is found from the binding saturation value of P_o calculated from equilibrium dialysis of pure protein (Fig. 2). These two measurements show that each polypeptide of molecular weight of 35,000 binds one arabinose molecule.

The molecular weight of the native protein was measured by sucrose-gradient

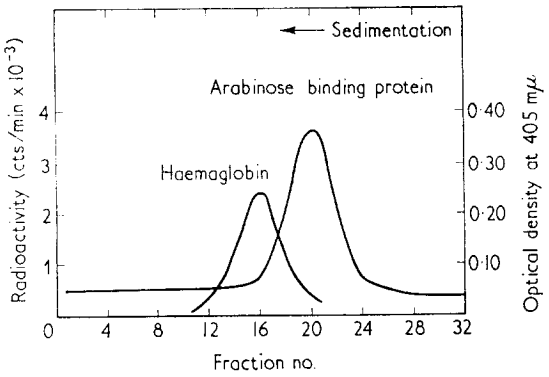

FIG. 3. Sedimentation velocity of arabinose binding protein.
Purified arabinose binding protein at 0·8 mg/ml. was dialyzed 6 hr against 20 ml. buffer A with [^{14}C]arabinose (3·5 mc/m-mole) at $3{\cdot}5\times10^{-7}$ M at 4°C. After dialysis, 0·10 ml. was layered on a 5-ml. linear gradient from 10 to 30% glycerol in a uniform concentration of arabinose at the same concentration and specific activity as was used in dialysis. A 3-mg sample of haemoglobin was sedimented in parallel. Centrifugation was 14 hr at 65,000 rev./min in the Spinco SW65 rotor. After centrifugation, 2-drop fractions were counted in Bray's scintillation fluid or diluted before reading optical density.

sedimentation to determine whether the protein is an oligomer. The binding relationship $Bd = P_o - K(Bd/F)$ is equivalent to: $Bd/F = P_o/(K + F)$; thus when the concentration of free arabinose throughout the gradient is 7×10^{-7} M and that of the binding protein is 10^{-5} M, then the concentration of radioactive arabinose at the position of the protein will be 10 times its concentration elsewhere. Figure 3 shows the results of such a sedimentation and the position of haemoglobin which sedimented in parallel. An S-value of 3·2 s was found for binding protein by comparison to haemoglobin whose S-value was taken to be 4·3 s. This corresponds to a molecular weight of 35,000 to 40,000 for the binding protein by the relationship $M \propto S^{3/2}$ for globular proteins. Together these measurements show the molecule is a single polypeptide chain of molecular weight about 35,000 having one arabinose binding site.

4. Studies on Arabinose Permeation

(a) *Uptake of arabinose by cells*

Permeation measurements are best done in a strain which cannot metabolize arabinose. An arabinose isomerase mutant fulfills this requirement. Figure 4(a) shows the uptake of arabinose in an isomerase mutant, strain 3, both uninduced and induced by growth in the presence of arabinose (see Materials and Methods for further details of the measurements). The quantity of arabinose taken up, plotted as a function of the concentration factor, shows first-order dependence upon arabinose concentration with an apparent K_m of 5×10^{-5} M. However, when the constitutive strain 56 (constitutive enzyme level is the same as fully induced wild type) is used, the quantities of arabinose taken up are not characteristic of a single transport system, as shown in Figure 3(b). The uptake appears to result from the combined effects of two transport systems; one of low capacity but high affinity, dissociation constant $K_m = 3\times10^{-6}$ M, and one of high capacity but lower affinity, $K_m = 5\times10^{-5}$ M. The dissociation constant of binding protein at 37°C is 2×10^{-6} M. The binding protein might therefore be part of the high-affinity permeation system.

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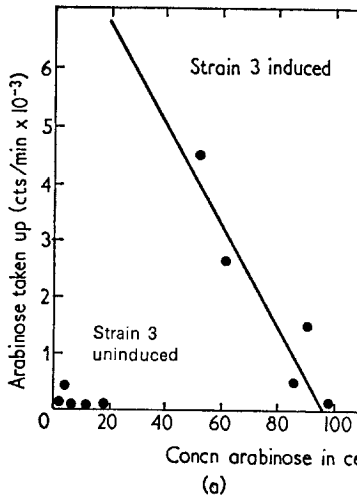

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(b) Arabinose uptake by strain 56 a
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$5{\cdot}2\times10^{-5}$, 7×10^{-5} and 1×10^{-4} M.
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The failure to observe the high-affinity transport system in the wild-type strain induced by growth in arabinose-containing medium results from arabinose retained in the cells. When the constitutive strain is grown in the presence of arabinose at the same concentration used to induce the wild-type strain, only first-order uptake is seen (Fig. 4(b)). Most likely, small amounts of arabinose are retained in the cells grown in the presence of arabinose despite the extensive washing given to the cells before the uptake measurement. This then decreases the specific activity of the radioactive arabinose in the permease measurement, the dilution being the most pronounced at the lowest concentrations of arabinose.

