THE PATHWAY FOR L-GALACTONATE CATABOLISM IN *ESCHERICHIA COLI* K-12

Ronald A. COOPER
Department of Biochemistry, University of Leicester, Leicester LE1 7RH, England

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1. Introduction

In studies on the catabolism of aldonic sugar acids by enteric bacteria [1] we showed that D-galactonate was catabolised in *Escherichia coli* to give pyruvate and glyceraldehyde 3-phosphate by a pathway that involved the sequential action of galactonate dehydratase (EC 4.2.1.6), 2-oxo-3-deoxygalactonate kinase (EC 2.7.1.58) and 2-oxo-3-deoxygalactonate 6-phosphate aldolase (EC 4.1.2.21). These enzymes were inducible and appeared to constitute a single catabolic operon [2].

The bacterial utilisation of L*-galactonate does not appear to have been studied and since our preliminary observations indicated that *E. coli* could grow readily on L-galactonate we set out to identify the reactions involved. This paper describes the pathway for L-galactonate catabolism in *E. coli* K-12.

2. Materials and methods

The bacteria used during the course of this work were: the *E. coli* strains, NCTC 9001 (neotype), B, C, Crookes, W and the K-12 derivatives K10, X289 [3], A1201 [4]; *Salmonella typhimurium* LT-2; *Enterobacter aerogenes* FG24.

Bacteria were grown aerobically in appropriately supplemented minimal medium 63 [5] with the appropriate hexose (10 mM) or glycerol (20 mM) as carbon source. The liquid media were solidified when necessary by the incorporation of 1.6% Oxoid bacteriological agar. Phage P1vir-mediated transduction was carried out by standard procedures [5].

For the preparation of cell-free extracts bacteria from 100 ml medium were harvested in the late logarithmic phase of growth, suspended in 4 ml 50 mM sodium/potassium phosphate buffer (pH 6.8) containing 9% (v/v) glycerol and disrupted by exposure to ultrasonic oscillations in an MSE 100-W ultrasonic disintegrator (operating at 7–8 μm peak-to-peak amplitude) at 0°C for 1 min. The suspensions were centrifuged at 150 000 × *g* for 1.5 h at 4°C to remove cell debris and the particulate $NADH_2$ oxidase activity. Soluble protein was measured by the biuret method [6] using crystalline bovine serum albumin as the standard.

The assay for L-galactonate oxidoreductase (EC 1.1.1.?) contained in 1 ml: sodium/potassium phosphate buffer (pH 6.0) (80 μmol), NAD (3 μmol), L-galactonate (25 μmol) and bacterial extract (100 μg protein). The increase in A_{340} concomitant with the formation of $NADH_2$ was monitored in a recording spectrophotometer. Altronate oxidoreductase (EC 1.1.1.58) was assayed spectrophotometrically at 340 nm in a system that contained in 1 ml: imidazole-HCl buffer (pH 7.5) (80 μmol), $MgSO_4$ (5 μmol), $NADH_2$ (0.15 μmol), tagaturonate (2 μmol) and bacterial extract (2 μg protein). The endogenous rate of $NADH_2$ oxidation was measured and then the reaction was started by addition of the tagaturonate. The decrease in A_{340} concomitant with the reduction of tagaturonate was measured in a recording spectrophotometer. Use was made of the very high activity of altronate oxidoreductase in crude extracts to measure uronic acid isomerase (EC 5.3.1.12) activity. For this, the system was identical to that for the assay

* Where no stereo-isomeric form is indicated the sugars are of the D-series

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of altronate oxidoreductase but with galacturonate (5 μmol) instead of tagaturonate and with more bacterial protein (20 μg). The tagaturonate produced by the action of the uronic acid isomerase was reduced by the endogenous altronate oxidoreductase and the concomitant decrease in A_{340} was measured. Since addition of more altronate oxidoreductase, in the form of crude extract from a uronic acid isomerase-negative mutant grown on L-galactonate, did not increase the rate of the reaction, it appeared that the endogenous altronate oxidoreductase provided an adequate excess. All the enzyme assays were carried out at 30°C and 1 unit of enzyme activity is defined as that amount of enzyme that transforms 1 μmol substrate/min.

