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The metabolism of 4-deoxy-4-fluoro-D-glucose in *Pseudomonas putida**

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ABSTRACT

The synthesis of 4-deoxy-4-fluoro-D-[U-^{14}C]glucose from D-[U-^{14}C]galactose is reported. A 24-h incubation of *Pseudomonas putida* with 4-deoxy-4-fluoro-D-[U-^{14}C]glucose gives 95 ± 5% release of fluoride and 4.8 ± 0.2% of the initial radioactivity as $^{14}CO_2$. After centrifugation, Dowex-1 [borate 2^-] column chromatography of the cell supernatant, which amounts for 52.4 ± 1.3% of the initial radioactivity, allows the isolation of a major radioactive metabolite. By ^{13}C- and ^{1}H-n.m.r. spectroscopy and by mass spectrometric analysis, this metabolite is identified as 2,3-dideoxy-D-*glycero*-pentonic acid. Extensive dialysis of the remaining cell pellet, followed by sonication and appropriate centrifugation, allows isolation of a cell envelope fraction with 0.4 ±0.05% of the initial radioactivity. Gel filtration of this sodium dodecylsulphate solubilised fraction shows all the radioactivity to be in a large molecular weight peptidoglycan–protein complex (> 400 000 daltons). Following lysozyme treatment, this complex now elutes from the same column with a lower molecular weight (< 14 000 daltons). The radioactivity of the peptidoglycan complex is shown to be due to the presence of aspartate, threonine, and glutamate.

INTRODUCTION

Reports of deoxyfluoro sugars in which biochemical cleavage of the carbon–fluorine bond occurs are not numerous. They include the action of *α*- and *β*-glucosidases on glycosyl fluorides[1] and the metabolism of 3-deoxy-3-fluoro-D-glucose in *Locusta migratoria*[2,3]. Our earlier studies on the metabolism of 4-deoxy-4-fluoro-D-glucose (4FG) in *Pseudomonas putida* demonstrated that the sugar was not oxidized but was extensively defluorinated (95%) by whole cells of this organism[4]. The specificity of this defluorination is illustrated by the fact that 3-deoxy-3-fluoro-D-glucose is oxidized in *P. putida*, with retention of the C–F bond, to 3-deoxy-3-fluoro-D-gluconic acid and 3-deoxy-3-fluoro-D-*arabino*-2-hexulosonic acid[5]. It was also shown that the defluorination of 4FG was markedly inhibited by prior treatment of the cells with chloramphenicol or when the cells were grown on succinate instead of glucose. Furthermore, fluoride release was almost completely prevented when cells were incubated with 4FG in the presence of glucose, gluconate, or D-*arabino*-2-hexulosonic acid. Based on these results and preliminary cell envelope fractionation studies, it was suggested, therefore,

* Abbreviations: 4FG, 4-deoxy-4-fluoro-D-glucose; D-[U-^{14}C]-4FG, 4-deoxy-4-fluoro-D-[U-^{14}C]glucose; SDS, sodium dodecylsulphate; f.a.b., fast atom bombardment.

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that the defluorination may be due to the presence of an inducible/repressible outer-membrane protein[4].

Further studies to elucidate the site of defluorination were undertaken with tritium-labeled 4FG. Incubation of *P. putida* with 4-deoxy-4-fluoro-D-[6-^{3}H]glucose[6], however, gave an unexpected extensive loss of tritium as tritiated water[7].

In view of this extensive loss of radioactivity from tritiated 4FG, we have now re-examined the interaction of 4FG with *P. putida* using a carbon-14 labeled form of the sugar. In this communication we wish to report: (*i*) The synthesis of D-[U-^{14}C]-4FG. (*ii*) The incubation of *P. putida* with D-[U-^{14}C]-4FG for 24 h, followed by cell fractionation and an analysis of the distribution of the radioactive components. (*iii*) The isolation and structural elucidation of a major non-fluorinated metabolite. A preliminary account of some of these results has appeared elsewhere[8].

MATERIALS AND METHODS

General. — Crystalline D-[U-^{14}C]-4FG (specific activity, 10 600 d.p.m.·μmol^{-1}) was obtained, as outlined below, by modification of previously published methods[9,10] for the synthesis of 4FG. D-[U-^{14}C]-galactose (specific activity, 57 μCi·μmol^{-1}) was from Amersham Corp. (Amersham, U.K.). The sodium salt of 2,3-dideoxy-D,L-*glycero*-pentonic acid was prepared as described in the following section from 5-hydroxy-4-valerolactone[11]. Diethylaminosulphur trifluoride (DAST) and 4-pentenoic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). SDS, Bio-Gel A-1.5m (200–400 mesh A.S.T.M. wet mesh), and glass chromatography columns were from Bio-Rad Laboratories (Richmond, CA). Dowex-1-X8 [Cl^-] (200–400 dry mesh A.S.T.M.) anion exchange resin, Dowex50W-X8 [H^+] (200–400 dry mesh A.S.T.M.) cation-exchange resin, and enzymes were obtained from Sigma Chemical Co. (St. Louis, MO). Scintillation-grade toluene, 2,5-diphenyloxazole (PPO), and 1,4-bis(5-phenyloxazol-2-yl)-benzene (POPOP) were obtained from BDH Chemicals, Ltd. (Toronto, Ont.), and scintillation vials were purchased from Packard Instrument Canada, Ltd. (Mississauga, Ont.). Thin-layer chromatography (t.l.c.) was carried out on Silica Gel-60 sheets (Merck, Darmstadt, Germany) using 3:3:1 (v/v) ethyl acetate–acetic acid–water and a 1:1 (v/v) sulphuric acid–ethanol spray, with subsequent charring for 20 min at 140° for detection. Melting points were determined with a Fisher–Johns melting point apparatus and are uncorrected.

