The Metabolism of D-Arabinose by *Salmonella typhimurium*

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INTRODUCTION

Little is known about the use of pentoses and pentitols by *Salmonella typhimurium* apart from a report by Gutnick *et al.* (1969) with *S. typhimurium* LT-2. Old (1977) showed that only D-ribose, L-arabinose and L-fucose were used as sole carbon sources by wild-type strains representative of the primary biotypes of Duguid *et al.* (1975). Although D-arabinose was not used as sole carbon source by wild-type strains, D-Ara⁺ mutants were isolated from all strains incubated for 2 to 3 days in peptone water containing D-arabinose (Old, 1977). This work compares the mechanism of D-arabinose utilization by D-Ara⁺ *S. typhimurium* strains with those previously established for other enterobacteria.

METHODS

Bacteria. *Salmonella typhimurium* strains were selected from the series of Duguid *et al.* (1975). Strains s844 (biotype 1a), s1836 (biotype 17a) and s2764 (biotype 25e) used L-fucose as sole carbon source and fermented D-arabinose in peptone water cultures (Old, 1977). Strain s2626 (biotype 17bf) did not utilize L-fucose as sole carbon source and did not ferment D-arabinose.

Media. Peptone water was 1.5% (w/v) Bacto-peptone (Difco) containing, when required, 0.5% (w/v) D-arabinose. The two mineral salts media used were the medium of Le Blanc & Mortlock (1971a) and the basal medium of Davis & Mingioli (1950), subsequently referred to as MMA and MMB respectively. Casein hydrolysate medium (CH) was MMA supplemented at 5 g l⁻¹ with casein hydrolysate (Nutritional Biochemicals, Cleveland, Ohio, U.S.A.). Carbon sources were added to all media at 5 g l⁻¹.

Isolation of mutants. D-Ara⁺ mutants were isolated from s844, s1836 and s2764 by culture of the wild-type strains in MMB plus D-arabinose in aerated, shaken cultures at 37 °C for 48 h. D-Ara⁺ mutants, s844.da1, s1836.da1 and s2764.da1, were of the same full biotype as their parent strains. Mutants s2626.lf1, s2626.lf2 and s2626.lf6, utilizing L-fucose as sole carbon source, were isolated from s2626 after prolonged culture in peptone water plus L-fucose.

Culture conditions. Bacteria were assayed for D-arabinose enzyme activities after growth under three cultural conditions: (i) aerated, shaken cultures grown at 37 °C to late-exponential phase in 20 ml CH with or without L-fucose or D-arabinose; (ii) aerobic, static cultures grown in 120 ml peptone water plus D-arabinose (in 5 ml portions) for 24 h at 37 °C; (iii) aerated, shaken cultures grown in 25 ml MMA plus D-arabinose for 24 h at 37 °C.

Growth was followed in side-arm flask cultures on a Klett-Summerson photocolormeter (Klett Manufacturing Co., New York, U.S.A.) with a red (660 nm) filter.

Bacteria were harvested by centrifuging at 12000 g for 10 min at 4 °C, washed in 10 ml distilled water and suspended in 10 mM-Tris/HCl (pH 7.5) buffer containing 1 mM-mercaptoethanol and 0.1 mM-MgCl₂.

Preparation of extracts. Bacteria were disrupted at 4 °C by two 15 s pulses in a Biosonik sonicator with an interval of 20 s and then centrifuged at 27000 g for 15 min.

Enzyme activities. L-Fucose isomerase (D-arabinose isomerase; EC 5.3.1.3) activity was determined by using purified ribitol dehydrogenase (EC 1.1.1.56) from *Klebsiella pneumoniae* strain w-70 to catalyse the reduction of L-fuculose (D-ribulose), as described by Oliver & Mortlock (1971b). L-Fuculokinase

(D-ribulokinase; EC 2.7.1.51) activity was determined by measuring ADP formation with the pyruvate kinase-lactate dehydrogenase coupled system, as described by Le Blanc & Mortlock (1971*a*) and Oliver & Mortlock (1971*a*).

The protein content of cell-free extracts was measured by the method of Gornall, Bardawill & David (1949).

Other assays. Glycollate was measured by its reaction with chromotropic acid (Le Blanc, 1970). D-Arabinose was determined by the orcinol method (Mejbaum, 1939).

Chemicals. L-Fucose and D-ribulose were prepared as described by Le Blanc & Mortlock (1971*a*). All other chemicals and reagents were from commercial sources.

