Determination of effector molecules in L-arabinose-induced bulge formation and lysis of *Escherichia coli* IFO 3545

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(Received 6 September 1990; revised 2 January 1991; accepted 2 January 1991)

L-Ribulose 5-phosphate (L-Ru5P) was identified as the primary effector molecule of L-arabinose-induced bulge formation in *Escherichia coli* IFO 3545 observed in nutrient broth with 5% (w/v) sodium chloride. Hyperinduction of L-arabinose isomerase was due to exogenous sodium chloride and the resulting alteration in the balance of the L-arabinose-metabolizing enzymes resulted in accumulation of L-Ru5P. L-Ru5P induced the lysis of an L-arabinose-negative, L-Ru5P 4-epimerase-less mutant, ara-207, even when directly added to the medium but was not active against the wild-type strain. Some L-arabinose-utilizing (L-arabinose-resistant) revertants of ara-207 were still sensitive to L-Ru5P, indicating the involvement of another mutation in L-Ru5P-sensitivity other than genetic lack of L-Ru5P 4-epimerase. Among the various pentose phosphate esters tested, only L-Ru5P could induce lysis of ara-207. The lytic activity of L-Ru5P was attributed to its effect on bacterial sugar nucleotide metabolism which caused secondary accumulation of uridine 5'-diphosphate galactose (UDPGal), which provoked lysis induction.

Introduction

L-Arabinose can induce the characteristic morphological change of bulge formation, highly reminiscent of a penicillin-induced bulge (Schwarz *et al.*, 1969; Spratt, 1975), in *Escherichia coli* IFO 3545 grown under hypertonic condition with 5% (w/v) sodium chloride (Tanaka *et al.*, 1986). Bulge formation was observed in mutant ara-207, defective for L-ribulose 5-phosphate (L-Ru5P) 4-epimerase, in the absence of sodium chloride but not in mutants defective for either L-arabinose isomerase or L-ribulokinase (Tanaka *et al.*, 1988a). These findings strongly suggested that osmotic treatment of *E. coli* cells with 5% (w/v) sodium chloride could regulate the level of activity of an L-arabinose-metabolizing enzyme (i.e. repression or inhibition of L-Ru5P 4-epimerase). However, it is doubtful whether environmental osmotic conditions can produce differential control of individual L-arabinose-metabolizing enzymes,

since the genetic expression of such enzymes is coordinated by the same control region in the L-arabinose operon (Cleary & Englesberg, 1974; Colomé *et al.*, 1977; Englesberg *et al.*, 1965). In the present study, the L-arabinose-induced morphological change of *E. coli* IFO 3545 was further characterized with respect to the relationship between accumulation of an effector molecule and osmoregulation of the L-arabinose metabolizing enzymes.

Our previous study also suggested that L-Ru5P might be an effector molecule of L-arabinose-induced morphological change and lysis through its ability to inhibit bacterial cell wall synthesis (Tanaka *et al.*, 1988a). We applied HPLC analysis (Mengin-Lecreux *et al.*, 1983; Payne & Ames, 1982) to detect peptidoglycan (PG) precursors such as uridine 5'-diphosphate *N*-acetylglucosamine (UDPGlcNAc) and its derivatives which may accumulate due to inhibition of PG synthesis. Although the above substances were not detectable in abnormal quantities, HPLC analysis revealed overproduction of uridine 5'-diphosphate galactose (UDPGal) in ara-207 cells grown with L-Ru5P. The present report describes the lytic effect of L-Ru5P on ara-207 and its effect on secondary accumulation of UDPGal which is closely involved with the lytic events of *E. coli* (Yarmolinsky *et al.*, 1959) and *Salmonella* sp. (Fukasawa & Nikaido, 1961).

Abbreviations: L-Ru5P, L-ribulose 5-phosphate; UDPGal, uridine 5'-diphosphate galactose; PG, peptidoglycan; UDPGlcNAc, uridine 5'-diphosphate *N*-acetylglucosamine; D-Xu5P, D-xylulose 5-phosphate; D-G3P, D-glyceraldehyde 3-phosphate; D-R5P, D-ribose 5-phosphate; LPS, lipopolysaccharide; GlcNAc, *N*-acetylglucosamine; UDPG, uridine 5'-diphosphate glucose; D-Ru5P, D-ribulose 5-phosphate; D-R1P, D-ribose 1-phosphate; D-Gal1P, D-galactose 1-phosphate.

