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Identification of the AraE transport protein of *Escherichia coli*

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1. Two arabinose-inducible proteins are detected in membrane preparations from strains of *Escherichia coli* containing arabinose–H^+ (or fucose–H^+) transport activity; one protein has an apparent subunit relative molecular mass (M_r) of 36000–37000 and the other has M_r 27000. 2. An *araE* deletion mutant was isolated and characterized; it has lost arabinose–H^+ symport activity and the arabinose-inducible protein of M_r 36000, but not the protein of M_r 27000. 3. An *araE*$^+$ specialized transducing phage was characterized and used to re-introduce the *araE*$^+$ gene into the deletion strain, a procedure that restores both arabinose–H^+ symport activity and the protein of M_r 36000. 4. *N*-Ethylmaleimide inhibits arabinose transport and partially inhibits arabinose–H^+ symport activity. 5. *N*-Ethylmaleimide modifies an arabinose-inducible protein of M_r 36000–38000, and arabinose protects the protein against the reagent. 6. These observations identify an arabinose-transport protein of *Escherichia coli* as the product of the *araE*$^+$ gene. 7. The protein was recognized as a single spot staining with Coomassie Blue after two-dimensional gel electrophoresis.

There are two transport systems for arabinose in *Escherichia coli* (Novotny & Englesberg, 1966; Schleif, 1969; Brown & Hogg, 1972). One is specified by the gene *araE* (Englesberg *et al.*, 1965; Hogg & Englesberg, 1969; Bachmann *et al.*, 1976), and transports arabinose by a sugar–H^+ symport mechanism (Daruwalla, 1979). The other, designated *araF* (Brown & Hogg, 1972), involves a binding protein (Schleif, 1969; Parsons & Hogg, 1974) and is probably energized by a phosphorylated product of glycolysis (Daruwalla, 1979). Expression of the transport genes is highly induced by arabinose through positive interaction with the product of *araC* (Englesberg *et al.*, 1965; Heffernan *et al.*, 1976); *araC* maps at 1 min on the *E. coli* genetic map with *araA*, *araB* and *araD*, the structural genes for the isomerase, kinase and epimerase involved in cytoplasmic metabolism of arabinose (Englesberg *et al.*, 1965). By contrast, *araE* maps near *thyA* at about 61 min (Brown & Hogg, 1972; Daruwalla, 1979; the present paper) and *araF* maps near *his* at about 44 min (Heffernan *et al.*, 1976), a separation which facilitates genetic manipulation of the transport proteins. Presumably these proteins are membrane-bound, although the *araF* lesion has been correlated with the loss of a periplasmic binding protein (Brown & Hogg, 1972; Heffernan *et al.*, 1976).

This paper identifies a protein component of the *araE* arabinose-transport system of *E. coli*. This was made possible by the isolation of an *araE* deletion mutant, and by the construction of an *araE*$^+$ lysogen. The protein product of the *araE* gene has an apparent subunit relative molecular mass (M_r) of 36000–38000, as determined by SDS/polyacrylamide-gel electrophoresis of membrane proteins from the various *E. coli* strains. Detection of the *araE* gene product among the partially resolved membrane proteins was accomplished by dual-isotope labelling of induced and uninduced cultures, or by substrate protection against reaction with *N*-ethylmaleimide. These biochemical techniques have previously aided the identification of the lactose-transport protein of *E. coli* and the dicarboxylate-transport proteins of *Bacillus subtilis* (see, e.g., Carter *et al.*, 1968; Jones & Kennedy, 1969; Fournier & Pardee, 1974; reviewed by Wilson & Smith, 1978), but we have combined them with two-dimensional non-equilibrium pH-gradient gel electrophoresis (O'Farrell *et al.*, 1977) to resolve the *araE* gene product as a single protein detected by staining with Coomassie Blue.

Throughout this paper arabinose signifies the L-isomer and fucose signifies the D-isomer.

Abbreviation used: SDS, sodium dodecyl sulphate.

Methods

Genetical methods

Conjugation and bacteriophage P1-mediated

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transduction were performed as described by Miller (1972). Bacteriophage λ techniques were those of Gottesman & Yarmolinsky (1968), Shimada *et al.* (1972) or Kaiser & Masuda (1970). We are grateful to Drs. M. E. Gottesman, R. W. Hogg, P. Oliver and B. M. Wilkins for supplying strains.

Growth conditions

For arabinose–H^+ symport experiments all strains were grown overnight to about 0.6mg dry mass/ml in 400ml of basal salts medium (Henderson *et al.*, 1977) in a 500ml flask. The following carbon sources were used: strains SB5314 and JM1647, 22.5mM-glycerol; strain JM1637, 22.5mM-sodium succinate (pH7.0). L-Lysine was also added to JM1637 cultures at a concentration of 80μg/ml. Arabinose (1mM) was used as inducer for strain SB5314 and 4mM-arabinose for strains JM1637 and JM1647.

In other experiments the strains were grown overnight in 100ml of basal salts medium in a 250ml flask. The cells were harvested at about 0.6mg dry mass/ml and resuspended in 2ml of basal salts medium. This was used to inoculate two parallel cultures to a concentration equivalent to 0.1mg dry mass/ml, which were grown for two generations with the same carbon source and additions as described above.

Inhibition of arabinose-promoted pH changes by N-ethylmaleimide

The initial rate of arabinose–H^+ symport was measured as described by Henderson *et al.* (1977) and Daruwalla (1979), except that *N*-ethylmaleimide was added to the bacterial suspension at the concentrations shown 10min before the addition of arabinose. The alkalification of the cell suspension on addition of *N*-ethylmaleimide was overcome by titrating the suspension to pH6.6 shortly before the arabinose–H^+ symport measurements were made. Control measurements without the addition of *N*-ethylmaleimide were made with separate portions of cells both during the course of the measurements and at the end, since the rate of symport was observed to decline over the experimental period of 3–4h. Interpolation of these control measurements was used to calculate the percentage inhibition of the initial rate of symport by treatment with *N*-ethylmaleimide.

Equilibration assay of transport activity

These experiments were performed essentially as described by Winkler & Wilson (1966).

A 400ml culture of bacteria was grown overnight and harvested at 0.6mg dry mass/ml. The pellet was resuspended in 400ml of 150mM-KCl/5mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/30mM-NaN_3 (pH6.5), and incubated at 37°C for 1h. The cells were again harvested, and resuspended in the same buffer to 2.5mg dry mass/ml.

The cell suspension was divided into 1ml portions, which were incubated with or without 1mM-*N*-ethylmaleimide for 15min at 25°C. Unlabelled arabinose was then added to a final concentration of 20mM, and allowed to equilibrate at 25°C for a further 30min.

The incubations were terminated by centrifugation for 2min in an Eppendorf microfuge; each was resuspended in 1ml of 150mM-KCl/5mM-Hepes/30mM-NaN_3 (pH6.5).

[1-^{14}C]Arabinose was added (0.5mM final concn., 1.25Ci/mol), and at the subsequent indicated times 0.1ml samples were filtered and washed with approx. 4ml of arabinose-free buffer. The radioactivity retained in the filtered bacteria was measured as described by Henderson *et al.* (1977).

Preparation of membranes containing radioisotope-labelled proteins

The procedure for preparing membranes was adapted from the method of Archer *et al.* (1978).

Two 100ml cultures in 250ml flasks were inoculated to 0.15mg dry mass/ml by using an overnight culture of the appropriate strain (see above). Only one culture contained arabinose as inducer. After growth for one generation, 100μCi of ^{3}H-labelled amino acid mixture (TRK. 440; The Radiochemical Centre, Amersham, Bucks., U.K.) was added to one culture and 50μCi of ^{14}C-labelled amino acid mixture (CFB. 104; The Radiochemical Centre) was added to the other. After growth for a further generation into late exponential phase (to about 0.6mg dry mass/ml), the cultures were harvested.

The bacteria from both the induced and uninduced cultures (about 0.2g dry mass) were mixed by resuspending in 15ml of 0.2M-Tris/HCl (pH8.0) and spheroplasts were prepared as follows (Witholt *et al.*, 1976). The same volume of 0.2M-Tris/HCl (pH8.0)/1M-sucrose/1mM-EDTA was added to the suspension followed by 4mg of lysozyme and 30ml of deionized water after 90 and 135s respectively. The suspension was then stirred for 30min at room temperature, when spheroplast formation was verified by phase-contrast microscopy.

The spheroplasts were sedimented (47000*g*, 20min, 4°C), and lysed by resuspension in 30ml of water with homogenization. After re-centrifugation the supernatant was stored at −20°C, and the precipitate resuspended in 30ml of 1mM-EDTA (pH8.0), and incubated at 37°C for 30min to release extrinsic proteins (Archer *et al.*, 1978). This was centrifuged (47000*g*, 20min, 4°C) and the supernatant discarded. The pellet was placed in a freezing mixture of solid CO_2/96% (v/v) ethanol for

3 min, and subsequently thawed at room temperature. After washing in water, the freeze–thaw procedure was repeated.

The membranes were washed once more in 40 ml of water, and twice in 40 ml of 0.1 M-sodium phosphate (pH 7.2)/1 mM-2-mercaptoethanol. They were finally resuspended in 0.1 M-sodium phosphate (pH 7.2)/1 mM-2-mercaptoethanol at 4–8 mg of protein/ml and stored at −20°C.

