Metabolism of 3-deoxy-3-fluoro-D-mannose and 4-deoxy-4-fluoro-D-mannose by *Saccharomyces cerevisiae* S288C

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Incubation of *Saccharomyces cerevisiae* S288C with 4-deoxy-4-fluoro-D-[1-¹⁴C]-mannose resulted in the formation of three metabolites that were characterized as 4-deoxy-4-fluoro-D-[1-¹⁴C]mannose 1,6-bisphosphate, 4-deoxy-4-fluoro-D-[1-¹⁴C]mannose 6-phosphate and GDP-4-deoxy-4-fluoro-D-[1-¹⁴C]mannose. In addition, radioactive material was incorporated into a particulate fraction composed primarily of cell-wall polysaccharides. Compared with the 4-fluoro sugar, 3-deoxy-3-fluoro-D-[1-¹⁴C]mannose was not transported into yeast cells as well, and its conversion into sugar nucleotide was much less efficient. Metabolites that were isolated after incubation with the 3-fluoro analogue were identified as 3-deoxy-3-fluoro-D-[1-¹⁴C]mannose 1,6-bisphosphate, 3-deoxy-3-fluoro-D-[1-¹⁴C]mannose 6-phosphate and GDP-3-deoxy-3-fluoro-D-[1-¹⁴C]mannose. Little radioactivity was transferred into the cell-wall fraction.

Deoxy and deoxyfluoro sugars can substitute for natural monosaccharides in a variety of enzymic reactions (Ciba Foundation Symposium, 1972). Such sugar analogues have attracted interest as potential anti-tumour (Bessell *et al.*, 1973; Bernacki *et al.*, 1977) or anti-viral agents (Scholtissek, 1975), as inhibitors or modifiers of protein glycosylation (Schwarz & Datema, 1980) and as probes for monitoring metabolic rates in different regions of the brain (Sokoloff *et al.*, 1977; Reivich *et al.*, 1979). Compounds that possess significant biological activity generally are phosphorylated and converted into a sugar nucleotide derivative *in vivo*. Analogues of D-glucose and D-mannose modified at C-2, specifically 2-deoxyglucose, 2-deoxy-2-fluoro-D-glucose and 2-deoxy-2-fluoro-D-mannose, have been employed extensively for studies of glycoprotein biosynthesis and function (Schwarz & Datema, 1980). Each is phosphorylated efficiently and metabolized further to the uridine and guanosine nucleotide derivatives (Schmidt *et al.*, 1974, 1978).

The metabolism of D-glucose analogues modified at C-3 or C-4 has been examined (Bessell & Thomas, 1973*a,b*; Taylor *et al.*, 1976; Romaschin & Taylor, 1981), but little is known about C-3- or C-4-substituted mannose derivatives. The recent syntheses of 3-deoxy-D-arabino-hexose (3-deoxy-D-mannose) (Scensny *et al.*, 1982), 4-deoxy-D-lyxo-hexose (4-deoxy-D-mannose) (Rasmussen, 1980),

Abbreviation used: h.p.l.c., high-pressure liquid chromatography.

3-deoxy-3-fluoro-D-mannose and 4-deoxy-4-fluoro-D-mannose (Rasmussen *et al.*, 1983) in unlabelled and 1-¹⁴C-labelled forms make it practicable to study the metabolic fate and biological activities of these compounds. In the present paper the activation of 3-deoxy-3-fluoro-D-mannose and 4-deoxy-4-fluoro-D-mannose to the sugar 6-phosphate and GDP-sugar derivatives by *Saccharomyces cerevisiae* S288C *in vivo* is described.