RESULTS

Extracts of wild-type D-Ara⁻ *S. typhimurium* strains and their D-Ara⁺ mutants grown in CH medium plus L-fucose showed L-fucose isomerase and L-fuculokinase activities. The isomerase utilized either L-fucose or D-arabinose as substrate and the kinase was active with either L-fucose or D-ribulose as substrate (Table 1). As these activities were not induced in D-Ara⁺ bacteria grown in CH medium without L-fucose, the mutation allowing their growth on D-arabinose could not be due to the constitutive synthesis of these enzyme activities.

Incubation in CH medium plus D-arabinose induced these activities in D-Ara⁺ bacteria but not in D-Ara⁻ bacteria. The levels of induction were low but this may have been because the bacteria grew well enough on the casein hydrolysate to keep the levels of enzyme low due to a poorer induction rate with D-arabinose incubation. None of the wild-type *S. typhimurium* strains grown in peptone water plus D-arabinose (without aeration) contained detectable amounts of L-fucose isomerase or L-fuculokinase activities whereas extracts from D-Ara⁺ bacteria similarly grown had appreciable levels of both. The enzymes were also demonstrated in D-Ara⁺ bacteria grown in MMA medium plus D-arabinose.

The ratio of the specific activity of the isomerase with L-fucose as substrate to that with D-arabinose as substrate was 1.8 to 2.0, and the ratio of the specific activities of the kinase with L-fucose and D-ribulose as substrates was 2.8 to 3.0 (Table 1). These ratios were similar regardless of whether the enzymes were induced in D-Ara⁻ bacteria by L-fucose or in D-Ara⁺ bacteria by L-fucose or D-arabinose (Table 1; unpublished observations). When tested at saturating substrate levels, the reaction velocity of the isomerase with L-fucose was about twice that with D-arabinose and that of the kinase with L-fucose was nearly three times that with D-ribulose. The combined reaction rates with L-fucose and D-arabinose as substrates (or with L-fucose and D-ribulose) were not additive. The observations are consistent with the proposal that a single isomerase and a single kinase are each involved with the oxidation of two substrates.

The typical D-Ara⁺ mutant s2764.da1 was grown on MMA medium plus D-arabinose in aerated shaken culture, and at intervals during the exponential growth period, samples of the growth medium were assayed for the presence of glycollate and D-arabinose. The concentration of D-arabinose declined over a 9 h period and in the first 6 h of growth there was a parallel accumulation of glycollate. Thereafter the concentration of glycollate declined and after 9 h glycollate and D-arabinose were present in only trace amounts.

Salmonella typhimurium s2626 was unusual among the wild-type strains tested in that it did not utilize L-fucose as sole carbon source. Extracts of s2626 grown on CH medium with or without L-fucose showed no detectable levels of isomerase or kinase activities. The L-fucose-1-phosphate aldolase status of the strain was not determined. Extracts of L-fucose-utilizing mutants of this strain (s2626.lf1, -lf2 and -lf6) grown in CH medium plus L-fucose showed L-fucose (D-arabinose) isomerase and L-fucose (D-ribulose) kinase activities; the specific activity ratios for their respective substrates were similar to those already described. Thus, the acquisition of the ability to grow on L-fucose was accompanied by the ability to synthesize both isomerase and kinase enzymes in the presence of L-fucose.

Table 1. *Isomerase and kinase specific activities in cell-free extracts of wild-type Salmonella typhimurium strains and D-arabinose-utilizing mutants*Specific enzyme activities are expressed as $\mu\text{mol NADH oxidized min}^{-1} (\text{mg protein})^{-1}$.

Strain no.*	Growth medium†	Isomerase		Kinase	
		D-Arabinose	L-Fucose	D-Ribulose	L-Fucose
s844(-)	CH	< 0.007‡	< 0.009	< 0.003	< 0.003
s844(-)	L-Fuc.CH	0.484	0.938	0.108	0.296
s844.da1(+)	CH	< 0.018	< 0.019	< 0.005	< 0.005
s844.da1(+)	L-Fuc.CH	0.305	0.643	0.066	0.173
s1836(-)	CH	< 0.003	< 0.010	< 0.006	< 0.006
s1836(-)	L-Fuc.CH	0.782	1.240	0.086	0.321
s1836.da1(+)	CH	< 0.003	< 0.009	< 0.003	< 0.003
s1836.da1(+)	L-Fuc.CH	0.570	1.040	0.158	0.406
s2764(-)	CH	< 0.007	< 0.005	< 0.004	< 0.004
s2764(-)	L-Fuc.CH	0.564	1.060	0.118	0.300
s2764.da1(+)	CH	< 0.010	< 0.007	< 0.005	< 0.005
s2764.da1(+)	L-Fuc.CH	0.542	1.024	0.142	0.400

* (+) or (-) indicates ability to utilize D-arabinose as sole carbon source.