Methods

Bacterial strains. *Escherichia coli* IFO 3545 (Tanaka *et al.*, 1986) and its mutant with a defect in L-Ru5P 4-epimerase, ara-207 (Tanaka *et al.*, 1988a), were used. L-Arabinose-resistant revertants of ara-207 were isolated as described below. An overnight culture (100 µl) of ara-207 in 3% NB (5 g beef extract, 15 g polypeptone, 5 g K_2HPO_4 l⁻¹; pH 7.0) was inoculated into 5 ml of 1% NB (the concentration of each component was one-third of that in 3% NB) containing 6.7 mM-L-arabinose and grown with shaking at 37 °C for more than 7 h until complete lysis was observed. After centrifugation of the lysate at 8000 r.p.m. for 10 min, the resulting pellet was washed with 0.8% sodium chloride and plated onto 1% NB agar containing 6.7 mM-L-arabinose. Colonies were picked after overnight incubation at 37 °C and considered as L-arabinose-resistant revertants of ara-207.

Media and growth conditions. Unless stated otherwise, precultivation was done with 3% NB and the bacterial growth or morphological change was examined in 1% NB according to our method described previously (Tanaka *et al.*, 1986, 1988a). Utilization of L-arabinose was examined using M-9 mineral salts medium (Tanaka *et al.*, 1988a).

Assay of L-arabinose-metabolizing enzymes. Arabinose-metabolizing enzymes such as L-arabinose isomerase, L-ribulokinase and L-Ru5P 4-epimerase were assayed as described previously (Tanaka *et al.*, 1988a). Cells grown in hypertonic medium containing 5% (w/v) sodium chloride were washed and resuspended in 100 mM-Tris/HCl buffer, pH 7.5, supplemented with 5% (w/v) sodium chloride prior to disruption by sonication. One unit of each enzyme activity was defined as the amount of enzyme that catalysed the conversion of 1 µmol substrate min⁻¹ under the assay conditions.

Assay of L-arabinose metabolites. Cells of *E. coli* IFO 3545 (wild-type) were grown in 500 ml 1% NB [supplemented with or without 5% (w/v) sodium chloride] containing 6.7 mM-L-arabinose. Samples of the bacterial cell suspension (100 ml) were withdrawn and harvested by centrifugation at several stages of cultivation. After washing twice with 5% (w/v) sodium chloride, cell pellets were suspended in 5 ml distilled water. L-Arabinose metabolites were then extracted by vigorously shaking the cell suspension with 50 µl toluene at 30 °C for 15 min. The above extraction procedure was as effective as that with trichloroacetic acid reported by Englesberg *et al.* (1962) and even more useful in that the supernatant obtained after removing the cell pellet could be employed directly in the following assays. Total pentose phosphate esters such as L-Ru5P and D-xylulose 5-phosphate (D-Xu5P) were colorimetrically determined by a modified o-aminodiphenylacetic acid method (Tanaka *et al.*, 1988b). Enzyme coupling reactions were employed for specific determination of D-Xu5P and D-glyceraldehyde 3-phosphate (D-G3P), respectively, as described below. After heating the sample at 100 °C for 5 min and removing the resulting precipitate by centrifugation, each supernatant (1.4 ml) was incubated with a mixture of 30 µmol sodium glutathione, 1.2 µmol thiamin pyrophosphate, 12 µmol $MgCl_2 \cdot 6H_2O$, 1.5 µmol NAD^+ , 12 µmol D-ribose 5-phosphate (D-R5P), 1 unit transketolase, and 5 units D-G3P dehydrogenase in 1.9 ml 30 mM-glycylglycine (pH 8.5) at 30 °C. D-G3P was measured by following the increase in absorbance at 340 nm in a control reaction which was run without transketolase and D-R5P. D-Xu5P was measured from the difference in absorbances at 340 nm obtained in the above two reactions. In the present study, total pentose phosphate esters detected during L-arabinose metabolism were found to consist mainly of L-Ru5P; the amount of D-Xu5P measured enzymically was negligible. L-Arabinose was determined according to the method of Timell *et al.* (1956). Thin-layer chromatography was performed using silica gel plates (Merck Kieselgel 60) and butanol/propionic acid/water (10:5:7, by vol.) as the solvent. After development twice at room temperature, sugar phosphate esters were located by spraying with a mixture consisting of 5 ml 60% (v/v) perchloric acid,

10 ml 1 M-HCl, 25 ml 4% (w/v) ammonium molybdate and 60 ml distilled water, followed by heating at 100 °C and ultraviolet irradiation.