Protein concentrations were determined by the method of Lowry *et al.* (1951) using bovine serum albumin (Fraction V, Sigma) as standard.

Solubilization of the cytoplasmic membrane by Sarkosyl (sodium lauryl sarkosinate)

Membranes prepared by the full procedure described above were resuspended at 4 mg of protein/ml in 0.1 M-sodium phosphate (pH 7.2)/1 mM-2-mercaptoethanol/2%-Sarkosyl (NL-97)/1 mM-EDTA. Samples (300 μl) of this suspension were incubated at 37°C for 20 min and the incubation was terminated by centrifugation (100000 **g**, 10 min, 25°C) in a Beckman Airfuge. The supernatant was removed and 100 μl was incubated for 15 min at 37°C with SDS dissolving buffer (final volume 133 μl). The precipitate was similarly dissolved in 100 μl of SDS dissolving buffer. The proteins in each dissolved sample (50 μl) were separated by SDS/polyacrylamide (15%)-gel electrophoresis.

Modified preparation of membranes for two-dimensional electrophoretic analysis and N-ethyl[2,3-^{14}C]-maleimide labelling

Bacteria were grown as described for dual-isotope labelling, except that no radioisotopes were added, and each culture and the subsequent membrane preparations were maintained separately throughout.

The membrane preparation was obtained as described above, with the omission of the EDTA incubation and freeze-thaw stages; after lysis of spheroplasts the suspension was centrifuged (47000 **g**, 20 min, 4°C), and the pellet washed four times in 0.1 M-sodium phosphate (pH 7.2)/1 mM-2-mercaptoethanol. The final pellet was resuspended to a protein concentration of 6–8 mg/ml in 0.1 mM-sodium phosphate (pH 7.2)/1 mM-2-mercaptoethanol, and stored at −195°C.

Treatment of isolated membranes with N-ethyl[2,3-^{14}C]maleimide

Membranes prepared in parallel from induced and uninduced cultures of strain SB5314 by the modified method described in the previous section were allowed to thaw at 25°C, and washed twice in 30 ml of 0.1 M-sodium phosphate (pH 6.5)/1 mM-EDTA to remove 2-mercaptoethanol. They were then resuspended at 4 mg of protein/ml in 0.1 M-sodium phosphate (pH 6.5)/1 mM-EDTA. Portions of each suspension (uninduced or induced) were incubated with 1 mM-*N*-ethyl[2,3-^{14}C]maleimide (0.92 Ci/mol; The Radiochemical Centre) in a volume of 200 μl for 15 min at 25°C, the incubation being stopped by the addition of 0.1 ml of 2-mercaptoethanol.

The membranes were sedimented by centrifugation for 5 min in an Eppendorf microfuge, washed once in 0.5 ml of 0.1 M-sodium phosphate (pH 6.5)/1 mM-EDTA, and finally dissolved in 100 μl of SDS dissolving buffer. After incubation of this solution for 15 min at 37°C, a 50 μl sample was loaded for separation of the proteins by SDS/polyacrylamide (15%)-gel electrophoresis.

Labelling of 'Kaback' vesicles with N-ethyl[2,3-^{14}C]maleimide

Vesicles prepared by the method of Kaback (1971) corresponding to 8 mg of protein were removed from liquid N_2, and thawed at 25°C. They were sedimented and resuspended at 4 mg of protein/ml in 200 μl portions in 0.1 M-potassium phosphate (pH 6.5)/25 mM-$MgSO_4$. Substrate protection was attempted by the addition of 50 mM-arabinose to indicated samples, and energization was achieved by including 25 mM-ascorbate (pH 6.5)/125 μM-phenazine methosulphate, and bubbling the suspension with O_2. After addition of 1 mM-*N*-ethyl[2,3-^{14}C]maleimide (0.9 Ci/mol) the samples were incubated at 25°C for 15 min, when the reaction was terminated with 0.1 ml of 2-mercaptoethanol.

The vesicles were sedimented (Eppendorf microfuge, 5 min, 4°C) and washed twice in 0.1 M-sodium phosphate (pH 6.6), before resuspension in 100 μl of SDS dissolving buffer; 50 μl was loaded for separation of proteins by SDS/polyacrylamide (15%)-gel electrophoresis.

Labelling of 'Kaback' vesicles with N-ethyl[2,3-^{14}C]maleimide after prior protection by arabinose during exposure to unlabelled N-ethylmaleimide

Vesicles were removed from liquid N_2 and thawed at 25°C before washing twice in 0.1 M-potassium phosphate (pH 6.6) (Eppendorf microfuge, 10 min, 4°C). The pellet was resuspended at 4 mg of protein/ml in 250 μl samples in 0.1 M-potassium phoshate (pH 6.6)/20 mM-$MgSO_4$/1 mM-*N*-ethylmaleimide. Arabinose (100 mM) was included in one sample to afford protection from *N*-ethylmaleimide modification. These were incubated at 25°C for 15 min.

The vesicles were centrifuged (Eppendorf microfuge, 10 min, 4°C), and washed three times in 0.1 M-potassium phosphate (pH 6.6) before resuspension in 250 μl of 0.1 M-potassium phosphate (pH 6.6)/20 mM-$MgSO_4$/1 mM-*N*-ethyl[2,3-^{14}C]maleimide (0.9 Ci/mol). The suspensions were incu-

bated at 25°C for 1h, and the reaction was terminated by the addition of 100μl of 2-mercaptoethanol. The vesicles were sedimented (Eppendorf microfuge, 10min, 4°C) and washed twice in 0.1M-sodium phosphate (pH6.6) before solubilization in 200μl of SDS dissolving buffer by incubation at 37°C for 15min. A 50μl sample of this was loaded for SDS/polyacrylamide (15%)-gel electrophoresis.

Electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed in 15%-acrylamide gels by the method of Laemmli (1970) with the modifications of Blattler *et al.* (1972), in a slab-gel system (Studier, 1973). The composition of SDS dissolving buffer (four times the final concentration) was 4% (w/v) sodium dodecyl sulphate, 40% (v/v) glycerol, 0.58M-2-mercaptoethanol, 40mM-Tris/HCl, pH7.2, 0.005% (w/v) Bromophenol Blue. The following markers were used: bovine serum albumin (M_r 68000); ovalbumin (M_r 43000); carbonic anhydrase (M_r 29000); lysozyme (M_r 14400). A solution was made containing each protein at 0.2mg/ml; this was boiled for 1min with SDS dissolving buffer and 30μl was loaded in at least two tracks. Electrophoresis was carried out at 20mA constant current (60–250V) for 5–6h. There was a linear relationship between log M_r and distance migrated.

Two-dimensional electrophoresis was performed as described by O'Farrell *et al.* (1977) by using pH3.5–10 Ampholines (LKB) in the first-dimension tube gels (350V for 6h). They were then equilibrated in 5ml of SDS dissolving buffer (O'Farrell *et al.*, 1977) for 2h before loading for the second dimension. SDS/polyacrylamide slab gels for the second dimension contained 15% acrylamide, and were made and run as described for the one-dimensional system. Two second-dimension gels were run in parallel (40mA constant current, 60–250V) by staggering the bevelled plates, and hence the loading positions, by 0.5cm.

The final pH gradient in the first dimension gels, determined as described by O'Farrell (1975), was essentially linear, ranging from pH4.3 to 8.5.

Processing of gels after electrophoresis

Upon completion of SDS/polyacrylamide-gel electrophoresis, the gel was removed from the apparatus, and placed in fixing and staining solution [0.97g of Coomassie Blue R/l, 44% (v/v) methanol, 9% (v/v) acetic acid] overnight. The gel was transferred to 7% (v/v) methanol/5% (v/v) acetic acid solution for destaining, which was carried out at 37°C on a shaking water-bath (24rev./min) for 12h. The wet gel was photographed, and dried on to Whatman no. 1 filter paper by using a Bio-Rad slab-gel drier.

To measure radioactivity in the dried gel, each track was cut into 2mm or 1mm sections, and these were dissolved in 0.4ml of 100-vol. H_2O_2 in scintillation vials. After incubation at 60°C for 12h, these were counted for radioactivity in 16ml of scintillant [67% (v/v) xylene/33% (v/v) Triton X-114 containing 3g of diphenyloxazole/litre].

Analysis of dual-isotope labelled samples

Each vial was counted for radioactivity in a Packard 3385 liquid-scintillation spectrometer by using five channels: channel 1 detected ^3H optimally and ^{14}C inefficiently; channel 2 detected ^{14}C alone; channel 3 detected ^{14}C optimally and ^3H inefficiently; channels 4 and 5 were used to detect quenching of the external standard by using the manufacturer's preset conditions.

Two sets of six vials, one containing [^3H]leucine, and the other [^{14}C]leucine in the ranges of radioisotope found in the experimental samples, were included to allow the calculation of the ^{14}C and ^3H in channels 1 and 3. To avoid quenching errors, these and six vials without added isotope (to measure background radiation) were made up each containing a slice from the non-radioactive region of the dried gel and incubated with H_2O_2 in parallel with the experimental samples.