2-Oxo-3-deoxygluconate 6-phosphate was estimated enzymically [7]. L-Galactonolactone was purchased from Sigma (London) and converted to sodium L-galactonate by titration with NaOH. Tagaturonate was prepared as in [8]. Other biochemicals and enzymes were purchased from Boehringer (London).

3. Results and discussion

When tested on solid media various *E. coli* strains including the neotype strain NCTC 9001, strain K-12 derivatives, strain W and strain Crookes grew readily on L-galactonate as sole source of carbon and energy. However, *E. coli* strain C grew very poorly, whilst *E. coli* strain B, *Salmonella typhimurium* strain LT-2 and *Enterobacter aerogenes* strain FG24 failed to grow.

In liquid medium at 37°C the doubling time for growth of the *E. coli* K-12 strain K10 on L-galactonate was 75 min, only slightly longer than the doubling times on other sugars such as glucose (64 min), galacturonate (66 min), D-galactonate (68 min) and gluconate (69 min).

Since it was possible that the catabolic pathway for L-galactonate was like that for D-galactonate but with intermediates of the opposite stereochemical configuration, extracts were prepared from L-galactonate-grown cells and assayed for pyruvate production in a system identical to that described for D-galactonate [1] but with L-galactonate as substrate. However, no pyruvate was produced, suggesting that L-galactonate catabolism involved reactions chemically distinct from those for D-galactonate catabolism.

A possible sequence for L-galactonate catabolism was suggested by consideration of the structure of L-galactonate. Figure 1 shows that L-galactonate may be converted by 1-step reactions to any of 3 compounds, galacturonate, tagaturonate or altronate, whose catabolism is already well understood, fig.2, [9]. This possibility was tested in two ways.

(i) An *E. coli* K-12 strain, A1201, defective in 2-oxo-3-deoxygluconate 6-phosphate aldolase (EC 4.1.2.14) and consequently unable to grow on galacturonate, glucuronate or gluconate [4] was found to be unable to grow on L-galactonate although shown to grow normally on D-galactonate [1]. When galacturonate-positive transductants of strain A1201 were selected they had simultaneously regained the ability to grow on L-galactonate, gluconate and glucuronate.

Mutants such as strain A1201 that lack 2-oxo-3-deoxygluconate 6-phosphate aldolase activity grow normally on glycerol but addition of gluconate, galacturonate or glucuronate to such cells growing on glycerol leads to the accumulation of 2-oxo-3-deoxygluconate 6-phosphate and consequent inhibition of growth [4,10,11]. Likewise, when 10 mM L-galactonate was added to strain A1201 growing on glycerol, growth was rapidly inhibited and 2-oxo-3-deoxy-

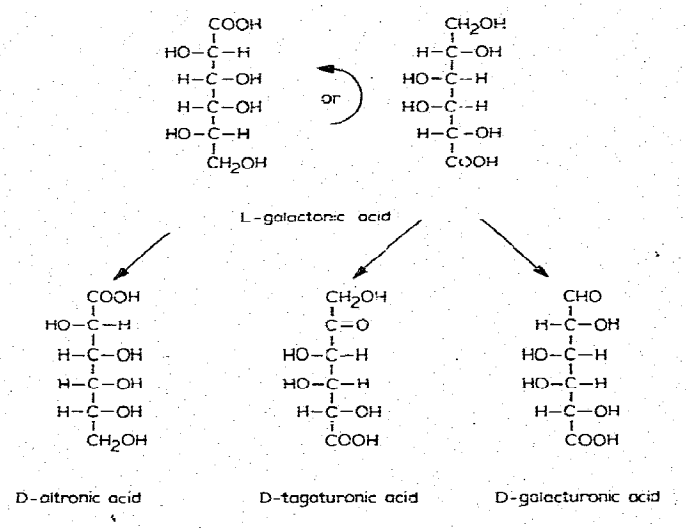

Fig.1. Structural relationships between L-galactonate, D-altronate, D-tagaturonate and D-galacturonate.