Incorporation of radioactive precursors into macromolecular cell wall fractions. An overnight culture of wild-type strain or ara-207 (100 µl) was inoculated into 5 ml 1% NB and grown with shaking at 30 °C. For the assay of lipopolysaccharide (LPS) synthesis, D-[1-¹⁴C]galactose (50 mCi mmol⁻¹; 1.85 GBq mmol⁻¹) was added to a final activity of 0.5 µCi ml⁻¹ to the growing culture at an OD₆₆₀ of 0.1. Cell samples (200 µl) were withdrawn at 5 min intervals and poured into 1 ml cold 1.32 M-NH₄OH containing 6.6 mM-MgCl₂. Alkali-insoluble precipitates were collected on glass microfibre filters (Whatman GF/C) and radioactivity on the filters was counted with 10 ml toluene-based scintillation fluid in a Packard Tri-Carb 460 scintillation counter as reported by Ichimura *et al.* (1987). N-Acetyl[1-¹⁴C]glucosamine ([1-¹⁴C]GlcNAc) (50 mCi mmol⁻¹; 1.85 GBq mmol⁻¹) was added to a final activity of 0.05 µCi ml⁻¹ as the precursor of both PG and LPS synthesis. Cell samples (200 µl) were withdrawn and poured into 1 ml cold 10% (w/v) trichloroacetic acid, and then acid-insoluble precipitates were collected and their radioactivity measured according to the above method.

Assay of intracellular level of UDPGal and uridine 5'-diphosphate glucose (UDPG). Overnight cultures (1 ml each) of the wild-type strain and ara-207 as well as several L-arabinose-resistant revertants of ara-207 were inoculated into 50 ml portions of 1% NB supplemented or not with 2 mM-L-Ru5P and then grown with shaking at 30 °C for 4 h. Cells were harvested by centrifugation and suspended in 1 ml distilled water. The supernatant obtained after shaking the cell suspension with 20 µl toluene at 30 °C for 15 min and removing the cell debris by centrifugation was analysed for its content of UDPGal and UDPG by HPLC on an ODS reverse-phase column (6 × 150 mm). The operating conditions were as follows: isocratic elution at room temperature with 0.6 M-(NH₄)₂HPO₄; flow rate, 1 ml min⁻¹; detector sensitivity at 260 nm, 0.02 AUFS.

Chemicals. L-Arabinose was purchased from Wako Pure Chemical Co. (Japan). L-Ribulose was prepared by chemical isomerization of L-arabinose as described previously (Tanaka *et al.*, 1988a). L-Ru5P was prepared by enzymic phosphorylation of L-ribulose using L-ribulokinase according to our method reported previously (Tanaka *et al.*, 1988a). Alcohols of L-Ru5P were prepared by reduction with NaBH₄ as described below. L-Ru5P (260 µmol) dissolved in 1 ml distilled water was incubated with 5 ml 0.1% NaBH₄ overnight at 25 °C. Amberlite IR 120 resin (H⁺) was added to decompose excess NaBH₄ and the supernatant obtained after removing the resin was evaporated to dryness. The resulting precipitate was dissolved in methanol and repeatedly evaporated with methanol *in vacuo*. The final syrup was used as a mixture of L-arabitol 5-phosphate and L-ribitol 5-phosphate. This preparation displayed no reducing power in the reaction with Fehling's solution, indicating complete reduction of carbonyl groups. L-Arabitol and a mixture of L-arabitol and L-ribitol were prepared following the same method, using L-arabinose and L-ribulose, respectively. The following chemicals and enzymes were purchased from Sigma: D-ribulose 5-phosphate (D-Ru5P), D-Xu5P, D-R5P, D-G3P, D-ribose 1-phosphate (D-R1P), transketolase and D-G3P dehydrogenase. D-[1-¹⁴C]galactose (50 mCi mmol⁻¹) and [1-¹⁴C]GlcNAc (50 mCi mmol⁻¹) were products of American Radiolabelled Chemicals Inc.