(b) *Isolation of mutants with reduced arabinose uptake but normal arabinose binding protein*

Two similar approaches were tried to isolate mutants unable to concentrate arabinose. One was based on the arabinose sensitivity of strains lacking the ribulose phosphate epimerase (Boyer, Englesberg & Weinberg, 1962) and the other upon the arabinose sensitivity in low-phosphate medium of strains lacking the arabinose isomerase (Englesberg, 1966). The inability to concentrate arabinose intracellularly should relieve the arabinose inhibition particularly in the case of the isomerase mutant where any arabinose leaking into the cell would not be trapped in phosphorylated compounds.

Mutants of the constitutive strain 56 lacking isomerase were selected to be resistant to arabinose inhibition. Of 100 such mutants, approximately half were mutations in

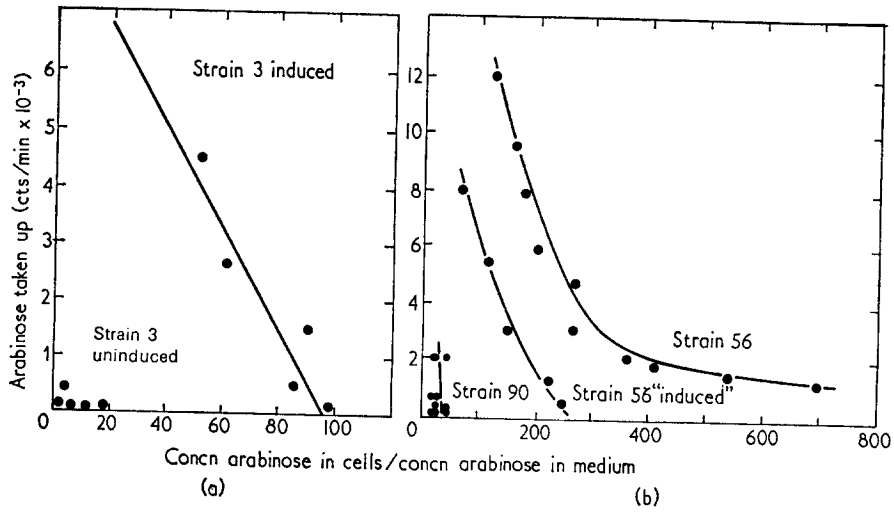

FIG. 4. Arabinose uptake.

(a) Arabinose uptake by strain 3 uninduced and induced. For induction, strain 3 was grown 4 hr in M9 medium containing 0·2% glycerol, 0·2% arabinose and 1% casein hydrolysate. Before the uptake measurement both cultures were filtered and rinsed with medium at room temperature lacking arabinose. After further growth for 10 min, the cells were added to tubes containing radioactive arabinose, 3·5 mc/m-mole, at final concentrations of $2{\cdot}2\times10^{-6}$, $5{\cdot}5\times10^{-6}$, $1{\cdot}4\times10^{-5}$, $3{\cdot}5\times10^{-5}$ and 7×10^{-5} M.

(b) Arabinose uptake by strain 56 and 56 "induced" and strain 90. The same conditions were used here as were used in (a), see also Materials and Methods. For strains 56 and 90 arabinose was used at concentrations of $2{\cdot}1\times10^{-6}$, $3{\cdot}5\times10^{-6}$, $5{\cdot}6\times10^{-6}$, 7×10^{-6}, $1{\cdot}4\times10^{-5}$, $3{\cdot}5\times10^{-5}$, $5{\cdot}2\times10^{-5}$, 7×10^{-5} and 1×10^{-4} M. The dissociation constants for the two parts of strain 56 uptake are 5×10^{-5} and 3×10^{-6} M.

the *C* gene, and the other half were still constitutive, contained normal amounts of the arabinose binding protein, but were not able to concentrate arabinose as well as their parent. One of these, strain 90, was studied in detail. This mutation to arabinose resistance does not co-transduce with threonine or leucine markers. The ability of the strain to concentrate is decreased to approximately 50-fold in contrast to approximately 500 in its parent, as shown in Figure 4(b). Despite the fact that the uptake of arabinose in the strain is more temperature dependent than in the parent, the arabinose binding protein is present in normal quantities and binds arabinose as tightly as that from the parent at 4, 24 and 37°C.

The arabinose resistant mutants of the constitutive strain lacking epimerase 306 were of the same nature as those found from the constitutive strain lacking isomerase. In this case a diploid homozygous for the arabinose region was used to give stronger selection for mutations from outside the threonine–leucine region, since it has been suggested that the permease gene maps elsewhere on the chromosome (Englesberg *et al.*, 1965). Of 50 mutants resistant to arabinose, none showed less ability to concentrate arabinose than those found from the strain lacking isomerase.