The data were processed on the Cambridge IBM 370/165 computer, with a program written in FORTRAN. This calculated the amount of ^3H and ^{14}C present in each vial after correction for background radiation, and expressed these as a percentage of the total of each radioisotope in all the experimental samples. The difference between these percentages was plotted against the apparent M_r of the proteins in the gel slice.

Results

Isolation of an araE deletion mutant

The grown of strains carrying the temperature-sensitive allele of *fda* (which specifies fructose bisphosphate aldolase, EC 4.1.2.13; Böck & Neidhardt, 1966) is inhibited by sugars at 40°C, but the same sugars are growth substrates at 30°C. Mutants selected for their ability to grow on a gluconeogenic carbon source at 40°C in the presence of a sugar have lost the ability either to take up the sugar or to convert it into intracellular sugar phosphate (Jones-Mortimer & Kornberg, 1976; Henderson *et al.*, 1977). The isolation by this technique of arabinose-resistant mutants from an *araE*$^+$ *araF*$^+$ strain yielded a class of mutant that remained able to grow (albeit at a slightly lower rate) at 30°C with arabinose as sole carbon source. The lesion was 45% co-transducible with *thyA* [60.5min on the *E. coli* linkage map (Bachmann *et al.*, 1976)], as shown by the frequent correction of both lesions simultaneously by transduction with bacteriophage P1

Table 1. *E. coli strains used*

Strain	Genotype	Origin or reference
JM173	*lacX74 thyA*	Henderson *et al.* (1977)
JM1448	*fda*^{ts} *galP thyA galK* (λdgal^{+}) *his gnd*$^{\Delta}$ *ilv thr leu ptsM mgl ptsF ptsG mel*	Roberton *et al.* (1980)
JM1456	as JM1448, but *araE lysA galR*$^{\Delta}$	Arabinose resistant on glycerol
JM1501	as JM1456, but λ^{-}	See the text
JM1593	*araE araF tonB trp*$^{\Delta}$	T1 resistant, λ^{-}, from RS1E^{-}F^{-}
JM1629	*araE lysS galR*$^{\Delta}$ *araF thyA his ilv*?	JM1501 × JM1593
JM1630	as JM1501, but *thy*$^{+}$	P1.K10 × JM1501
JM1637	*lacX74 araE lysA galR*$^{\Delta}$	P1.JM1630 × JM173
JM1641	as JM1629, but (λd*araE*$^{+}$ *lysA*$^{+}$)(λcI*857*)	See the text
JM1647	as JM1637, but (λd*araE*$^{+}$ *lysA*$^{+}$)(λcI*857 S7*)	See the text
K10	wild type	Laboratory stock
RS1E^{-}F^{-}	*araE araF*	R. W. Hogg
SB5314	*araA*	E. Englesberg
# 72	*att*λ$^{\Delta}$ (λcI*857*)	Shimada *et al.* (1972)

grown on a wild type strain. Therefore this type of mutation is not in *araA*, *araB* or *araC* which map at 1 min (Bachmann *et al.*, 1976), but could be in *araE*, which maps between *serA* and *argA* (Isaacson & Englesberg, 1964) and is co-transducible with *thyA* (Brown & Hogg, 1972). If so, the second arabinose-transport system coded by *araF* which maps near *his* at 44 min (Heffernan *et al.*, 1976), could not have been functioning under the conditions of the mutant selection. The transduction data also suggested that the affected gene must lie very close to *lysA*, since values from 27% (Bukhari & Taylor, 1971) to 80% (Taylor & Trotter, 1967) have been reported for co-transduction of *thyA* and *lysA*.

To obtain an *araE* deletion mutant we therefore selected for the simultaneous appearance of arabinose-resistance and a requirement for added lysine. Strain JM1448 (Table 1) was plated on glycerol in the presence of arabinose (5 mM) and lysine (80 μg/ml) at 40°C. One colony, strain JM1456, out of about 500 examined, required lysine for growth and would not revert to grow in the absence of lysine. Strain JM1456 was cured of its λ prophage by heteroimmune superinfection (Kaiser & Masuda, 1970) with bacteriophage λ *imm21 b2* to yield strain JM1501. This was then transduced to Thy^{+} with bacteriophage P1 grown on strain K10. Bacteriophage P1 grown on this recombinant (strain JM1630) was used to transduce strain JM173 to Thy^{+}, selecting in the presence of lysine. A lysine-requiring recombinant from this cross, strain JM1637, was shown to be *galR* [constitutive for galactose–H^{+} symport and 2-deoxygalactose uptake; see Henderson & Giddens (1977)], unlike strain JM173 or the strain in which the lesion was originally isolated. That the mutation abolishes the activity of three genes of unrelated function (*araE*, *galR* and *lysA*) indicates that it is a deletion. This strain, JM1637, was used as the arabinose-transport (*araE*)-deficient strain in subsequent experiments. Since this strain had retained the ability to transport and metabolize arabinose (results not shown) it presumably remains *araF*$^{+}$.

Re-introduction of araE^{+} *into strain JM1637 by using specialized transducing phage*

To obtain a strain in which an *araE*$^{+}$ specialized transducing phage could readily be detected, strain JM1501 (Table 1) was mated with an F^{-} phenocopy of strain JM1593 (a λ^{-}, *tonB trp* derivative of strain RS1E^{-}F^{-}) and an *araE lysA thyA araF* recombinant (strain JM1629) obtained. Strain 72, which has λcI*857* integrated between *lysA* and *thyA* (Shimada *et al.*, 1972), was thermally induced and yielded 8 × 10^{6} plaque-forming units (p.f.u.)/ml. This lysate (1 ml), 0.1 ml of a lysate of λcI*857* (2.5 × 10^{10} p.f.u./ml) as helper and 1 ml of a $MgSO_4$-treated (Shimada *et al.*, 1972) suspension of strain JM1629 were mixed and incubated at 37°C for 20 min. Samples were plated on suitably supplemented minimal medium to select for lysine-positive or thymine-positive transductants, with lactate as carbon source at 30°C. Colonies were tested for the unselected phenotypes, and of 80 lysine-positive transductants 14 were arabinose-positive. None of 53 thymine-positive transductants was arabinose-positive, nor had any transductant simultaneously become thymine- and lysine-positive. The gene order must therefore be:

*araE lysA att*λ *thyA*

One of the lysine-positive arabinose-positive transductants, strain JM1641, was shown to behave as a typical λd*araE*$^{+}$ *lysA*$^{+}$, λ dilysogen, though the ratio of effective transducing particles to p.f.u. was low.

Thermal induction of strain JM1641 yielded a lysate which was used (at the lowest multiplicity of infection compatible with obtaining transductants) together with λcI857 S7 as helper to infect strain JM1637, and lysine-positive progeny were selected. One of these (strain JM1647) was shown to be lysogenic both for λd*araE*$^+$ *lysA*$^+$ and for λcI857 S7. This strain was used as the arabinose-transport (*araE*$^+$) positive strain in subsequent experiments.

Confirmation of the sugar-transport phenotypes in E. coli strains

Measurement of the initial rate of H^+ uptake when arabinose is added to an energy-depleted suspension of bacteria is a specific assay for the AraE transport phenotype (Daruwalla, 1979). By this criterion *E. coli* strain SB5314 contained a highly inducible *araE* gene product, but strain JM1637 did not (Table 2). The failure of expression of *araE* in strain JM1637 may be due to impaired accumulation of the inducer as well as the genetic deletion described above. The alternative substrate for the AraE system, fucose, did elicit an H^+ uptake into strain JM1637, presumably because the *galP* gene had become derepressed; both fucose and galactose are substrates for this transport system (Rotman *et al.*, 1968), and consistently galactose also promoted H^+ uptake into strain JM1637 (results not shown).

With energy-depleted cells of glycerol-grown arabinose-induced strain JM1647, the addition of arabinose gave an extensive acidification owing to metabolism, which obscured any H^+ uptake. This problem has been encountered with other strains of *E. coli* (Daruwalla, 1979). However, fucose did promote H^+ uptake, albeit at a rate 10–20% of that observed with strain SB5314 (Table 2). The possibility that fucose was entering on the galactose–H^+ symport system was eliminated by the failure of galactose to promote an alkaline pH change (result not shown), and by the observation that fucose–H^+ symport was inducible by arabinose (Table 2).

2-Deoxy-D-galactose is reported to be a substrate of the galactose–H^+ but not the arabinose–H^+ symport system of *E. coli* (Henderson & Giddens, 1977). Radioisotope-labelled 2-deoxy-D-galactose was taken up by both arabinose-induced and uninduced strain JM1637 to a concentration of 3.7 nmol/mg per 2 min, about four times higher than obtained with arabinose-induced or uninduced strains SB5314 or JM1647. This supports the deduction that the *galP* gene is derepressed in strain JM1637, but not in strain SB5314 or JM1647.

All these observations confirm the genetic constitution of the strains as described in the previous section.

Identification of arabinose-inducible membrane proteins by dual-isotope labelling

^3H- or ^{14}C-labelled amino acids were added to each of two cultures of *E. coli* strain SB5314 growing on glycerol, one containing arabinose as inducer. After approximately two generations of growth, the cultures were mixed and a single membrane preparation was made (see the Methods section). A sample was subjected to SDS/polyacrylamide-gel electrophoresis; the gel was dried, cut into 1 mm slices and the ^3H- and ^{14}C-content of each slice determined (see the Methods section).