Results

Effect of sodium chloride on L-arabinose metabolism

An L-arabinose-induced morphological change was first observed in the *E. coli* IFO 3545 wild-type strain along

Fig. 1. Course of L-arabinose metabolism in *E. coli* IFO 3545 wild-type strain grown at 30 °C in 500 ml of 1% NB containing 66.7 mM-L-arabinose in the presence (open symbols) or absence (filled symbols) of 5% (w/v) sodium chloride. At the times indicated, cell suspensions were withdrawn and used for assays of growth (○, ●), L-arabinose remaining in the medium (□, ■) and L-Ru5P accumulated in the cells (△, ▲), as described in the text. The amounts of L-Ru5P are expressed as total pentose phosphate esters detected in the extracts of toluene-treated cells obtained from 1 ml culture.

with inhibition of bacterial cell division under hyper-tonic conditions with 5% (w/v) sodium chloride (Tanaka *et al.*, 1986). Under these conditions, the presence of sodium chloride was expected to affect the bacterial L-arabinose metabolism so that the effector molecule accumulated within the cells. As shown in Fig. 1, a pentose phosphate ester (L-Ru5P) was detected by the modified *o*-aminodiphenylacetic acid test at a significant level during growth of the wild-type strain in 1% NB containing 66.7 mM-L-arabinose and 5% (w/v) sodium chloride. An enzymic assay could detect D-Xu5P but the amount obtained from cells of 1 ml culture was less than 5 nmol, being negligible compared to the total pentose phosphate ester. The major positive component in the colorimetric reaction assay was identified by thin-layer chromatography to be L-Ru5P. However, the amount of L-Ru5P detected was less than that of L-arabinose taken up from the medium. This indicated that accumulation of L-Ru5P did not depend on complete inhibition or repression of L-arabinose metabolism as was the case for the mutant with a genetic defect in L-Ru5P 4-epimerase.

Accumulation of L-Ru5P could result from an alteration in the balance of L-arabinose-metabolizing enzymes such as L-arabinose isomerase, L-ribulokinase and L-Ru5P 4-epimerase. Therefore assays were done for these enzymes using cell-free extracts at several stages of growth. In the absence of sodium chloride, the highest activities of L-arabinose isomerase, L-ribulokinase and L-Ru5P 4-epimerase were detected during the exponential growth phase (4 h) and decreased with further cultivation, as shown in Table 1. L-Arabinose isomerase was detected at an apparently higher level than the other two enzymes at 16 h of cultivation with 5% (w/v) sodium chloride. The activity of this enzyme was slightly repressed at 40 h of cultivation but was still much higher than those of the other two enzymes. The assay could not

be done for the sample from 4 h cultivation with 5% (w/v) sodium chloride because of poor growth. From the biochemical viewpoint, hyperinduction of L-arabinose isomerase was the most probable cause of the accumulation of L-Ru5P arising from treatment of *E. coli* with sodium chloride.

Lytic effect of L-Ru5P

L-Ru5P which had accumulated intracellularly was thought to be important in the bulge formation of wild-type cells. As shown in Fig. 2, however, L-Ru5P did not show any effect on the growth and morphology of the wild-type strain when added to the medium. In contrast, lysis of the L-Ru5P 4-epimerase-less mutant, *ara-207*, occurred after 4 h of cultivation in 1% NB plus L-Ru5P, as observed with its precursor, L-arabinose (Tanaka *et al.*, 1988a). The minimum concentration of L-Ru5P (2.0 mM) effective for lysis of *ara-207* was lower than that of L-arabinose (6.7 mM).

Microscopical observation revealed bulged and spheroplast-like cells prior to bacterial lysis. Thus, it was concluded that L-Ru5P was an effector of bulge formation and lysis of *E. coli* cells.

Sensitivity to the lytic effect of L-Ru5P

The wild-type strain was probably resistant to exogenously added L-Ru5P either because it could metabolize this compound by dephosphorylation to yield assimilable L-ribulose or via a constitutive level of L-Ru5P 4-epimerase (it is not known if L-Ru5P can serve as an inducer of the L-arabinose operon). Therefore, the relationship between L-Ru5P sensitivity and the genetic defect in L-Ru5P 4-epimerase was examined using 50 independently isolated L-arabinose-resistant revertants

Fig. 2. Effect of L-Ru5P on the growth of *E. coli* IFO 3545 wild-type strain (●) and mutant *ara*-207 (○, □, ■). Cells were grown with shaking at 30 °C in 5 ml of 1% NB supplemented with L-Ru5P at various concentrations.