(c) *Isolation of a permease-negative strain with reduced levels of arabinose binding protein*

None of the mutants selected for altered permeation was totally defective in arabinose uptake. Therefore a different approach was tried. A blind search for a mutant unable to bind arabinose was made through clones from a mutagenized culture. One candidate from 1200 was found to have one-tenth the amount of arabinose binding protein but the isomerase activity only slightly lower than that of its parent, showing that other arabinose genes were still expressed constitutively. Arabinose uptake measurements of this strain show it concentrates arabinose no more than threefold at 7×10^{-6} M. The partially purified binding protein showed no differences from that purified from strain 56 in the affinity for arabinose at 4, 24 and 37°C. Equilibrium dialysis measurements made on dinitrophenol-treated whole cells as well as on unfractionated extracts showed directly that the number of binding molecules per cell was reduced, thus eliminating the possibility that the binding protein was lost during purification. Despite the presence of arabinose isomerase, and presumably ribulokinase and epimerase, the colonies on tetrazolium arabinose plates were pink or red (red colonies are arabinose-negative, white indicates arabinose-positive) but not as deep a red as strains possessing non-leaky enzymic defects. The strain can grow slowly on minimal arabinose medium. The transport systems for many sugars use several proteins in common (Simoni *et al.*, 1967). Therefore this permease-negative mutant with lowered levels of binding protein could be deficient in such a protein. The strain was tested for its ability to grow on maltose, xylose, mannitol and lactose. Since it was carbohydrate-positive on indicator plates for these sugars, the defect is unlikely to affect a protein common to several transport systems.

5. Discussion and Summary

Regulation of gene expression in the arabinose system reflects positive control. At present we understand neither the mechanism of positive control, nor the reason a cell should possess both positive and negative control systems. We also understand very little about active transport in which extracellular substances are concentrated

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I thank Professors Walter Gilb
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in the cell. Therefore it is of great interest to isolate parts of the arabinose permease or control systems in order to examine their functioning by *in vitro* studies. The most obvious way to isolate either the permease or the product of the arabinose *C* gene, presumed to be a protein, is to look for proteins which bind arabinose. It has been shown that this method will work even for proteins present at low levels, for the *lac* repressor was isolated using as an assay the binding of its inducer iso-propyl-β-D-thiogalactoside (Gilbert & Müller-Hill, 1966). It is likely that arabinose and not a derivative of arabinose interacts with the *C* protein since cells unable to change arabinose to ribulose (*araA* mutants) are still inducible. A search was made for proteins binding arabinose, and indeed one was found. This was shown not to be the *C* protein, most directly by its presence in a strain deleting the *C* gene.

For two reasons it is not possible to identify this protein as the product of the arabinose *E* gene, the permease. First, the permeation system may consist of several proteins of which the binding protein purified here is only one, and the mutants that Englesberg used to define the permease gene may lie in another of the permeation proteins. The second is that the binding protein has not yet been shown to be directly involved in permeation. The same situation exists for the proteins that bind sulfate, leucine and galactose (Pardee, 1968). They too are of molecular weight about 35,000, can be removed from cells by osmotic shock, and bind one molecule of their substrate. As in the case of the arabinose binding protein isolated in this work, these proteins are assumed to be involved in permeation, but no direct proof yet exists.

A mutant was found with lowered levels of the arabinose binding protein. Apparently, with the same lesion the ability of the cells to concentrate arabinose was abolished. It therefore follows that the binding protein, if not itself part of the permease, at least is in the same operon as the permease.

The uptake of arabinose by cells did not follow a Michaelis–Menton concentration dependence. It is not possible to distinguish between two separate permeation systems, each with its own characteristic dissociation constant, or one complicated permeation system.

Many mutants were found with reduced ability to concentrate arabinose, but none had an altered arabinose binding protein. In these mutants, the uptake of arabinose was first order in arabinose concentration. This implies that these mutants have lost one of the permeation systems. Opposing this suggestion of two independent permeation systems is the fact that the lowered binding protein mutant had no permeation ability, suggesting that this mutant is defective in a component essential for any arabinose uptake. Neither the mutations giving defective uptake nor the mutation reducing the level of arabinose binding protein mapped in the threonine–leucine region of the chromosome, nor did they co-transduce with *serA*, a marker linked to the *E* gene in strain B/r (Englesberg *et al.*, 1965).

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Note added in proof: D-Fucose also interacts with the *C* protein, for its presence at 10^{-4} M reduces induction by arabinose at 4×10^{-4} M to 10% of normal in the permease-defective ribulokinase-defective strain 496.

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