The profile of the difference between ^3H-labelled induced and ^{14}C-labelled uninduced preparation is shown in Fig. 1(*a*); to eliminate the possibility of a differential isotope effect the experiment was repeated with ^{14}C-labelled amino acids in the induced culture (Fig. 1*b*). Both profiles show major inducible membrane proteins of apparent M_r 27000

Table 2. *Sugar-promoted alkaline pH changes with different strains of E. coli*

The methods of measuring pH changes are described by Henderson *et al.* (1977) and Daruwalla (1979). Growth conditions are described in the Methods section. Values are means ± S.E.M. on separate batches of cells (numbers in parentheses). A positive H^+-efflux indicates that the added sugar was metabolized.

E. coli strain	Sugar added	Initial rate of H^+-uptake (nmol/min per mg dry mass)	H^+-efflux
SB5314 (arabinose-induced)	Arabinose	4.69 ± 2.57 (5)	–
	Fucose	9.02 ± 4.45 (2)	–
SB5314* (uninduced)	Arabinose	0.03 ± 0.02 (3)	–
	Fucose	0 (3)	–
JM1637 (arabinose-induced)	Arabinose	0 (3)	+
	Fucose	1.17 ± 0.24 (3)	–
JM1647 (arabinose-induced)	Arabinose	0 (4)	+++
	Fucose	1.38 ± 0.37 (4)	–
JM1647 (uninduced)	Fucose	0 (2)	–

* Henderson *et al.* (1975).

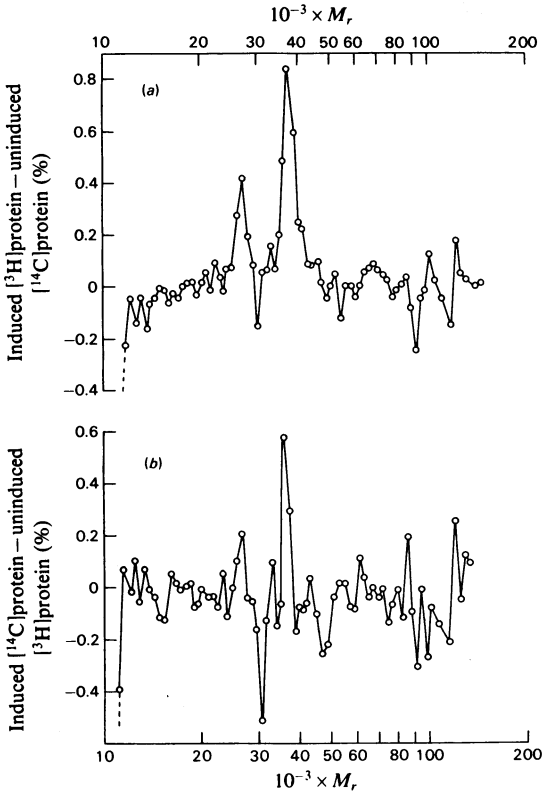

Fig. 1. *Dual-isotope labelling of E. coli strain SB5314*
(*a*) An arabinose-induced and an uninduced culture of *E. coli* SB5314 were grown in parallel on glycerol. After growth for one generation, 100μCi of ^{3}H-labelled amino acid mixture was added to the induced culture and 50μCi of ^{14}C-labelled amino acid mixture to the uninduced culture; growth was concontinued for another generation to late exponential phase (to about 0.6mg dry mass/ml). The bacteria from the two cultures were harvested, pooled, and a single membrane preparation was made (see the Methods section). The membranes (0.1mg of protein) were incubated for 15min at 37°C in SDS dissolving buffer (total volume 133μl) and two 50μl samples were analysed by SDS/polyacrylamide (15%)-gel electrophoresis. After drying, each track was cut into 1mm sections and incubated with 0.4ml of H_2O_2 at 60°C for 12h before liquid-scintillation counting. The difference between the percentages of radioisotope in each section is plotted against the apparent relative molecular mass. In this Figure the results from two separate tracks have been averaged. (*b*) The experiment was performed as in (*a*), but 50μCi of ^{14}C-labelled amino acid mixture was added to the induced culture and 100μCi of ^{3}H-labelled amino acid mixture to the uninduced culture.

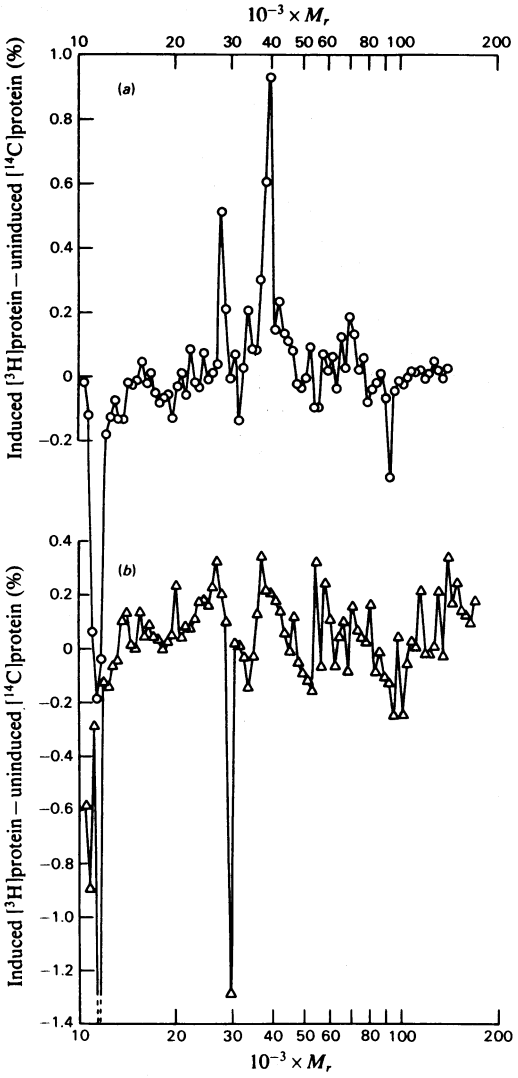

Fig. 2. *Sarkosyl (NL-97) extraction of dual-isotope labelled membrane(s) of E. coli SB5314*
Membranes prepared as described in Fig. 1(*a*) were incubated at 37°C for 20min in 2%-Sarkosyl (NL-97)/1mM-EDTA/0.1M-sodium phosphate, pH 7.2. Insoluble material was separated by centrifugation (100000*g*, 10min, 25°C). (*a*) The supernatant was removed and 100μl was incubated with SDS dissolving buffer at 37°C for 15min before 50μl was loaded for SDS/polyacrylamide (15%)-gel electrophoresis. (*b*) The precipitate was similarly dissolved in 100μl of SDS dissolving buffer and 50μl was loaded for electrophoresis.

and 36000–37000, and a negatively induced or repressed protein of M_r 30000. These peaks occurred reproducibly in three different dual-isotope-labelled preparations from induced/uninduced cultures of *E. coli* SB5314. Other minor peaks were observed, but not consistently in all preparations.

The solubilization of the proteins before electrophoresis was routinely achieved by incubation in SDS dissolving buffer at 37°C for 15min (see the

Methods section). If instead the incubation was at 100°C for 1 min, the same profile was seen, except that the inducible peak at M_r 36000 was distorted and obscured by a major protein (16–20% of the total protein) of M_r 37000–40000. This solubilization and M_r value are characteristic of an outer-membrane protein (Kadner & Bassford, 1978), an identification confirmed by the following procedure. The labelled membrane preparation was treated at 37°C for 20 min with 2% Sarkosyl (NL-97), a detergent which selectively solubilizes cytoplasmic membrane proteins of *E. coli* (Filip *et al.*, 1973). The residue was sedimented, and the extract and the residue were each incubated for 15 min at 37°C in SDS dissolving buffer before SDS/polyacrylamide-gel electrophoresis. The Sarkosyl extract had essentially the same profile (Fig. 2) as that shown in Fig. 1. The residue contained mostly outer-membrane protein, although this did not migrate into the gel unless the sample was boiled (results not shown). The arabinose-inducible peaks migrated at slightly higher M_r values (Fig. 2), perhaps because some Sarkosyl molecules remained associated with the SDS-solubilized proteins. The repressed (negatively induced) peak of M_r 30000 appeared not to be extracted with Sarkosyl (Fig. 2). These results imply that the arabinose-inducible proteins are associated with the inner membrane of *E. coli*.

It is this elimination of the dominant outer-membrane protein that prevented distortion of the protein migration and permitted detection of the arabinose-inducible protein of M_r 36000. The two arabinose-inducible proteins could be the products of several genes *araA* (arabinose isomerase, EC 5.3.1.3, which is inactive in *E. coli* SB5314), *araB* (L-ribulokinase, EC 2.7.1.16), *araD* (L-ribulose 5-phosphate-4-epimerase, EC 5.1.3.4), *araF* or *araE*. The first three are cytoplasmic enzymes of reported M_r values 50000, 55000 and 33000 (Bloch *et al.*, 1980) that are unlikely to be retained in the membrane preparation. Similarly, the product of the *araF* gene is a periplasmic protein of reported M_r 34000 (Quiocho *et al.*, 1977) that is unlikely to persist in a membrane preparation (see also Kaback, 1972); however, there may well be arabinose-inducible membrane-bound protein(s) associated with the binding protein-dependent *araF* transport system. Since there was very little binding protein activity in glycerol-grown *E. coli* SB5314 as assayed by the method of Hogg & Englesberg (1969) (activity was present in succinate-grown cells), expression of the *araF* gene is repressed in glycerol-grown cells, probably through catabolite repression (Magasanik, 1961; Perlman & Pastan, 1971; Kornberg & Jones-Mortimer, 1977). *E. coli* strain SB5314 has the genotype *araA* and lacks a functional arabinose isomerase, so that arabinose is a gratuitous inducer; this results in hyperinducibility of *araE* as assessed by the initial rate of H^+–arabinose symport (Daruwalla, 1979).