Table 1. Levels of *L*-arabinose-metabolizing enzymes in *E. coli* IFO 3545

Cells of wild-type *E. coli* were grown in 500 ml of 1% NB containing 66.7 mM-*L*-arabinose or 66.7 mM-*L*-arabinose and 5% sodium chloride at 30 °C. At the times indicated, samples (100 ml) were withdrawn and enzyme activities were assayed as described in the text.

Cultivation time (h)	5% Sodium chloride	Enzyme activity* [units (mg protein) ⁻¹]		
		Isomerase	Ribulokinase	4-Epimerase
4	—	0.212	0.034	0.076
16	—	0.109	0.031	0.032
16	+	0.519	0.049	0.046
40	—	0.046	0.011	0.016
40	+	0.394	0.020	0.008

* Isomerase, *L*-arabinose isomerase; ribulokinase, *L*-ribulokinase; 4-epimerase, *L*-Ru5P 4-epimerase.

Table 2. Characteristics of *L*-arabinose-resistant revertants of *ara*-207

Strain	Utilization of <i>L</i> -arabinose	Specific activity of <i>L</i> -Ru5P 4-epimerase [units (mg protein) ⁻¹]	<i>L</i> -Ru5P* (mM)
Wild-type	+	0.076	> 20
<i>ara</i> -207	—	0.000	2
<i>ara</i> ^r -1	+	0.047	> 20
<i>ara</i> ^r -4	+	0.047	2
<i>ara</i> ^r -18	+	0.023	> 20
<i>ara</i> ^r -21	+	0.025	2

* Minimum concentration of *L*-Ru5P effective for lysis.

of *ara*-207. All of them were found to utilize *L*-arabinose and thus could grow without any morphological change in 1% NB supplemented with *L*-arabinose. Table 2 shows the intracellular levels of *L*-arabinose-metabolizing enzymes in some of these revertants, indicating apparent reversion of *L*-Ru5P 4-epimerase activity. Interestingly, 24 revertants, including *ara*^r-4 and *ara*^r-21, retained the same sensitivity to *L*-Ru5P as *ara*-207, showing lysis when grown with 2 mM-*L*-Ru5P. This indicated that the *L*-Ru5P sensitivity of *ara*-207 did not depend on a genetic lack of *L*-Ru5P 4-epimerase but on some mutation at a gene locus other than *araD*, the structural gene of the enzyme.

Structure-sensitivity relationship

We examined whether the lytic activity mediated by *L*-Ru5P on *ara*-207 could be observed with other sugar phosphate esters or whether it was specific for *L*-Ru5P. Other pentose phosphate esters such as *D*-Ru5P, *D*-R5P and *D*-R1P were not effective even at 20 mM, indicating the existence of stereochemical specificity in the lytic activity of *L*-Ru5P. After complete reduction of the carbonyl group, the resulting alcohols of *L*-Ru5P such as *L*-arabitol 5-phosphate and *L*-ribitol 5-phosphate still retained lytic activity at 5.0 mM. However, the corresponding sugar alcohols lacking the phosphate moiety were inactive. The phosphate moiety was therefore considered to be essential for the lytic activity of *L*-Ru5P.

Effect of *L*-Ru5P on incorporation of radioactive precursors into macromolecular cell wall fractions

We tried to determine whether the lytic activity of *L*-Ru5P was due to inhibition of PG or LPS synthesis. No clear inhibition by 2 mM-*L*-Ru5P could be detected in the assay for incorporation of [¹⁴C]GlcNAc into acid-insoluble material (*ara*-207 incorporated approximately 6000 c.p.m. during 15 min incubation with or without *L*-Ru5P). *L*-Ru5P did, however, inhibit incorporation of *D*-[¹⁴C]galactose into LPs of *ara*-207, as shown in Fig. 3. Apparent *L*-Ru5P-induced inhibition of LPS synthesis was also observed in the wild-type strain, which was resistant to lytic activity of *L*-Ru5P. Inhibition of *D*-[¹⁴C]galactose incorporation seemed to depend on a decrease in the specific radioactivity of labelled UDPGal, an intermediate of LPS synthesis, rather than on the inhibition of LPS synthesis itself (see below).