Materials and methods

Materials

Saccharomyces cerevisiae S288C was obtained from Dr. Gerald Fink, Cornell University. Yeast autolysate, GDP-mannose, hexokinase (type F-300), mannose 1-phosphate, fructose 1,6-bisphosphate, 2-deoxy-D-glucose, fructose bisphosphatase, glucose phosphate isomerase, phosphofructokinase and alkaline phosphatase (type VII-S) were purchased from Sigma Chemical Co. Phosphodiesterase (from *Crotalus durissus terrificus*) was supplied by Boehringer Mannheim. [U-¹⁴C]Mannose (sp. radioactivity 325 mCi/mmol) and 2-deoxy-D-[1-¹⁴C]-glucose (sp. radioactivity 52 mCi/mmol) were obtained from New England Nuclear. The syntheses of unlabelled and 1-¹⁴C-labelled (sp. radioactivity 51.1 mCi/mmol) 3-deoxy-3-fluoro-D-mannose, 3-deoxy-3-fluoro-D-glucose, 4-deoxy-4-fluoro-D-mannose and 4-deoxy-4-fluoro-D-glucose are described elsewhere (Rasmussen *et al.*, 1983).

H.p.l.c. and u.v.-absorption spectrophotometry

H.p.l.c. separations were performed on a Partisil-10 SAX (Whatman) column with the use of a Laboratory Data Control high-pressure liquid chromatograph. Compounds were eluted with 200 mM-KH₂PO₄, pH 3.4, at a flow rate of 1.1 ml/min and detected with a u.v. detector operating at 254 nm. U.v.-absorption spectra were recorded on a Hewlett-Packard 8450A spectrophotometer.

Cell growth and labelling procedure

S. cerevisiae S288C cells were grown to mid-exponential phase (17–20 h) on a shaker at 28°C in a medium consisting of 0.2% (NH₄)₂SO₄, 2% glucose and 0.75% yeast autolysate (Biely *et al.*, 1972). The yeast cells were collected by centrifugation (6000 g, 20 min) and washed three times with ice-cold 50 mM-KH₂PO₄. The washed cells (2.7 g) were resuspended in 5 ml of 50 mM-KH₂PO₄ and mixed thoroughly with 2.5 mg of 3-deoxy-3-fluoro-D-[1-¹⁴C]mannose or 4-deoxy-4-fluoro-D-[1-¹⁴C]mannose (sp. radioactivity 0.66 mCi/mmol). The resulting mixture (7.5 ml) was incubated at 28°C with stirring.

For transport studies, 1.5 ml samples were withdrawn and centrifuged (6000 g, 20 min). After removal of the supernatant, the cells were washed twice with 5 ml of 50 mM-KH₂PO₄, then resuspended in 50 mM-KH₂PO₄, and the radioactivity of a portion was counted in aqueous scintillation fluid. For metabolism studies, the labelled cells were washed three times with 20 ml of 50 mM-KH₂PO₄, resuspended in 2 ml of the phosphate buffer, and then lysed by three freeze-thaw cycles with liquid N₂. After particulate matter was removed by centrifugation (9000 g, 20 min), the supernatant was combined with an equal volume of 95% (v/v) ethanol and centrifuged to remove precipitated protein. The resulting supernatant (fraction D, Scheme 1) was analysed by paper chromatography in solvent A. Each pellet fraction was washed with 50 mM-KH₂PO₄ until no radioactivity was observed in the washes. For radioactivity counting, cell-wall material was resuspended in phosphate buffer and the ethanol-precipitated pellet was dissolved in 1% sodium dodecyl sulphate.

Enzymic synthesis of [1-¹⁴C]fructose 1,6-bisphosphate and [1-¹⁴C]fructose analogues