From all these considerations it is likely that one of the major inducible membrane proteins in this strain is the *araE* gene product, and the following experiments were designed to confirm this.

Identification of the araE gene product by using the deletion mutant JM1637

The dual-isotope-labelling technique was applied to the *araE* deletion mutant JM1637. An arabinose-inducible protein of M_r 29000 was present and also one of M_r 63000–66000, but no component of M_r 36000 appeared (Fig. 3). This result was obtained when the organism was grown on either glycerol or succinate, when the labels were reversed, and under a variety of solubilization conditions.

The M_r 36000 protein reappeared in strain JM1647, the lysogenic strain in which *araE* is reincorporated into the genetic background of strain JM1637 (Fig. 4). This indicates that the protein of apparent M_r 36000 is the product of the *araE* gene, although possibly *araE* specifies an enzyme for the incorporation of the protein into the membrane.

Identification of the araE gene product by two-dimensional gel electrophoresis

Staining with Coomassie Blue showed about 70 bands on the one-dimensional polyacrylamide gels, but there was not a clear difference between the staining of induced and uninduced membrane preparations of strain SB5314 at positions corre-

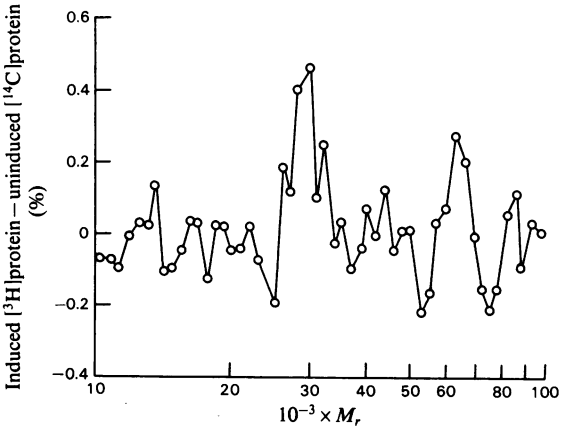

Fig. 3. *Dual-isotope analysis of E. coli JM1637 membrane proteins*

Membranes from *E. coli* JM1637 grown on succinate ± arabinose were analysed by the dual-isotope method as described in Fig. 1(*a*), except that the SDS/polyacrylamide gel was cut into 2 mm sections.

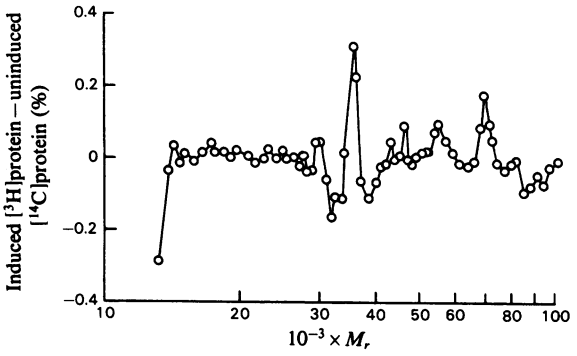

Fig. 4. *Dual-isotope analysis of E. coli JM1647 membrane proteins*

Membranes from *E. coli* JM1647 grown on glycerol ± arabinose were analysed by the dual-isotope method as described in Fig. 1(*a*), except that the SDS/polyacrylamide gel was cut into 2mm sections.

sponding to M_r 27000, 30000 or 36000. This may be because these proteins do not stain with Coomassie Blue, or because they migrate at the same position as other staining proteins. We attempted to resolve single proteins by using the two-dimensional technique of non-equilibrium pH-gradient electrophoresis (O'Farrell *et al.*, 1977). This technique suffers from a lack of reproducibility between gels; sophisticated computer analysis can compensate for this, but none of the methods available has gained universal acceptance (Alexander *et al.*, 1980). Therefore, to provide a control for each arabinose-induced membrane preparation two second-dimension gels were processed in parallel, the second containing an identically prepared membrane preparation from uninduced or deletion strains.

When stained with Coomassie Blue, the pattern of proteins derived from arabinose-induced SB5314 membranes was very similar to the pattern from an uninduced preparation. About 90 corresponding single spots could be resolved (Figs. 5*a* and 5*b*). Calibration of the second dimension gel with molecular-weight standards showed one extra protein in the range of M_r 34000–38000 in two separate induced preparations (Figs. 5*a* and 5*b*). Within experimental error the M_r was 36000. The exact position and relative intensity of the spot varied, but other differences between induced and uninduced preparations were not reproducible.

Although the position of the M_r-36000 protein corresponded to a pH of about 6.4, its isoelectric point cannot be deduced because the technique does not allow equilibration in the first dimension.

The protein absent from two different membrane preparations of uninduced strain SB5314 was also absent from two induced preparations of the deletion mutant JM1637 (Fig. 5*d*). Since arabinose-induced strain JM1637 had to be grown on succinate with lysine as an auxotrophic requirement, strain JM1647 grown under identical conditions was used for the control membrane preparation; once again the protein of M_r 36000 was present only in the JM1647 preparation (Fig. 5*c*). To confirm the inducibility of the proposed *araE* gene product in strain JM1647, three separate parallel preparations were made of arabinose-induced and uninduced JM1647 grown on glycerol, and these were analysed by the two-dimensional technique. In each case a spot was seen in the induced preparation that was absent from the uninduced preparation at the predicted position (Figs. 5*e* and 5*f*). However, the intensity of the induced spot in Fig. 5(*e*) was weaker than that shown in Fig. 5(*c*); this may be due to the use of different growth substrates.

The inducibility of the M_r-36000 protein by arabinose, its disappearance in the *araE* deletion mutant JM1637, and reappearance when *araE*$^+$ was re-introduced on λ phage in strain JM1647, identify this protein as the *araE* gene product. Potentially the isoelectric focusing followed by gel filtration provide a basis for its purification.

It has been reported that the arabinose-binding protein of *E. coli*, the *araF* gene product, has an apparent M_r of 38000 (Parsons & Hogg, 1974), although more precise methods gave M_r 33100 (Hogg & Hermondson, 1977). Since the M_r of the protein that we have identified as the *araE* gene product is in this range, we have used the two-dimensional separation technique to determine whether the proteins are different.

E. coli strain SB5314 was grown on succinate with arabinose as inducer. The binding protein was isolated and assayed exactly as described by Hogg & Englesberg (1969). A sample (15μg of protein) was then solubilized and processed in the two-dimensional system as described for the membrane preparation. About eight spots were revealed by staining with Coomassie Blue. The major one migrated to a position corresponding to M_r 33000 within experimental error, but its location was distinct from that observed for the *araE* gene product, presumably because the isoelectric points of the proteins are different.

This observation, together with the absence of binding-protein activity from glycerol-grown arabinose-induced strain SB5314, in which AraE activity was high (Table 2), confirms that the *araE* and *araF* gene products are different.

Inhibition of arabinose-promoted pH changes by N-ethylmaleimide

To assess the inhibitory effect of *N*-ethylmaleimide on arabinose–H$^+$ symport activity, the initial