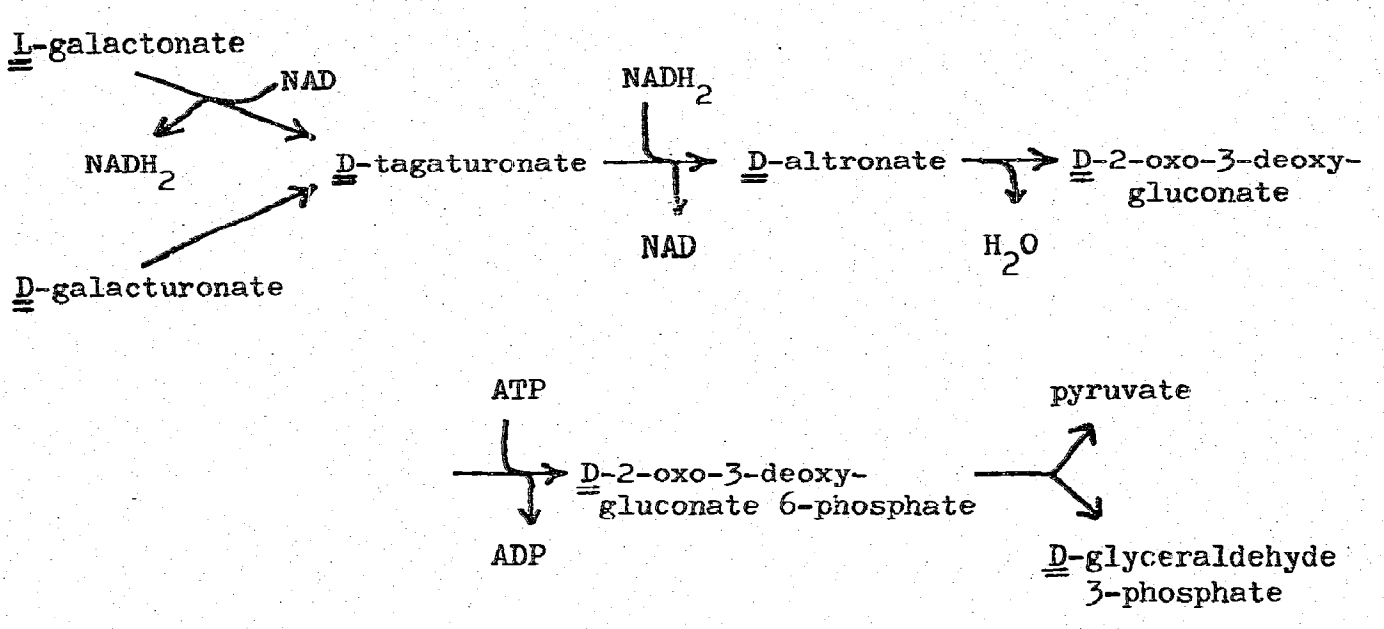

Fig.2. Schematic representation of the route for galacturonate and L-galactonate catabolism.

gluconate 6-phosphate (0.5 μmol/ml) was found to be present in the growth medium [10,11].

(ii) Extracts prepared from wild-type cells grown on glycerol plus L-galactonate were found to have uronic acid isomerase and altronate oxidoreductase activities at least 100-fold higher than those in extracts prepared from glycerol-grown wild-type cells and equivalent in activity to wild-type cells grown on glycerol plus galacturonate (table 1). These findings support the view that L-galactonate is degraded via the galacturonate catabolic sequence.

To identify the point of entry of L-galactonate into the galacturonate catabolic sequence mutants were induced with ethylmethane sulphonate and those defective in growth on galacturonate but unaffected in their growth on gluconate were selected by standard procedures [5]. One such mutant, strain CO133, lacking uronic acid isomerase was unable to grow on either galacturonate or glucuronate but grew normally on gluconate. It also grew on L-galactonate, albeit more slowly (doubling time 165 min) than the parental strain K10. However, strain CO133 also grew slowly on tagaturonate, suggesting that there was a further defect in addition to uronic acid isomerase. Since the gene for uronic acid isomerase is known to

Table 1
Induction of uronic acid isomerase and altronate oxidoreductase in *E. coli* K-12 strain K10 and its L-galactonate oxidoreductase negative mutant, strain CO 131

Growth substrate	K 10		CO 131	
	Uronic acid isomerase[a]	Altronate oxidoreductase[a]	Uronic acid isomerase[a]	Altronate oxidoreductase[a]
glycerol	0	0.06	0	0.05
glycerol + L-galactonate	1.27	8.76	0.57	3.68
glycerol + galacturonate	0.57	12.1	n.d.	n.d.