A reaction mixture containing 3-deoxy-3-fluoro-D-[1-¹⁴C]glucose or 4-deoxy-4-fluoro-D-[1-¹⁴C]glucose (20 × 10⁶ c.p.m.; 50 μl), 1 M-Tris/HCl buffer, pH 7.5 (40 μl), 1 M-MgCl₂ (13 μl), ATP (4 mg), hexokinase (40 units), glucose phosphate isomerase (25 units) and phosphofructokinase (25 units) was adjusted to pH 7.5 with 2 M-Tris base. After incubation at 37°C for 24 h, this solution was treated with an equal volume of ethanol, and the precipi-

tated protein was removed by centrifugation. The ethanol-soluble reaction products were separated by paper chromatography in solvent A. More than 80% of the total radioactivity co-migrated with standard fructose 1,6-bisphosphate. Treatment of this material with alkaline phosphatase yielded 3-deoxy-3-fluoro-D-[1-¹⁴C]fructose (*R*_{3-F-Man} 0.88, solvent B) or 4-deoxy-4-fluoro-D-[1-¹⁴C]fructose (*R*_{4-F-Man} 0.85, solvent B).

Paper chromatography

Chromatographic separations were performed at room temperature on Whatman 3MM paper in a descending direction with solvent A [ethanol/1 M-ammonium acetate, pH 7.5 (5:2, v/v)], solvent B [ethyl acetate/pyridine/water (8:2:1, by vol.)] or solvent C [ethyl acetate/pyridine/water (3.6:1:1.15, by vol.)]. After development, chromatograms were cut into 1 cm strips and the radioactivities counted. Reference compounds were detected with u.v. light, molybdate reagent (Hanes & Isherwood, 1949), or liquid-scintillation counting of radioactivity.

Chemical and enzymic studies

Radiolabelled compounds were eluted from the chromatograms with water, and the solutions were concentrated to a volume of 25–50 μl with a stream of N₂. After treatment with acid, alkaline phosphatase, phosphodiesterase or fructose bisphosphatase by using standard procedures (Grier & Rasmussen, 1982), the resulting products were analysed by paper chromatography. Particulate fraction B (Scheme 1) isolated after labelling of yeast cells with 4-deoxy-4-fluoro-D-[1-¹⁴C]mannose was hydrolysed with 2 M-HCl at 100°C for 1 h.

Radioisotopic analysis

The radioactivities of all labelled samples were counted in a Beckman LS-100 liquid-scintillation spectrometer. The radioactivities of paper strips were counted in 10 ml of a scintillation 'cocktail' containing 4.0 g of 2,5-diphenyloxazole and 100 mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene/l of toluene. Aqueous samples were suspended in 5 ml of Aqueous Counting Scintillant (Amersham International) and their radioactivities counted.

Results

Incubation of yeast cells with 3-deoxy-3-fluoro-D-[1-¹⁴C]mannose or 4-deoxy-4-fluoro-D-[1-¹⁴C]mannose in 50 mM-KH₂PO₄ resulted in a time-dependent uptake of analogue (Fig. 1). The rate of transport of each fluoro sugar was significantly lower than that of 2-deoxy-D-[1-¹⁴C]glucose under the same conditions. Fractionation of the radio-labelled material retained by the cells (Scheme 1) demonstrated that most of the radioactive product

synthesized from either analogue was soluble in 50% (v/v) ethanol (Table 1).

Analysis by paper chromatography (solvent *A*) of

Fig. 1. *Transport of 1-¹⁴C-labelled sugars into yeast cells*
Cells were incubated with 2-deoxy-D-[1-¹⁴C]-glucose, 4-deoxy-4-fluoro-D-[1-¹⁴C]mannose or 3-deoxy-3-fluoro-D-[1-¹⁴C]mannose (see the Materials and methods section). Samples were withdrawn from the incubation mixture and centrifuged. After the cells had been washed with 50 mM-KH₂PO₄, portions of the phosphate washes and the cell pellet were solubilized and their radioactivities counted.

the ethanol supernatant (fraction D, Scheme 1) from the incubation with 4-deoxy-4-fluoro-D-[1-¹⁴C]-mannose revealed four radioactive fractions (Fig. 2). The chromatographic mobilities of the four components corresponded to those expected for a hexose 1,6-bisphosphate, GDP-hexose, hexose monophosphate and hexose. In addition, 2.6% of the product radioactivity was present in a particulate fraction (fraction B) that presumably consisted of cell-wall mannan and glucan (Schmidt *et al.*, 1978). Acid hydrolysis of this material yielded a ¹⁴C-labelled product that co-migrated with 4-deoxy-4-fluoro-D-mannose during paper chromatography in solvent *B* (Fig. 3*f*).