Effect of *L*-Ru5P on intracellular level of UDPGal

The concentration of UDPGlcNAc and its derivatives was within the normal range in *ara*-207 cells grown with *L*-Ru5P. In contrast, UDPGal was detected at a higher

Fig. 3. Incorporation of D-[1-¹⁴C] galactose into the LPS fraction of *E. coli* IFO 3545 wild-type strain (□, ■) and mutant *ara*-207 (○, ●). The growing cultures in 1% NB were supplemented with D-[1-¹⁴C] galactose at OD₆₆₀ of 0.1 and further incubated with (open symbols) or without (filled symbols) 2 mM-L-Ru5P at 30 °C. At the times indicated, cell samples were withdrawn and the radioactivity in the alkali-insoluble fractions determined.

Table 3. Effect of L-Ru5P on the level of UDPGal and UDPG in several strains of *E. coli* IFO 3545

Strain	Addition of L-Ru5P	Intracellular level [nmol (mg wet cells) ⁻¹]	
		UDPGal	UDPG
Wild-type	none	0.08	0.08
	2 mM	0.04	0.03
<i>ara</i> -207	none	0.90	0.03
	2 mM	13.50	0.13
<i>ara</i> ^r -1	2 mM	0.13	0.08
<i>ara</i> ^r -4	2 mM	4.59	0.00
<i>ara</i> ^r -18	2 mM	0.04	0.00
<i>ara</i> ^r -21	2 mM	2.26	0.00

level in *ara*-207 than in wild-type cells and the level was significantly higher when the cells were grown with 2 mM-L-Ru5P, as shown in Table 3. Overproduction of UDPGal was also observed in revertants of *ara*-207 with L-Ru5P sensitivity such as *ara*^r-4 and *ara*^r-21. L-Ru5P did not, however, affect the level of UDPGal in the wild-type strain, as in *ara*^r-1 and *ara*^r-18, which were resistant to the lytic activity of L-Ru5P. UDPG 4-epimerase-less mutants of *E. coli* K12 (Yarmolinsky *et al.*, 1959) and *Salmonella* sp. (Fukasawa & Nikaido, 1961) can accumulate UDPGal when grown with D-galactose, and UDPGal plays an essential role in D-galactose-induced lysis of these mutant strains. The lytic activity of L-Ru5P on

ara-207 could be attributed to its effect on another pathway of UDPGal synthesis involving epimerization of UDPG, hence causing overproduction of UDPGal.

The apparent rate of LPS synthesis decreased in response to dilution of labelled UDPGal formed from D-[1-¹⁴C]galactose, when unlabelled UDPGal was provided. This finding suggested that UDPGal was also involved in L-arabinose-induced bulge formation of wild-type cells under hypertonic conditions with 5% (w/v) sodium chloride. Thus, the cellular UDPGal level was examined in bulged cells of the wild-type strain obtained at 48 h of cultivation, when L-Ru5P was detected at almost the maximum level (Fig. 1). Unexpectedly, HPLC analysis did not reveal accumulation of a detectable amount of UDPGal. UDPGal might be further metabolized to a compound more closely involved with the morphological change of *E. coli* cells under the above conditions.

Discussion

In *E. coli*, L-arabinose metabolism proceeds via the reactions of L-arabinose isomerase, L-ribulokinase and L-Ru5P 4-epimerase. The structural genes of these enzymes (*araBAD*) constitute a typical gene cluster which functions as an operon, and their expression is coordinated by the *araC* gene product (Lobell & Schleif, 1990). In the present study, osmotic treatment of *E. coli* cells with 5% (w/v) sodium chloride was found to maintain the fully induced level of L-arabinose isomerase when both L-ribulokinase and L-Ru5P 4-epimerase were repressed. Missense mutations of *araB* coding for L-ribulokinase have been known to result in hyperinducibility of L-arabinose isomerase coded for by the adjacent gene *araA* (Katz & Englesberg, 1971). The fully induced level of this enzyme is believed to depend on relief of self catabolite repression due to lack of L-arabinose metabolites as catabolite repressors. In support of this, we have isolated an L-arabinose-negative mutant of *E. coli* IFO 3545 (*araB*) showing hyperinducibility of L-arabinose isomerase (Tanaka *et al.*, 1988a). However, the osmoregulation of an individual L-arabinose-metabolizing enzyme could not be explained simply by an effect on transcriptional control in the above mutant cells. As shown in Table 1, self catabolite repression was apparently functioning for both L-ribulokinase and L-Ru5P 4-epimerase in the presence of 5% (w/v) sodium chloride. A post-translational change, such as alteration in its turnover, may therefore be involved in sodium chloride-hyperinduction of L-arabinose isomerase in wild-type cells.

