Uridine Diphosphate α -D-Galactofuranose, an Intermediate in the Biosynthesis of Galactofuranosyl Residues

By A. GARCIA TREJO, G. J. F. CHITTENDEN, J. G. BUCHANAN and J. BADDILEY

Microbiological Chemistry Research Laboratory, School of Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, U.K.

(Received 11 February 1970)

An increasing number of oligo- and poly-saccharides from a variety of micro-organisms have been shown to contain D-galactose in the 1,4-furanose ring form. It is noteworthy that several of the type-specific substances of *Pneumococcus* spp. contain this unit (Roberts, Buchanan & Baddiley, 1963; Rao, Buchanan & Baddiley, 1966; Rao, Watson, Buchanan & Baddiley, 1969). More recently a galactofuranosyl residue has been found in the O-antigenic portion of a lipopolysaccharide (Berst *et al.* 1969). Other micro-organisms known to produce polysaccharides containing such residues include *Mycoplasma mycoides* var. *mycoides* (Plackett & Buttery, 1964), *Gibberella fujikuroi* (Siddiqui & Adams, 1961), *Peltigera horizontalis* (Lindberg, Silander & Wachtmeister, 1964), *Bacteroides symbiosus* (Reeves, Latour & Lousteau, 1964) and *Penicillium chrysogenum* (Myazaki & Yadomae, 1969). Galactocarolose, an extracellular β -D-(1 $\rightarrow$ 5)-linked polygalactofuranose, produced by *Penicillium charlesii* G. Smith (Haworth, Raistrick & Stacey, 1937; Gorin & Spencer, 1959) was the first polysaccharide found to be composed of these units. So far little is known about the biosynthesis of galactofuranosyl residues, but if they are transferred to polymer chains from nucleotide intermediates in the manner usual for glycoside biosynthesis then their origin presents a particularly interesting problem, as the only known nucleotide containing galactose is UDP-galactopyranose.

Investigations on the biosynthesis of galactocarolose have now led to the isolation and characterization of a new nucleotide containing galactofuranose and this has been shown to be an intermediate for the introduction of these units into the polymer. Hexofuranose nucleotides were hitherto unknown.

A cell-free enzyme preparation from finely ground mycelia of *Penicillium charlesii* G. Smith, grown for 14 days on the Raulin-Thom medium, was prepared by extraction with ice-cold 0.2M-potassium phosphate buffer, pH 6.8 (1ml/g wet wt. of mycelia); it contained about 1.5mg of protein/ml (Lowry, Rosebrough, Farr & Randall, 1951). The extract utilized either [U - ^{14}C]glucose or [U - ^{14}C]glucose 1-phosphate for the synthesis of galactocarolose

(see Table 1). Addition of UTP to the incubation mixtures enhanced the incorporation of radioactivity into the product; [U - ^{14}C]galactose was not utilized. It was already known that whole cells of the organism do not utilize free galactose to produce galactocarolose (Gander, 1960).

The polysaccharide produced was characterized by its complete hydrolysis by 0.15M-sulphuric acid for 90min at 100°C to free galactose (Clutterbuck, Haworth, Raistrick, Smith & Stacey, 1934) and by its degradation to arabinose (Gorin & Spencer, 1959). Both products were identified by comparison with authentic samples by paper chromatography in propan-1-ol-ethyl acetate-water (7:1:2, by vol.) (Baar & Bull, 1953). No polymannan, also formed by the organism, was detected. Addition of GTP to the incubation mixtures yielded material that was not hydrolysed under the above conditions and amounted to about 10% of the total labelled product. Hydrolysis with 1.5M-sulphuric acid for 90min at 100°C, followed by paper chromatography, gave mannose.

Addition of excess of Zn^{2+} or F^{-} ions to the incubations inhibited polysaccharide synthesis and a new product was formed; this was purified by paper chromatography in ethanol-M-ammonium acetate, pH 7.5 (Paladini & Leloir, 1952). It possessed an absorption maximum at 265nm and had a similar mobility ($R_{adenosine}$ 0.49) to UDP-galactose ($R_{adenosine}$ 0.44). It was readily hydrolysed in dilute acid, undergoing about 50% decomposition to free galactose when chromatographed in the system ethanol-M-ammonium acetate, pH 3.8 (Paladini & Leloir, 1952). Under the same conditions UDP-glucose and UDP-galactose remained unaffected. This marked lability in acid is consistent with the presence of a galactofuranosyl residue in the nucleotide.