Paper-chromatographic analysis (solvent *A*) of the ethanol supernatant from the incubation of yeast cells with 3-deoxy-3-fluoro-D-[1-¹⁴C]mannose revealed the presence of five radioactive fractions (Fig. 4), four of which possessed mobilities similar to those of the components observed in the 4-deoxy-4-fluoro-D-mannose reaction mixture. Only a small amount of radioactivity was incorporated into the cell-wall fraction (Table 1).

The radioactive products from the incubations with the 3-fluoro or the 4-fluoro analogue were eluted from the appropriate regions of the chromatogram and characterized by a combination of chemical and enzymic methods (Table 2 and Figs. 3 and 5).

Scheme 1. *Fractionation of yeast cells after incubation in 50 mM-KH₂PO₄ containing 4-deoxy-4-fluoro-D-[1-¹⁴C]mannose or 3-deoxy-3-fluoro-D-[1-¹⁴C]mannose*
For full experimental details see the Materials and methods section.

Table 1. *Distribution of radioactivity after labelling yeast cells with 3-deoxy-3-fluoro-D-[1-¹⁴C]mannose or 4-deoxy-4-fluoro-D-[1-¹⁴C]mannose*

Cells were labelled for 4.5 h with fluoro [¹⁴C]sugar. Labelling conditions and fractionation procedures are described in the Materials and methods section. In each case over 90% of the radioisotope added was recovered.

Fraction	Labelling with 3-F-[¹⁴ C]Man		Labelling with 4-F-[¹⁴ C]Man	
	10 ⁻³ × Radioactivity (c.p.m.)	Radioactivity recovered (% of total)	10 ⁻³ × Radioactivity (c.p.m.)	Radioactivity recovered (% of total)
A	16 700	80	8150	40
B	40	0.3	304	1.5
C	38	0.3	47	0.2
D	4000	19	11 600	58

Fig. 2. *Paper-chromatographic profile of fraction D after labelling of yeast cells with 4-deoxy-4-fluoro-D-[1-¹⁴C]-mannose*

Yeast cells were incubated with 4-deoxy-4-fluoro-D-[1-¹⁴C]mannose for 4.5 h at 28°C. After fractionation of the labelled cells as outlined in Scheme 1, fraction D (125 000 c.p.m.) was analysed by paper chromatography in solvent A.

Characterization of 4-deoxy-4-fluoro-D-[1-¹⁴C]-mannose metabolite peaks

Peak-I material (Fig. 2) was converted into a hexose monophosphate by mild acid hydrolysis and into a free monosaccharide by reaction with alkaline phosphatase. This behaviour is consistent with the chemical properties expected of a sugar 1,6-bisphosphate (Schmidt *et al.*, 1974). The released

monosaccharide component was characterized by descending paper chromatography in solvents A and B. In each case it co-migrated with 4-deoxy-4-fluoro-D-mannose. Inasmuch as solvent B clearly resolves hexoses from their deoxy and deoxyfluoro analogues (Schmidt *et al.*, 1978; $R_{4-F-Man}$ values 0.38 for mannose, 0.72 for 3-deoxymannose, 0.55 for 4-deoxymannose and 1.0 for 3-deoxy-3-fluoromannose), it appears highly likely that the metabolite is a fluoro sugar. Solvent B also separates 4-deoxy-4-fluoro-D-mannose from 4-deoxy-4-fluoro-D-glucose ($R_{4-F-Man}$ 0.87) and 4-deoxy-4-fluoro-D-fructose ($R_{4-F-Man}$ 0.85). Thus the paper-chromatographic mobility of the monosaccharide component of peak I provides strong support for its assignment as 4-deoxy-4-fluoro-D-mannose. The observation that peak-I material was not a substrate for fructose biphosphatase under conditions that converted 65% of enzymically synthesized (see the Materials and methods section) 4-deoxy-4-fluoro-D-[1-¹⁴C]fructose into a hexose monophosphate further argues against peak-I material being a fructose derivative. Presumably 4-deoxy-4-fluoro-D-[1-¹⁴C]mannose 1,6-bisphosphate, an intermediate in the phosphomannomutase reaction, accumulates by dissociating from the enzyme surface during catalysis. A similar observation has been made for the 1,6-bisphosphate derivative of 2-deoxy-D-glucose (Schwarz & Schmidt, 1976).