† Casein hydrolysate/salts medium alone (CH) or with L-fucose (L-Fuc.CH).

‡ <, No enzyme activity was detected, but must be less than the stated amount which is the lower limit of the sensitivity of the assays.

Furthermore, the L-Fuc⁺ mutants, unlike s2626, fermented D-arabinose in 2 to 3 days in peptone water cultures.

DISCUSSION

There are two different pathways for D-arabinose metabolism by enterobacteria (Mortlock, 1976). In D-Ara⁺ *K. pneumoniae*, the mutation allowing D-arabinose utilization is associated with the constitutive synthesis of an L-fucose isomerase acting on L-fucose and D-arabinose (Camyre & Mortlock, 1965; Oliver & Mortlock, 1971*a, b*). Isomerization of D-arabinose to D-ribulose by L-fucose isomerase induces synthesis of D-ribulokinase which phosphorylates D-ribulose to produce D-ribulose 5-phosphate (Mortlock & Wood, 1964*a, b*). Further metabolism takes place via the pentose phosphate pathway.

On the other hand, in D-Ara⁺ *Escherichia coli*, D-arabinose induces the synthesis of three enzymes normally associated with L-fucose catabolism, and all three display dual substrate activities. As in *K. pneumoniae*, L-fucose isomerase converts D-arabinose to D-ribulose (Le Blanc & Mortlock, 1971*b*) which is then converted to D-ribulose 1-phosphate by L-fuculokinase, to be cleaved by L-fuculose-1-phosphate aldolase to dihydroxyacetone phosphate and glycolaldehyde (Le Blanc & Mortlock, 1971*a, b*). D-Ara⁺ *K. pneumoniae* strains deficient in D-ribulokinase also metabolize D-arabinose by the D-ribulose 1-phosphate route to glycolaldehyde (Le Blanc & Mortlock, 1972).

Incubation of D-Ara⁺ *S. typhimurium* in the presence of L-fucose or D-arabinose induced the synthesis of L-fucose isomerase and L-fuculokinase which in other enterobacteria convert D-arabinose to D-ribulose 1-phosphate. The ratios of the specific activities of these enzymes for their substrates were similar regardless of whether their synthesis had been induced in D-Ara⁺ strains by growth in the presence of D-arabinose or L-fucose or in D-Ara⁻ strains by L-fucose. This suggested that in *S. typhimurium* these enzymes had dual substrate activities as noted in *E. coli* and *K. pneumoniae*. From our knowledge of the metabolism of D-arabinose by these latter bacteria, the most likely route of D-arabinose degradation by D-Ara⁺ *S. typhimurium* would seem to be via D-ribulose 1-phosphate.

Due to the presence of L-fuculose-1-phosphate aldolase, D-Ara⁺ *E. coli* and D-Ara⁺

D-ribulokinase-deficient *K. pneumoniae* form glycolaldehyde and dihydroxyacetone phosphate from D-ribulose 1-phosphate, and when grown on D-arabinose as sole carbon source, glycolic acid accumulates in the medium (Le Blanc & Mortlock, 1971*b*, 1972). D-Ara⁺ *S. typhimurium* grown similarly also accumulates glycolic acid. Thus, although we did not demonstrate the synthesis of L-fuculose-1-phosphate aldolase, its presence may be inferred if it is accepted that D-arabinose is metabolized via the D-ribulose 1-phosphate route.

Escherichia and *Salmonella* are considered to be more closely related to each other than either is to *Klebsiella* (Krieg & Lockhart, 1966). Because both *E. coli* and *S. typhimurium* lack a functional ribitol pathway (Le Blanc, 1970; Old, 1977) neither is likely to degrade D-arabinose by the D-ribulose 5-phosphate route used by most klebsiellae. The similar growth responses of *Escherichia* and *Salmonella* on pentoses and pentitols and the indication that both degrade D-arabinose by similar mechanisms are not surprising in view of their taxonomic relatedness by other criteria.

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