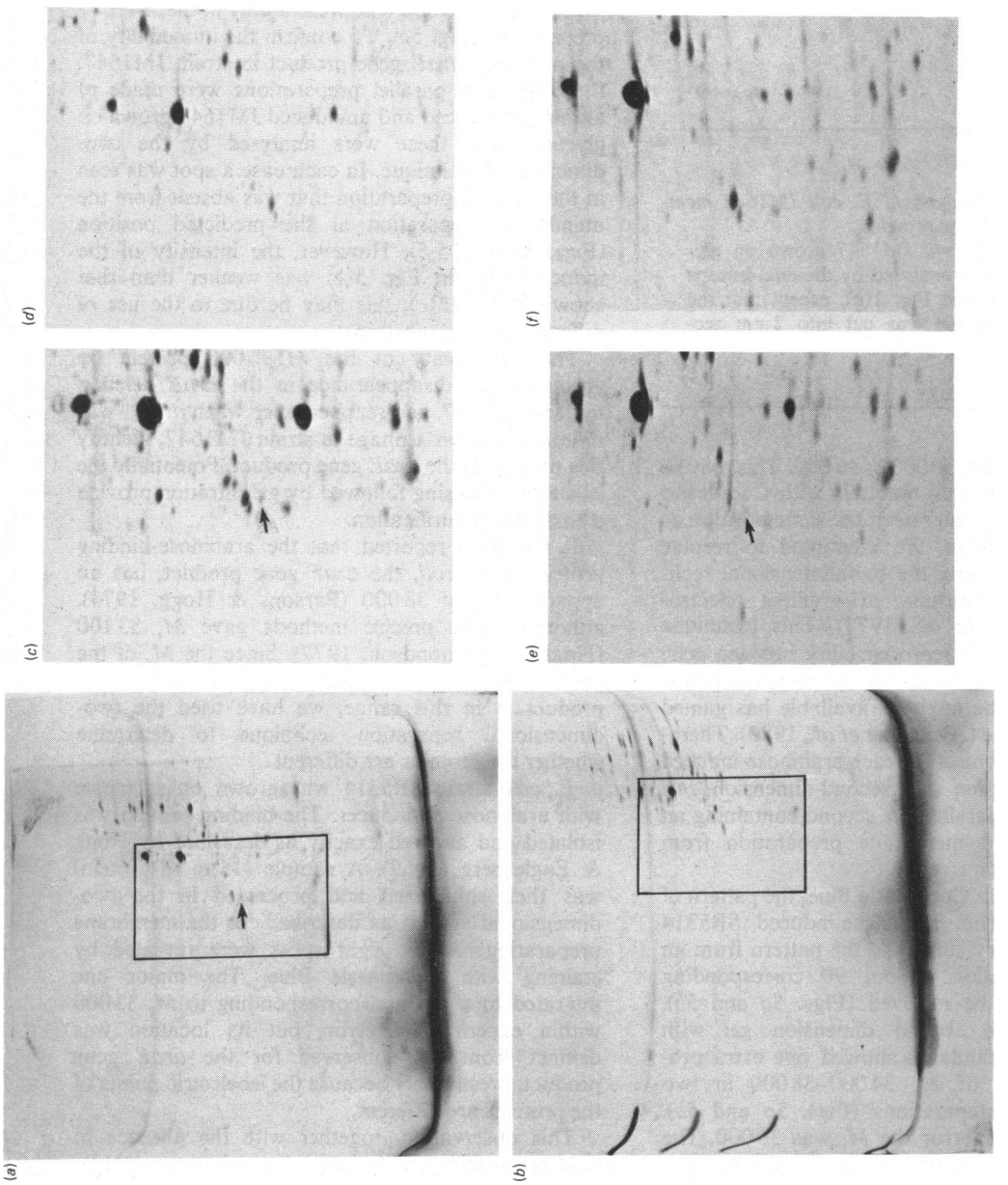

Fig. 5. *Two-dimensional electrophoresis of E. coli membrane proteins*

Two parallel cultures of *E. coli* were grown to late exponential phase (about 0.6 mg dry mass/ml). Each was harvested and two modified membrane preparations were made without pooling the bacteria (see the Methods section). Each membrane preparation (0.4 mg of protein) was analysed by two-dimensional electrophoresis, by using the non-equilibrium pH-gradient isoelectric focusing method of O'Farrell *et al.* (1977) in the first dimension and SDS/polyacrylamide (15%)-gel electrophoresis in the second. Proteins were stained with Coomassie Blue. (*a*) Strain SB5314, arabinose-induced; (*b*) strain SB5314, uninduced; (*c*) strain JM1647, arabinose-induced; (*d*) strain JM1637, arabinose-induced; (*e*) strain JM1647, arabinose-induced; (*f*) strain JM1647, uninduced. Glycerol was the growth substrate in (*a*), (*b*), (*e*) and (*f*); succinate was the growth substrate in (*c*) and (*d*).

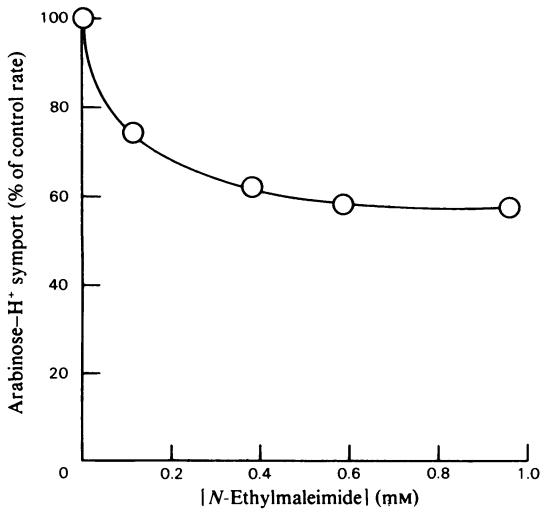

Fig. 6. *Inhibition of the initial rate of arabinose-H^+ symport by N-ethylmaleimide*

N-Ethylmaleimide was added at the concentrations shown to an anaerobic suspension of *E. coli* SB5314 (about 4mg dry mass/ml). Alkalification by *N*-ethylmaleimide was titrated back to pH6.6 with dilute HCl. After 10min incubation at 25°C, arabinose (7.5μmol) was added and the initial rate of sugar-promoted H^+ uptake measured (see the Methods section).

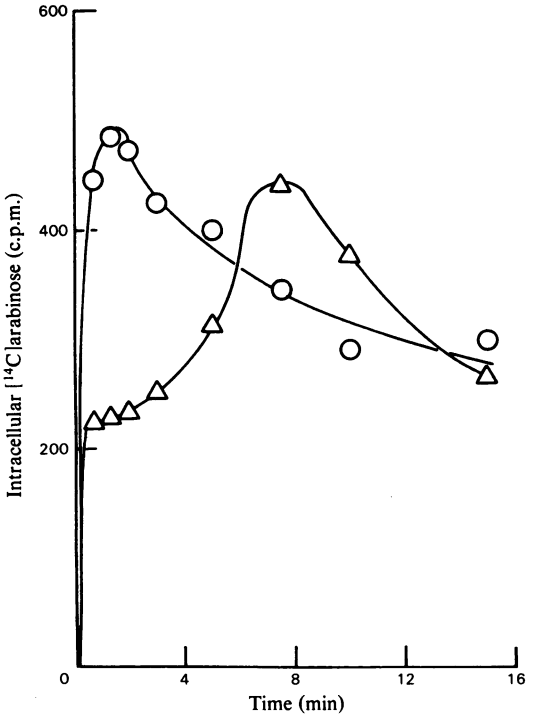

Fig. 7. *Measurement of the inhibition of arabinose counterflow by N-ethylmaleimide*

A de-energised suspension of *E. coli* SB5314 was incubated (△) with and (○) without 1mM-*N*-ethylmaleimide for 15min at 25°C. Unlabelled arabinose (20mM) was added, and the incubation continued for 30min. Each was harvested and resuspended in 150mM-KCl/5mM-Hepes/30mM-NaN_3, pH6.5, at 2.5mg dry mass/ml. [1-^{14}C]-Arabinose was added (0.5mM and 1.25Ci/mol) and at the indicated times 0.1ml samples were filtered and washed with approx. 4ml of arabinose-free buffer.

rates of arabinose-promoted alkaline pH changes were measured after incubating induced cells of strain SB5314 with various concentrations of *N*-ethylmaleimide. The disadvantage of this approach is that the intrepretation may be ambiguous; inhibition by *N*-ethylmaleimide may be caused by specific inactivation of the arabinose-transport proteins, or by unspecific damage to the membrane that increases proton permeability. The additions of *N*-ethylmaleimide did produce a slight alkalification of the suspension (not greater than 0.3pH unit), which was titrated back to pH6.6 before addition of arabinose. In fact, 1mM-*N*-ethylmaleimide inhibited the arabinose-promoted H^+ uptake by about 40% after 10min incubation at 25°C (Fig. 6). Greater inhibition was not observed when the incubation time was 15 or 20min.

Counterflow technique to verify N-ethylmaleimide inhibition of arabinose transport

The counterflow or equilibration method (Winkler & Wilson, 1966) was used to verify the inhibition of arabinose transport by *N*-ethylmaleimide, because it can distinguish between specific inhibition of a transport system and unspecific membrane damage. The technique involves loading de-energized induced *E. coli* SB5314 with unlabelled 20mM-arabinose, and examining the uptake of labelled sugar when resuspended in fresh buffer containing 0.5mM-[1-^{14}C]arabinose. The equilibration of internal and external sugar is characterized by an 'overshoot' of label accumulation, which is dependent on both the counterflow between these pools and leakage owing to the high intracellular sugar concentration (Winkler & Wilson, 1966). The uptake of [1-^{14}C]arabinose by *N*-ethylmaleimide-treated bacteria proceeded much more slowly than in the untreated control (Fig. 7). To maintain comparability with other experiments the measurements were performed at 25°C, although at this elevated temperature it is difficult to resolve the initial rate of appearance of intracellular radioisotope; conse-

quently there is an immediate appearance of internal radioisotope which is unaffected by previous incubation with unlabelled sugar (Winkler & Wilson, 1966).

The retarded influx of external radiolabelled sugar demonstrates inhibition of arabinose transort protein(s) by 1 mM-*N*-ethylmaleimide, corroborating the results obtained by measuring the initial rates of arabinose–H^+ symport. The appearance of a slow reversible influx under conditions of carrier inhibition (Fig. 7) demonstrates that 1 mM-*N*-ethylmaleimide does not significantly increase the permeability of the cytoplasmic membrane with respect to arabinose; such permeation would not occur to concentrations above the equilibrium value.

Labelling of membrane proteins with N-ethyl[2,3-^{14}C]maleimide

The inhibition of arabinose transport by *N*-ethylmaleimide was exploited to identify the *araE* gene product by using the radiolabelled reagent.