The primary metabolite formed by yeast cells when incubated with 4-deoxy-4-fluoro-D-mannose (peak II, Fig. 2) was identified as GDP-4-deoxy-4-fluoro-D-[1-¹⁴C]mannose. Mild acid hydrolysis of the material eluted from the peak-II region of the chromatogram yielded a monosaccharide that co-migrated with 4-deoxy-4-fluoro-D-mannose in solvents A, B and C (Figs. 3d and 5b). Incubation of peak-II material with phosphodiesterase produced a sugar monophosphate (Fig. 5c) that, on further treatment with alkaline phosphatase, was converted into a compound that co-chromatographed with 4-deoxy-4-fluoro-D-mannose (Fig. 5d). Peak-II material was unreactive with alkaline phosphatase

Fig. 3. Characterization of 4-deoxy-4-fluoro-D-[1- 14 C]-mannose metabolites

Products were eluted with water from appropriate regions of the chromatogram of 4-deoxy-4-fluoro-D-[1- 14 C]mannose metabolites (Fig. 1) and subjected to chemical and enzymic reactions as described in the Materials and methods section. The resulting products were analysed by paper chromatography in solvent B. (a) 4-Deoxy-4-fluoro-D-[1- 14 C]-mannose; (b) 4-deoxy-4-fluoro-D-[1- 14 C]glucose; (c) peak-IV material; (d) peak-II material treated with acid; (e) peak-III material treated with alkaline phosphatase; (f) cell-wall pellet treated with 2M-HCl; (g) 4-deoxy-4-fluoro-D-[1- 14 C]fructose 1,6-bisphosphate treated with alkaline phosphatase.

alone (Fig. 5e). These results are consistent with the chemical and chromatographic properties expected of a nucleoside diphosphate derivative of 4-deoxy-4-fluoro-D-[1- 14 C]mannose.

Several observations provided proof that peak-II

Fig. 4. Paper-chromatographic profile of fraction D after labelling of yeast cells with 3-deoxy-3-fluoro-D-[1- 14 C]-mannose

Yeast cells were incubated with 3-deoxy-3-fluoro-D-[1- 14 C]mannose for 4.5 h at 28°C. After fractionation of the labelled cells as outlined in Scheme 1, fraction D (75 000 c.p.m.) was analysed by paper chromatography in solvent A.

material was a GDP ester. H.p.l.c. analysis of peak-II material obtained after paper chromatography indicated the presence of several u.v.-active products but a single major radioactive peak (Fig. 6). The major u.v.-active metabolite had a retention time identical with that of GDP-mannose and was eluted in the same fraction as most of the radioactivity. The u.v.-absorption spectrum of this compound (Fig. 7) was characteristic of a guanine-containing nucleotide (Hurlbert, 1957; A_{280}/A_{260} 0.68 and λ_{max} 252 nm). The molar ratio of guanosine to 4-deoxy-4-fluoro-D-[1- 14 C]mannose was calculated to be 1.05:1.0 (assuming ϵ_{252} 13 700 M $^{-1}$ cm $^{-1}$ for guanosine at pH 7). Additional support for identifying the peak-II material as GDP-4-deoxy-4-fluoro-D-[1- 14 C]mannose was obtained by using a system studied *in vitro*. A crude yeast extract (Braell *et al.*, 1976) supplemented with hexokinase, GTP and glucose 1,6-bisphosphate converted 4-deoxy-4-fluoro-D-[1- 14 C]mannose into a product with chemical, enzymic and chromatographic properties identical with those of peak-II