Accumulation of L-Ru5P may depend on either a specific increase in the rate of its formation or a decrease in the rate of its epimerization to D-Xu5P. Our data

support the former idea, as follows. The fully induced level of L-arabinose isomerase contributed to the activation of L-ribulokinase by providing excess L-ribulose. The rate of L-Ru5P synthesis *in vivo* may be higher than expected from the activity of L-ribulokinase detected in the *in vitro* assay. Similarly, treatment of *E. coli* with sodium chloride causes an increase in the cellular ATP content, another substrate for L-Ru5P synthesis by L-ribulokinase (Ohwada & Sagisaka, 1987).

Sugar phosphate esters such as D-Gal1P and L-Ru5P are known to have other biological effects via exogenous administration of their precursors. In human galactosaemia, D-Gal1P can cause serious pathological effects due to genetic lack of D-Gal1P uridylyltransferase (Schwarz *et al.*, 1956). D-Gal1P is also known as an effector molecule of D-galactose-induced stasis of *E. coli* K12 with a genetic lack of the same enzyme (Kalckar *et al.*, 1959; Sundararajan, 1963; Yarmolinsky *et al.*, 1959) as is L-Ru5P in L-arabinose-induced stasis of *E. coli* B/r deficient in L-Ru5P 4-epimerase (Englesberg *et al.*, 1962). In the present work, the lytic effect of L-Ru5P was demonstrated when it was given to an L-Ru5P 4-epimerase deficient mutant, ara-207, of *E. coli* IFO 3545. It should be noted that the L-Ru5P sensitivity of ara-207 did not depend on a genetic lack of L-Ru5P 4-epimerase involved in the metabolism of L-Ru5P to D-Xu5P (Table 2). The L-Ru5P-induced growth inhibition in ara-207 was rather similar to the type of D-galactose sensitivity or D-galactose-induced bacteriolysis found in *E. coli* K12 and *Salmonella* sp. deficient in UDPG 4-epimerase (Yarmolinsky *et al.*, 1959; Fukasawa & Nikaido, 1961). A morphological change, resembling spheroplast formation, was found for the above mutant strains during intracellular accumulation of UDPGal. L-Ru5P-induced lysis of ara-207 was also accompanied by overproduction of UDPGal but differed from D-galactose-induced lysis by the formation of bulge(s) in a typical rod cell prior to the appearance of spheroplast-like cells and lysis (Tanaka *et al.* 1988a). In addition, UDPGal was not detectable during L-arabinose-induced bulge formation of wild-type cells, which occurred without apparent lysis or loss of turbidity (Fig. 1; Tanaka *et al.*, 1986). Bulge formation may be a morphological change specifically induced by L-Ru5P, being independent of the lytic process due to accumulation of UDPGal. Fukasawa & Nikaido (1961) investigated the mechanism of D-galactose (UDPGal)-induced lysis of *Salmonella* sp. by analysing the incorporation of hexosamine and diamino-pimelic acid as the major components of PG. They reported that no inhibition of PG synthesis was observed at least up to 5–10 min before lysis. No explanation has yet been found for the relationship between accumulation of UDPGal and the accompanying bacteriolysis of *E. coli* K12 and *Salmonella* sp.

UDPGal synthesis can occur via UDPG pyrophosphorylase, which yields UDPG from UTP and D-glucose 1-phosphate, and UDPG 4-epimerase in the absence of D-galactose. The data of Fig. 3 are consistent with elevated levels of UDPGal, derived from this alternative pathway, accounting for the depressed rates of incorporation into LPS of exogenous labelled galactose.

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