Parallel preparations of SB5314 membranes derived from induced and uninduced cultures were treated for 15 min with 1 mM-*N*-ethyl[2,3-^{14}C]maleimide as described in the Methods section. Each was subsequently analysed by SDS/polyacrylamide-gel electrophoresis, which showed a complex profile of *N*-ethyl[2,3-^{14}C]maleimide incorporation (Fig. 8). The profiles from the induced and uninduced preparations corresponded closely, except for an excess of induced material of M_r 36000, 42000 and 68000. Of these the M_r-36000 peak showed the highest *N*-ethyl[2,3-^{14}C]maleimide incorporation. Moreover, the appearance of the single point corresponding to a protein of M_r 36000 was confirmed with a separate membrane preparation. There did not appear to be a significant peak corresponding to M_r 27000. When 100 mM-arabinose was included during incubation of the membranes with the *N*-ethyl[2,3-^{14}C]maleimide, no significant decrease in the induced peaks was observed. This failure of arabinose to protect any of the induced proteins against modification prevented precise identification of the transport protein(s).

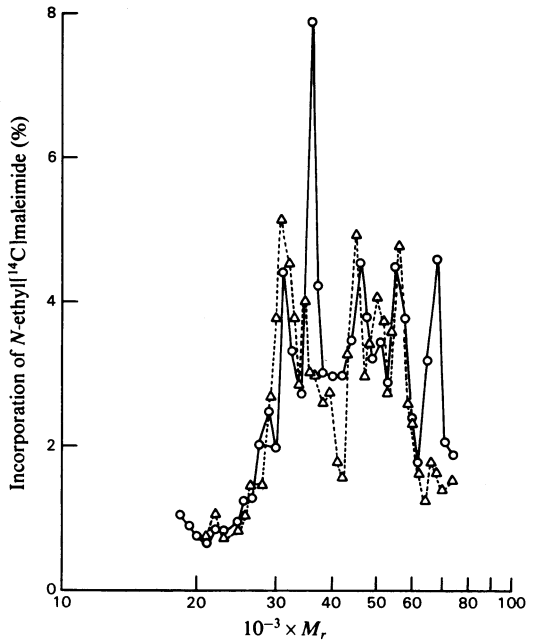

Fig. 8. *Labelling of E. coli SB5314 membranes with N-ethyl[2,3-^{14}C]maleimide*

Membranes were separately prepared by the modified method from induced (○) or uninduced (△) cultures of *E. coli* SB5314 (see the Methods section). These were washed and resuspended (4 mg of protein/ml) in 0.1 M-sodium phosphate/1 mM-EDTA. This was incubated for 15 min at 25°C with 1 mM-*N*-ethyl[2,3,-^{14}C]maleimide (0.92 Ci/mol). The membranes were washed once and dissolved at 37°C for 15 min in 100 μl of SDS dissolving buffer, of which 50 μl was analysed by SDS/polyacrylamide (15%)-gel electrophoresis. The dried gel was cut into 2 mm sections and radioactivity in each was measured and expressed as a percentage of the total radioactivity in all sections after correction for background radiation.

Arabinose protection against N-ethyl[2,3-^{14}C]maleimide modification of the proteins in Kaback vesicles

Kaback vesicles were used to attempt substrate protection of *N*-ethyl[2,3-^{14}C]maleimide modification of the AraE transport system. Vesicles have the advantages that their arabinose-transport activity can be checked before use, and the energized uptake of arabinose should only proceed via the AraE system, since the binding proteins are lost during the osmotic shock stage of preparation (Kaback, 1972). Membrane-bound components of the AraF system might be present, but the absence of the binding protein from glycerol-grown strain SB5314 (see above) argues against this.

Arabinose protected proteins of M_r 37000–38000 against labelling with *N*-ethyl[2,3-^{14}C]maleimide in energized Kaback vesicles (Fig. 9). Protection was much less apparent in unenergized vesicles. This may be due to a decreased K_m of the AraE carrier under these conditions, as described for the Lacy system (Wright & Overath, 1980). No significant protection was seen at M_r 42000 or 69000, the *N*-ethylmaleimide-labelled inducible proteins in isolated membranes. Also, no significant labelling or protection was seen at M_r 27000 (Fig. 9), the arabinose-inducible protein detected with dual-isotope labelling (Fig. 1).

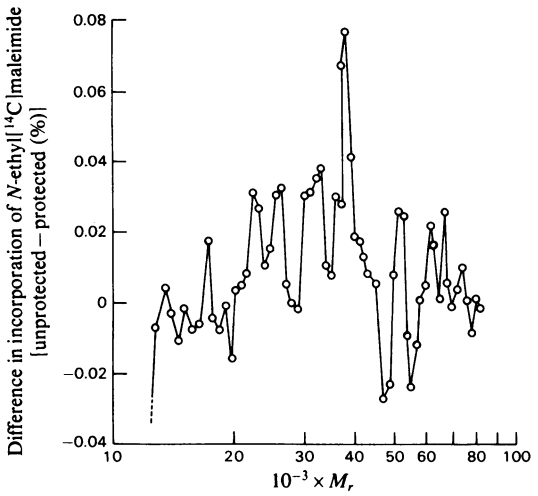

Fig. 9. *Arabinose protection of N-ethyl[2,3-^{14}C]maleimide labelling in energized 'Kaback' vesicles*

'Kaback' vesicles were incubated (4mg of protein/ml) in 0.2ml portions of 0.1M-potassium phosphate (pH6.5)/25mM-$MgSO_4$/1mM-*N*-ethyl[2,3-^{14}C]maleimide (0.9Ci/mol). In one portion 50mM-arabinose was added before the *N*-ethylmaleimide. Energization was achieved by including 25mM-ascorbate (pH6.5) followed by 125μM-phenazine methosulphate and bubbling the suspension with oxygen. Each portion of labelled vesicles was washed twice in 0.1M-sodium phosphate (pH6.6), analysed by electrophoresis and the radioactivity expressed as a percentage as described in Fig. 8. The difference in percentage incorporation between unprotected and protected vesicles is plotted against apparent relative molecular mass.

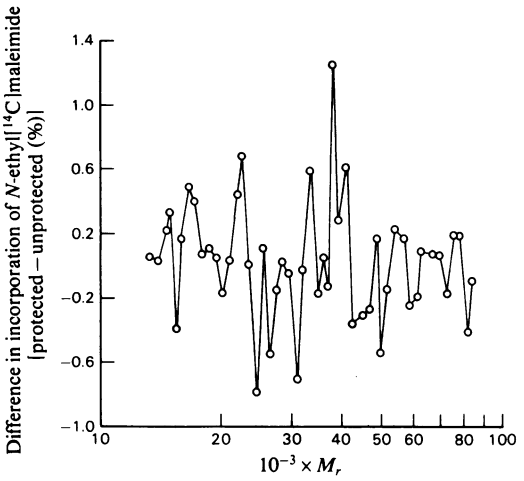

Fig. 10. *Arabinose protection against unlabelled N-ethylmaleimide modification of 'Kaback' vesicles before N-ethyl[2,3-^{14}C]maleimide labelling*

'Kaback' vesicles (4mg of protein/ml) were incubated in 200μl portions in 0.1M-sodium phosphate (pH6.6)/20mM-$MgSO_4$/1mM-*N*-ethylmaleimide at 25°C for 15min, with or without 100mM-arabinose. The incubation was stopped by the addition of 0.1ml of 2-mercaptoethanol. The vesicles were washed three times in 0.1M-sodium phosphate (pH6.6), and labelled with *N*-ethyl[2,3-^{14}C]maleimide as described in the legend to Fig. 9, except that no arabinose was added, and the vesicles were unenergized.

Labelling of Kaback vesicles with N-ethyl[2,3-^{14}C]-maleimide after prior protection by arabinose during exposure to N-ethylmaleimide

Since arabinose protected a protein of M_r 37000 against reaction with *N*-ethyl[2,3-^{14}C]maleimide, more specific labelling of this protein might be achieved by a procedure similar to that adopted by Jones & Kennedy (1969) to label the lactose 'M' protein. The vesicles were preincubated with unlabelled *N*-ethylmaleimide and arabinose; after their removal by stringent washing, *N*-ethyl[2,3-^{14}C]-maleimide was added. Even without energization, this produced a simpler profile of incorporation, with a major peak corresponding to a protein of M_r 38000 (Fig. 10), which was absent from the control preincubated in the absence of arabinose (Fig. 10). The minor peaks corresponded to proteins of M_r 17000, 22500, 33500 and 41000.

When fucose was used as protecting agent instead of arabinose, a more complex profile resulted. Again there was a major peak of M_r 36500, but there were other significant peaks of M_r 26000, 33000, 44000 and 52000. These require further investigation.

Discussion

We have concluded that the arabinose–H^+ transport protein is identifiable by its M_r between 36000 and 38000. The validity of this conclusion depends on it being the only protein that fits all of the very different experimental criteria: arabinose-inducible, present or absent in genetically modified strains, and protected by arabinose against covalent modification by *N*-ethylmaleimide. Its resolution on one-dimensional SDS/polyacrylamide gels was critically dependent on elimination of a predominant (outer-membrane) protein of M_r 37000–40000.