Table 2. Summary of the chemical and enzymic properties of the ^{14}C -labelled metabolites of 3-deoxy-3-fluoro-D-mannose and 4-deoxy-4-fluoro-D-mannose

Material from each metabolite peak was treated with acid or enzyme(s) as indicated. After the reaction, products were separated by paper chromatography and their mobilities were compared with those of standard compounds prepared by enzymic syntheses *in vitro*. The results of these analyses are tabulated below. Although small differences in mobility were observed for the sugar 6-phosphates and sugar 1-phosphates, these derivatives were not clearly distinguished by chromatography in solvent *A*. Results for the 4-deoxy-4-fluoro-D-[1- ^{14}C]mannose metabolites are shown in Figs. 2 and 5. Data for the 3-fluoro sugar were similar and are not shown.

Peak	Acid	Product of reaction			Assignment of metabolite
		Phosphodiesterase	Phosphodiesterase and alkaline phosphatase	Alkaline phosphatase	
4-F-Man metabolites					
I	4-F-Man- <i>P</i>			4-F-Man	4-F-Man-1,6- <i>P</i> ₂
II	4-F-Man	4-F-Man- <i>P</i>	4-F-Man	No reaction	GDP-4-F-Man
III	No reaction			4-F-Man + 4-F-Fru	4-F-Man-6- <i>P</i> + 4-F-Fru-6- <i>P</i>
3-F-Man metabolites					
V	3-F-Man- <i>P</i>			3-F-Man	3-F-Man-1,6- <i>P</i> ₂
VI	3-F-Man	3-F-Man- <i>P</i>	3-F-Man	No reaction	GDP-3-F-Man
VII	No reaction			3-F-Man	3-F-Man-6- <i>P</i>

material (results not shown). Replacement of GTP with ATP and UTP in this reaction mixture led to a nearly quantitative production of 4-deoxy-4-fluoro-D-[1- ^{14}C]mannose 6-phosphate, with no sugar nucleotide synthesis.

Peak-III material (Fig. 2), which migrated in the hexose monophosphate region of the chromatogram, consisted of hexose 6-phosphate(s), as judged by its stability to mild acid hydrolysis (1M-HCl, 100°C, 15 min). It was converted into a mixture of at least two monosaccharides by treatment with alkaline phosphatase (Fig. 3e). The minor component ($R_{4\text{-F-Man}}^0$ 0.85) migrated in solvent *B* as 4-deoxy-4-fluoro-D-[1- ^{14}C]glucose ($R_{4\text{-F-Man}}^0$ 0.87) and/or 4-deoxy-4-fluoro-D-[1- ^{14}C]fructose ($R_{4\text{-F-Man}}^0$ 0.85). The major component co-chromatographed with 4-deoxy-4-fluoro-D-[1- ^{14}C]mannose. Thus, although 4-deoxy-4-fluoro-D-mannose 6-phosphate can serve as a substrate for mannose phosphate isomerase under the conditions of the labelling experiment, it is primarily the mannose 6-phosphate analogue that accumulates.

Peak-IV material, which co-migrated with 4-deoxy-4-fluoro-D-[1- ^{14}C]mannose during paper chromatography in solvent *A* or *B* (Fig. 3c), appeared to be unchanged fluoro sugar.