Hydrolysis of the nucleotide in 0.1M-hydrochloric acid for 10min at 100°C gave galactose and UMP, identified by comparison with authentic samples. The u.v.-absorption spectrum of the UMP was identical with that of an authentic sample. That galactose was present in the furanose form was substantiated by limited oxidation with 0.08M-sodium metaperiodate for 30min at 0°C to yield

Table 1. *Substrates used for polymer synthesis*

In all experiments the enzyme extract (0.95 ml) was used and finally adjusted to 1 ml with water. In Expts. 1–9 the mixture contained [^{14}C]glucose (0.01 ml; 70×10^5 c.p.m./ml) and D-glucose (0.5 μmol), in Expts. 10–14 [^{14}C]glucose 1-phosphate (0.01 ml; 67×10^5 c.p.m./ml) and D-glucose 1-phosphate (0.5 μmol) and in Expt. 15 [^{14}C]galactose (0.05 ml; 80×10^4 c.p.m./ml); where appropriate nucleoside triphosphate (2 μmol), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (15 μmol) or NaF (5 μmol) was added. Mixtures were incubated for 1 h at 25°C, then heated for 2 min at 100°C, concentrated to about 0.25 ml *in vacuo* at 30°C, applied as bands to Whatman 3MM paper and chromatographed in ethanol–M-ammonium acetate, pH 7.5 (Paladini & Leloir, 1952). The polymer remained at the origin. The amount of incorporation into the polymer is expressed as a percentage of the total radioactivity on the chromatograms.

Expt. no.	Substrates	Polymer formed (% incorporation of labelled substrates)
1	[^{14}C]Glucose	49.0
2	[^{14}C]Glucose + UTP + ATP	68.0
3	[^{14}C]Glucose + UTP	82.0
4	[^{14}C]Glucose + ATP	0.0
5	[^{14}C]Glucose + UTP (enzyme boiled)	0.0
6	[^{14}C]Glucose + GTP	56.0
7	[^{14}C]Glucose + GTP + UTP	80.0
8	[^{14}C]Glucose + Zn^{2+} + UTP	8.0
9	[^{14}C]Glucose + F^- + UTP	14.0
10	[^{14}C]Glucose 1-phosphate	81.0
11	[^{14}C]Glucose 1-phosphate + UTP	89.0
12	[^{14}C]Glucose 1-phosphate + UTP (enzyme boiled)	0.0
13	[^{14}C]Glucose 1-phosphate + Zn^{2+} + UTP	10.0
14	[^{14}C]Glucose 1-phosphate + F^- + UTP	26.0
15	[^{14}C]Galactose + UTP	0.0
16	UDP-[^{14}C]galactofuranose*	76.0

* Initial radioactivity not measured owing to the instability of the compound.

[^{14}C]formaldehyde. This was characterized as the crystalline dimedone derivative. The radioactivity of this derivative (4.3×10^3 c.p.m.) represented about 12.5% of the total radioactivity of the nucleotide (3.35×10^4 c.p.m.) (calculated value for one carbon: 5.6×10^3 c.p.m.).

It is known that carefully controlled alkaline hydrolysis of sugar 1-pyrophosphates and nucleoside diphosphate sugars, having vicinal *cis*-hydroxyl groups, gives sugar 1:2-cyclic phosphates (Paladini & Leloir, 1952; Remy, Remy & Buchanan, 1955). Treatment of the nucleotide with 0.2M-potassium hydroxide for 2 h at 20°C, followed by neutralization and paper chromatography in ethanol–M-ammonium acetate, pH 7.5, gave a phosphate with R_F 0.60, in addition to UMP. This phosphate with 0.1M-sulphuric acid for 10 min at 100°C gave D-galactose 2-phosphate, R_{Ga1} 0.36 in propan-1-ol–aq. ammonia (sp.gr. 0.88)–water (6:3:1, by vol.) (Hanes & Isherwood, 1949), and galactose. The 2-phosphate was indistinguishable from an authentic sample (Chittenden, Roberts, Buchanan & Baddiley 1968) and represented about 80% of the radioactivity of the sample of cyclic phosphate. Sugar 1:2-cyclic phosphates are known to yield about 75% of the corresponding 2-phosphate on acid