The reproducibility of our molecular mass determinations was about $\pm$1000 in the region between 30000 and 40000, which accounts for the range of 36000–38000 determined for the *araE* gene product by using different labelling techniques. The slightly higher value obtained when Sarkosyl was present is discounted because there may be some retention of the detergent, which might retard migration.

The apparent molecular mass of the *lacy* gene product ('*lac* permease') has been reported to be 29000–33000 (reviewed by Wilson & Smith, 1978), as determined by gel filtration or SDS/polyacrylamide-gel electrophoresis of the detergent-solubilized protein. However, the recent sequencing of the *lacy*-gene DNA predicts an M_r of 46504 (Büchel *et al.*, 1980). This discrepancy could be explained by modification of the protein before insertion in the membrane, or by anomalous interaction of the highly hydrophobic protein with detergents (Ehring *et al.*, 1980). Consequently, although our estimate of the subunit M_r of the *araE* gene product in the region 36000–38000 is entirely reproducible, it may not be the true value.

Other protein bands appeared in some of the preparations, but not all. A minor band of M_r 68000–71000 (Figs. 1*a*, 2, 4 and 8) may represent a dimer of the *araE* gene product, although there was no evidence of the extensive aggregation reported for the *lacy* gene product (Teather *et al.*, 1978). Other bands of potential interest occurred at the apparent M_r values 27000 (Figs. 1*a*, 1*b* and 2*a*) and 41000–42000 (Figs. 8 and 10). Among other possibilities these may be products of the *araF* gene(s), and further work is required to identify their function. Of course, there may be other arabinose-transport proteins that our separation techniques have failed to expose. The dual-isotope labelling followed by one-dimensional electrophoresis would not reliably detect a protein comprising less than about 0.5% of the total protein in a preparation, and the two-dimensional technique was limited by the sensitivity of the Coomassie Blue staining. In the latter case, detection could be improved by using radioisotope-labelled proteins detected by fluorography (Bonner & Laskey, 1974; Laskey & Mills, 1975), or a recently described silver stain (Merril *et al.*, 1979).

The inhibition by *N*-ethylmaleimide was relatively weak (Fig. 7). This was nevertheless chosen as a suitable labelling reagent because the reaction product is not cleaved by 2-mercaptoethanol, and because it is available commercially in a radioisotope-labelled form. Protection by arabinose against *N*-ethylmaleimide could be demonstrated in Kaback vesicles, but not in osmotically lysed membrane preparations. The lysis procedure may denature the binding site, preventing substrate protection. Denaturation of the protein during this preparation and concomitant exposure of cysteine residues is evidenced by the extent of *N*-ethylmaleimide incorporation (Fig. 8), which is higher than in vesicles.

No precautions were taken against the activity of non-specific proteinases. The reproducibility of the protein profiles on both one- and two-dimensional electrophoresis, and when different preparations were made from one organism, argued against unspecific hydrolysis of the proteins during preparation or solubilization. However, it is quite possible that specific proteinase(s) may modify the protein after synthesis; this may occur as part of the process of insertion into the membrane. If so, the *araE* gene would code for a protein of higher M_r than the eventual membrane-bound product.

The results described in this work identify the *araE* transport protein. They provide the basis for amplification of the gene and large-scale purification of the gene product in order to determine its structure and the molecular mechanism of the transport process.

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References

Alexander, A., Cullen, B., Emigholtz, K., Norgard, M. V. & Monahan, J. J. (1980) *Anal. Biochem.* **103**, 176–183

Archer, A. B., Rodwell, A. W. & Rodwell, E. S. (1978) *Biochim. Biophys. Acta* **513**, 268–283

Bachmann, B. J., Low, K. B. & Taylor, A. L. (1976) *Bacteriol. Rev.* **40**, 116–167

Blattler, D. P., Garner, F., Van Slyke, K. & Bradley, A. (1972) *J. Chromatogr.* **64**, 147–155

Bloch, P. L., Phillips, T. A. & Neidhardt, F. C. (1980) *J. Bacteriol.* **141**, 1409–1420

Böck, A. & Neidhardt, F. C. (1966) *J. Bacteriol.* **92**, 464–469

Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88

Brown, C. E. & Hogg, R. W. (1972) *J. Bacteriol.* **111**, 606–613

Büchel, D. E., Gronenborn, B. & Müller-Hill, B. (1980) *Nature (London)* **283**, 541–545

Bukhari, A. I. & Taylor, A. L. (1971) *J. Bacteriol.* **105**, 988–998

Carter, J. R., Fox, C. F. & Kennedy, E. P. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **60**, 725–732

Daruwalla, K. R. (1979) Ph.D. Thesis, University of Cambridge

Ehring, R., Beyreuther, K., Wright, J. K. & Overath, P. (1980) *Nature (London)* **283**, 537–540

Englesberg, E., Irr, J., Power, J. & Lee, N. (1965) *J. Bacteriol.* **90**, 946–957

Filip, C., Fletcher, G., Wulff, J. L. & Earhart, C. F. (1973) *J. Bacteriol.* **115**, 717–722

Fournier, R. E. & Pardee, A. B. (1974) *J. Biol. Chem.* **249**, 5948–5954

Gottesman, M. E. & Yarmolinsky, M. B. (1968) *J. Mol. Biol.* **31**, 487–505

Heffernan, L., Bass, R. & Englesberg, E. (1976) *J. Bacteriol.* **126**, 1119–1131

Henderson, P. J. F., Dilks, S. N. & Giddens, R. A. (1975) *Proc. FEBS Meet. 10th*, 43–53
Henderson, P. J. F. & Giddens, R. A. (1977) *Biochem. J.* **168**, 15–22
Henderson, P. J. F., Giddens, R. A. & Jones-Mortimer, M. C. (1977) *Biochem. J.* **162**, 309–320
Hogg, R. W. & Engelsberg, E. (1969) *J. Bacteriol.* **100**, 423–432
Hogg, R. W. & Hermodson, M. (1977) *J. Biol. Chem.* **252**, 5135–5141
Isaacson, D. & Englesberg, E. (1964) *Bacteriol. Proc.* 113–114
Jones, T. H. D. & Kennedy, E. P. (1969) *J. Biol. Chem.* **244**, 5981–5987
Jones-Mortimer, M. C. & Kornberg, H. L. (1976) *Proc. R. Soc. London Ser. B.* **193**, 313–315
Kaback, H. R. (1971) *Methods Enzymol.* **22**, 99–120
Kaback, H. R. (1972) *Biochim. Biophys. Acta* **265**, 367–416
Kadner, R. J. & Bassford, P. J. (1978) in *Bacterial Transport* (Rosen, B. P., ed.), pp. 413–462, Marcel Dekker, New York and Basel
Kaiser, A. D. & Masuda, T. (1970) *Virology* **40**, 522–529
Kornberg, H. L. & Jones-Mortimer, M. C. (1977) in *Microbial Energetics* (Haddock, B. A. & Hamilton, W. A., eds.), pp. 217–240, Cambridge University Press, Cambridge
Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56**, 335–341
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Magasanik, B. (1961) *Cold Spring Harbor Symp. Quant. Biol.* **26**, 249–256
Merril, C. R., Switzer, R. C. & Van Keuven, M. L. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4335–4339
Miller, J. H. (1972) *Experiments in Molecular Genetics* Cold Spring Harbor Laboratory, Cold Spring Harbor
Novotny, C. P. & Englesberg, E. (1966) *Biochim. Biophys. Acta* **117**, 217–230
O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021
O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. (1977) *Cell* **12**, 1133–1142
Parsons, R. G. & Hogg, R. W. (1974) *J. Biol. Chem.* **249**, 3602–3607
Perlman, R. L. & Pastan, I. (1971) *Curr. Top. Cell. Regul.* **3**, 117–134
Quiocho, F. A., Gilliland, G. L. & Phillips, G. N. (1977) *J. Biol. Chem.* **252**, 5142–5149
Roberton, A. M., Sullivan, P. A., Jones-Mortimer, M. C. & Kornberg, H. L. (1980) *J. Gen. Microbiol.* **117**, 377–382
Rotman, B., Ganesan, A. K. & Guzman, R. (1968) *J. Mol. Biol.* **36**, 247–260
Schlief, R. (1969) *J. Mol. Biol.* **46**, 185–196
Shimada, K., Weissberg, R. A. & Gottesman, M. E. (1972) *J. Mol. Biol.* **63**, 483–503
Studier, F. W. (1973) *J. Mol. Biol.* **79**, 237–248
Taylor, A. L. & Trotter, C. D. (1967) *Bacteriol. Rev.* **31**, 332–353
Teather, R. M., Müller-Hill, B., Abrutsch, U., Aichele, G. & Overath, P. (1978) *Molec. Gen. Genet.* **159**, 239–248
Wilson, D. B. & Smith, J. B. (1978) in *Bacterial Transport* (Rosen, B. P., ed.), pp. 495–557, Marcel Dekker, New York and Basel
Winkler, H. H. & Wilson, T. H. (1966) *J. Biol. Chem.* **241**, 2200–2211
Witholt, B., Boekhout, M., Brock, M., Kingma, J., Van Heerikhuizen, H. & De Leij, L. (1976) *Anal. Biochem.* **74**, 160–170
Wright, J. K. & Overath, P. (1980) *Biochem. Soc. Trans.* **8**, 279–281