[a] Specific activity in μmol .min^{-1} .mg protein^{-1}

n.d., not determined

be located very close to the gene for altronate dehydratase [12] a likely explanation of the phenotype of strain CO133 is that it is partially defective in altronate dehydratase due to a polar effect of the mutation in the uronic acid isomerase gene.

A second mutant, strain CO 515, grew normally on glucuronate and gluconate but when grown on glucuronate at 37°C had < 10% of the normal altronate oxidoreductase activity. In liquid medium at 37°C it grew on galacturonate and L-galactonate, but at a slow rate (doubling times 360 min and 420 min, respectively), 20–25% of the rate of the parental organism, strain X289 (doubling times 68 min and 108 min for galacturonate and L-galactonate, respectively). On solid media at 42°C strain CO 515 failed to grow on either galacturonate or L-galactonate.

The results with strain CO 133 suggested that L-galactonate could not be converted to galacturonate exclusively (if at all) and the characteristics of strain CO 515 were consistent with the conversion of L-galactonate to tagaturonate. This conclusion was supported by the growth characteristics of other mutants known to be affected in specific steps of galacturonate utilisation. A strain RP1 [12] known to be defective in altronate dehydratase failed to grow on L-galactonate, whereas the uronic acid isomerase mutant, strain PB1 [12], grew normally on L-galactonate. The altronate oxidoreductase mutant, strain HR3 [12], grew slowly on galacturonate at 37°C but failed to grow at 42°C [12]. Likewise on L-galactonate it grew slowly at 37°C and failed to grow at 42°C.

Further support for this proposed conversion of L-galactonate to tagaturonate was provided by the identification, in L-galactonate-grown cells, of a previously unrecorded bacterial enzyme L-galactonate oxidoreductase. The enzyme utilised NAD^+ but not $NADP^+$ as coenzyme and was present in cells grown on L-galactonate, or on glycerol plus L-galactonate, at spec. act. 0.13 μmol $.min^{-1}$ $.mg\ protein^{-1}$. When the cells were grown on glycerol or galacturonate, no L-galactonate oxidoreductase activity could be detected, suggesting that it was an inducible enzyme.

The physiological significance of this L-galactonate oxidoreductase for growth on L-galactonate was further shown by study of mutants such as strain CO 131 that were unable to grow on L-galactonate but which still grew normally on galacturonate. No activity of L-galactonate oxidoreductase could be found in strain CO 131 after growth on glycerol plus L-galactonate. Similarly when transductants that had received the L-galactonate-negative phenotype were tested, they also were lacking L-galactonate oxidoreductase. However, the enzyme was present when L-galactonate-positive revertants of strain CO 131 were so grown and analysed.

That the defect in strain CO 131 was in L-galactonate oxidoreductase rather than in an L-galactonate uptake system was suggested by the finding that strain CO 131 grown on glycerol plus L-galactonate had high activity of uronic acid isomerase and altronate oxidoreductase (table 1). This implies that L-galactonate could enter the cells to serve as an inducer and since, in the absence of L-galactonate oxidoreductase, no tagaturonate would be formed it further suggests that L-galactonate itself can serve as an inducer of these enzymes as well as the previously recognised inducer, tagaturonate [13]. Since wild-type cells grown on galacturonate had no detectable L-galactonate oxidoreductase, tagaturonate cannot act reciprocally as an inducer of L-galactonate oxidoreductase.

Thus it seems that only 1 novel enzyme, the inducible L-galactonate oxidoreductase, is required to explain the catabolism of L-galactonate in *E. coli* K-12, since the subsequent reactions are those involved in the catabolism of galacturonate. If L-gulonate were to undergo an analagous reaction to that described here for L-galactonate, the product would be fructuronate, an intermediate in the degradation of glucuronate [9]. Since preliminary experiments suggest that L-galactonate oxidoreductase can also act on L-gulonate and since some *E. coli* K-12 strains can grow on L-gulonate as sole carbon and energy source, we are currently investigating this possible relationship between L-galactonate and L-gulonate catabolism.

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