Characterization of 3-deoxy-3-fluoro-D-[1- ^{14}C]mannose metabolite peaks

The enzymic and chemical analyses of the 3-deoxy-3-fluoro-D-[1- ^{14}C]mannose metabolites are summarized in Table 2 and described below. The data on which these results are based are similar to

those for the 4-fluoro analogue (Figs. 3 and 5) and are not shown. Mild acid hydrolysis of peak-V material (Fig. 4) produced a monophosphorylated sugar. Treatment of peak-V material with alkaline phosphatase yielded a product that co-migrated with 3-deoxy-3-fluoro-D-mannose during paper chromatography in solvents *A* and *B*. These results are consistent with peak-V material being 3-deoxy-3-fluoro-D-mannose 1,6-bisphosphate, because both 3-deoxy-3-fluoro-D-glucose ($R_{3\text{-F-Man}}^0$ 0.92) and 3-deoxy-3-fluoro-D-fructose ($R_{3\text{-F-Man}}^0$ 0.88) have different mobilities when chromatographed in solvent *B*.

Peak-VI material (Fig. 4) had a mobility similar to that of GDP-4-deoxy-4-fluoro-D-mannose during paper chromatography (solvent *A*) and exhibited the reactivity expected for GDP-3-deoxy-3-fluoro-D-[1- ^{14}C]mannose (Table 2). Treatment of this material with acid produced a compound that co-chromatographed with 3-deoxy-3-fluoro-D-mannose in solvents *A* and *B*. Peak-VI material was converted by phosphodiesterase into a hexose monophosphate that, on further incubation with alkaline phosphatase, yielded a monosaccharide that co-migrated with 3-deoxy-3-fluoro-D-mannose (solvents *A* and *B*). Incubation of peak-VI material with alkaline phosphatase alone produced no reaction. Peak-VI material was eluted from a paper chromatogram and purified by h.p.l.c. (results not shown). The product had a retention time identical with that of GDP-mannose. Analysis of its u.v.-absorption spectrum confirmed that it was a guanosine derivative (A_{280}/A_{260} 0.67 and λ_{max} 252 nm). The calculated molar ratio of guanosine to 3-deoxy-3-fluoro-D-[1- ^{14}C]-

Fig. 5. Characterization of peak-II material from yeast cells labelled with 4-deoxy-4-fluoro-D-[1- 14 C]mannose. Paper strips containing peak-II material were eluted with water and subjected to chemical and enzymic treatments as described in the Materials and methods section. After the reaction, products were analysed by paper chromatography in solvent *A*. (a) Peak-II material; (b) peak-II material treated with acid; (c) peak-II material treated with phosphodiesterase; (d) peak-II material treated with phosphodiesterase and alkaline phosphatase; (e) peak-II material treated with alkaline phosphatase.

mannose was 1.2:1.0. The overall yield of the GDP derivative was quite low.

Peak-VII material had the characteristics of a hexose monophosphate. It possessed the same chromatographic mobility (solvent *A*) as that of 3-deoxy-3-fluoro-D-[1- 14 C]mannose 6-phosphate synthesized *in vitro*. It was stable to mild acid hydrolysis and was converted by alkaline phosphatase into a monosaccharide that co-migrated with 3-deoxy-3-fluoro-D-mannose (solvents *A* and *B*).

Most of the 3-fluoro analogue taken up by the

Fig. 6. H.p.l.c. profile of peak-II material from yeast cells labelled with 4-deoxy-4-fluoro-D-[1- 14 C]mannose. Paper strips containing peak-II material (Fig. 1) were washed twice with diethyl ether and once with acetone before elution of 14 C-labelled material with water. The aqueous extract was examined by h.p.l.c. as described in the Materials and methods section. The effluent was monitored by its u.v. absorption at 254 nm (—) and by liquid-scintillation counting of the radioactivity (-----) of column fractions.

Fig. 7. U.v.-absorption spectrum of peak-II material (fraction D) from yeast cells labelled with 4-deoxy-4-fluoro-D-[1- 14 C]mannose.

Paper strips containing peak-II material (50000 c.p.m.) were eluted with water and purified by h.p.l.c. as described in the Materials and methods section. Purified peak-II material (retention time 5.5–6.0 min) was used for u.v. spectroscopy.

yeast cells was recovered as unphosphorylated products (peaks VIII and IX). Peak-VIII material has not been identified. Peak-IX material appears to be unchanged 3-fluoro sugar on the basis of its mobility in solvents *A* and *B*.