treatment. The other product is the labile 1-phosphate, which undergoes further hydrolysis to free sugar (Paladini & Leloir, 1952; Remy *et al.* 1955; Piras, 1963). The ready formation from the nucleotide of galactofuranose 1:2-cyclic phosphate indicates the presence of an α -linkage between the galactofuranose and phosphate, and as the other product of hydrolysis with alkali was UMP it follows that the nucleotide contains a galactofuranose 1-pyrophosphate grouping and must have the structure uridine diphosphate α -D-galactofuranose.

The nucleotide was capable of acting as a donor of galactofuranose units in the biosynthesis of galactocarolose. It was isolated by preparative paper chromatography at pH 7.5, eluted with water at pH 7.0 and treated with Dowex 50 (NH_4^+ form) ion-exchange resin, and the solution was then concentrated to a small volume by freeze-drying. Treatment of the product with the cell-free extract (see Table 1) resulted in 76% polysaccharide formation; the remaining radioactivity was located in the area corresponding to galactose, shown by paper chromatography. The galactose presumably arose through partial decomposition of the nucleotide during its isolation and purification.

A. G. T. thanks the British Council and I.N.I.C. (Mexico City) for financial support. The strain of *P. charlesii* G. Smith was purchased from the Commonwealth Mycological Institute, Kew, Surrey, U.K.

- Baar, S. & Bull, J. P. (1953). *Nature, Lond.*, **172**, 414.
- Berst, M., Hellerqvist, G. G., Lindberg, B., Lüderitz, O., Svensson, S. & Westphal, O. (1969). *Eur. J. Biochem.* **11**, 353.
- Chittenden, G. J. F., Roberts, W. K., Buchanan, J. G. & Baddiley, J. (1968). *Biochem. J.* **109**, 597.
- Clutterbuck, P. W., Haworth, W. N., Raistrick, H., Smith, G. & Stacey, M. (1934). *Biochem. J.* **28**, 94.
- Gander, J. E. (1960). *Archs Biochem. Biophys.* **91**, 307.
- Gorin, P. A. & Spencer, J. F. T. (1959). *Can. J. Chem.* **37**, 499.
- Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
- Haworth, W. N., Raistrick, H. & Stacey, M. (1937). *Biochem. J.* **31**, 640.
- Lindberg, B., Silvander, B. G. & Wachtmeister, C. A. (1964). *Acta chem. scand.* **18**, 213.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Myazaki, T. & Yadomae, T. (1969). *Chem. pharm. Bull., Tokyo*, **17**, 361.
- Paladini, A. C. & Leloir, L. F. (1952). *Biochem. J.* **51**, 426.
- Piras, R. (1963). *Archs Biochem. Biophys.* **81**, 404.
- Plackett, P. & Buttery, S. H. (1964). *Biochem. J.* **90**, 201.
- Rao, E. V., Buchanan, J. G. & Baddiley, J. (1966). *Biochem. J.* **100**, 801.
- Rao, E. V., Watson, M. J., Buchanan, J. G. & Baddiley, J. (1969). *Biochem. J.* **111**, 547.
- Reeves, R. E., Latour, N. G. & Lousteau, R. J. (1964). *Biochemistry, Easton*, **3**, 1248.
- Remy, C. N., Remy, W. T. & Buchanan, J. M. (1955). *J. biol. Chem.* **217**, 885.
- Roberts, W. K., Buchanan, J. G. & Baddiley, J. (1963). *Biochem. J.* **88**, 1.
- Siddiqui, I. R. & Adams, G. A. (1961). *Can. J. Chem.* **39**, 1683.