Discussion

The results presented in this paper demonstrate that 3-deoxy-3-fluoro-D-mannose and 4-deoxy-4-fluoro-D-mannose are taken up by yeast cells and metabolized *in vivo* to their GDP esters. The rate of transport of each fluoro sugar was considerably lower than that of 2-deoxyglucose. This result is consistent with the hypothesis that the transport of sugars in yeast cells is coupled to their phosphorylation (Franzosoﬀ & Cirillo, 1982). It is well established that the 3- and 4-hydroxy groups of glucose (and presumably mannose) are important for binding to hexokinase (Anderson *et al.*, 1978), and that modification of either of these groups decreases the rate of sugar phosphorylation (Bessell *et al.*, 1972). Several investigators have reported the release of F⁻ ion during incubations of whole cells with fluoro sugars (Romaschin & Taylor, 1981; Taylor *et al.*, 1976). On the basis of our chromatographic data, we see no evidence for the loss of fluoride from 4-deoxy-4-fluoro-D-mannose during incubation with yeast cells. It is possible that the unidentified 3-deoxy-3-fluoro-D-mannose metabolite in peak VIII is a defluorinated product, but the monosaccharide unit of the phosphorylated derivatives in peaks V, VI and VII exhibits the chromatographic behaviour expected of 3-deoxy-3-fluoro-D-mannose.

4-Deoxy-4-fluoro-D-mannose was metabolized in excellent yield to GDP-4-deoxy-4-fluoro-D-mannose. Although the 6-phosphate derivative of this fluoro sugar apparently is a substrate for mannose phosphate isomerase, most of the analogue was metabolized to the sugar nucleotide under the conditions of the labelling experiment. In addition, 4-deoxy-4-fluoro-D-mannose appeared to be transferred by mannosyltransferases into yeast mannan. In contrast with the 4-fluoro sugar, 3-deoxy-3-fluoro-D-mannose was transported into the cells at a lower rate and was converted into the GDP-sugar much less efficiently. Incorporation of this sugar into cell-wall material was extremely low.

Little is known about the specificity of three enzymes, namely phosphomannomutase, mannose phosphate isomerase and GDP-mannose pyrophosphorylase, that are vital to the metabolic fate of the mannose analogues. The results given in the present paper are suggestive of the relative importance of the 3- and 4-hydroxy groups of mannose for activity with these enzymes, but a more quantitative evaluation awaits studies with a better-defined

system *in vitro*. Preliminary results from studies with 3-deoxy-D-mannose and 4-deoxy-D-mannose indicate that fluorine is a much better substitute for the hydroxy group than is hydrogen. Under conditions similar to those with the fluoro sugars, no phosphorylated derivatives of 3-deoxy-D-[1-¹⁴C]-mannose were detected, and only very low concentrations of radiolabelled phosphorylated products were present after incubations with 4-deoxy-D-[1-¹⁴C]mannose (results not shown).

A number of sugar analogues that are activated to the level of the sugar nucleotide in yeast or mammalian cells have been found to inhibit or modify the normal glycosylation of proteins (Büchsel *et al.*, 1980; Schwarz & Datema, 1980; Sufrin *et al.*, 1980). A fraudulent sugar nucleotide can disrupt oligosaccharide assembly by any one of several mechanisms. It can inhibit glycosyltransferase reactions, alter nucleotide pools, prevent formation of the lipid-linked oligosaccharide dolichyl-*P*-*P*-GlcNAc₂Man₉Glc₃ that is required for protein glycosylation, or block addition of the terminal sugars of complex glycoproteins. The fact that 3-deoxy-3-fluoro-D-mannose and 4-deoxy-4-fluoro-D-mannose are metabolized to their GDP esters by yeast cells suggests that these analogues may have interesting effects on protein glycosylation.

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