*Synthesis of radiolabeled 4FG and 2,3-dideoxy-*D,L-glycero-*pentonic acid. Methyl α-*D-*[U-^{14}C]galactopyranoside monohydrate* (1). — To D-[U-^{14}C]galactose (200 μCi) was added unlabeled D-galactose (7 g, 38 mmol) and a 2% (w/v) solution of hydrogen chloride in absolute methanol (56 mL). After 8 h heating under reflux, the warm solution was neutralized by stirring with lead carbonate (8 g) for 3 h and filtered through a bed of Kieselguhr. The filtrate and methanol washings (200 mL) were evaporated *in vacuo* to a syrup which was mixed with water (2.0 mL). After standing for 24 h, the product 1 crystallised. The crystals were collected and washed with methanol (30 mL, chilled at 4°) to yield 2.1 g of product. The remaining mother liquor and methanol

washings were combined and dried by repeated evaporation *in vacuo* with methanol (4 × 100 mL) at 40–45°. The resulting syryp was submitted to an 8 h heating under reflux with a 2% solution of hydrogen chloride in methanol (56 mL), and **1** was isolated (1.3 g) as before. This recycling procedure was repeated twice to give a further quantity of product (1.95 g). The title compound **1** (total yield, 5.3 g, 64%) had a melting range 80–100° (Lit.[12], 75–99°).

*Methyl 2,3,6-tri-*O*-benzoyl-α-*D*-[U-^{14}C]galactopyranoside* (**2**). — Benzoyl chloride (9.6 mL, 82.0 mmol) was added dropwise to a stirred solution of **1** (3.6 g, 17 mmol) in anhydrous pyridine (110 mL) at −30° (dry ice–acetone bath). Stirring was continued for 2 h at 30°, for 24 h at 4°, and then for two days at room temperature. The product **2** was isolated as reported[13] and crystallised from ethanol. Yield, 6.0 g (69%); m.p. 138–140° (Lit.[13] 139–140°).

*Methyl 2,3,6-tri-*O*-benzoyl-4-deoxy-4-fluoro-α-*D*-[U-^{14}C]-glucopyranoside* (**3**). — To a stirred solution of **2** (4.8 g, 9.5 mmol) in methylene chloride (22 mL) at −40° (dry ice–acetone bath) was added diethylaminosulphur trifluoride (3.5 mL, 26.5 mmol), and the solution was allowed to warm to room temperature. After stirring for a further 28 h, the reaction mixture was cooled to 0° and quenched by the dropwise addition of methanol (15 mL), then poured into a saturated solution of sodium bicarbonate (100 mL). Extraction into methylene chloride (400 mL), followed by drying and concentration *in vacuo*, afforded a syrup which crystallised upon standing overnight. Recrystallisation from absolute ethanol (80 mL) after standing overnight gave **3** (3.2 g, 66%); m.p. 141–142° (Lit.[14], 139–140°).

*Methyl 4-deoxy-4-fluoro-α-*D*-[U-^{14}C]glucopyranoside* (**4**). — To a freshly prepared solution of sodium methoxide [prepared by addition of sodium (0.24 g, 10.4 mmol) to anhydrous methanol (4.1 mL)] was added dropwise to a stirred solution of **3** (3.2 g, 6.3 mmol) in anhydrous methanol (24 mL) at 0–4° (ice-bath). The reaction mixture was stirred for a further 16 h at 4° and then neutralized with Amberlite IR-120 [H^+] resin. The resin was removed by filtration and washed with methanol (2 × 50 mL). The filtrate and washings were concentrated *in vacuo* to a syrup which was extracted with a mixture of chloroform (100 mL) and water (100 mL). The aqueous layer was evaporated to dryness *in vacuo* to give **4** which was recrystallised from ethyl acetate–acetone. Yield, 1.0 g (81%); m.p. 123–124° (lit.[10], 129–130°).

*4-Deoxy-4-fluoro-*D*-[U-^{14}C]glucose* (**5**). — Compound **5** was obtained in higher yields than previously reported[9] by Dowex50W-X8 [H^+] treatment of **4** (1 g, 5.1 mmol)[10]. Yield, 0.75 g (78%); m.p. 188–191° alone or with admixture of authentic 4FG[9]. R_F 0.7 (ethyl acetate–acetic acid–water); specific activity, 10 600 ± 100 d.p.m.·μmol^{-1}.

*Sodium 2,3-dideoxy-*D,L*-glycero-pentonate* (**6**). — 5-hydroxy-4-valerolactone (1.0 g, 8.6 mmol), prepared from 4-pentenoic acid[11], was dissolved in water (100 mL). A 5.0-mL aliquot of this solution was heated to 70–80° and titrated against 0.2M sodium hydroxide to a light pink phenolphthalein end point which was stable for 1 h at this temperature. To the remaining non-titrated solution of the lactone was added the proportionate volume of 0.2M sodium hydroxide to attain the above end-point. This solution was heated for 1 h at 80° and allowed to cool overnight. After extraction of the

solution with chloroform (2 × 100 mL), concentration of the aqueous layer *in vacuo* (bath temperature, 40–45°) gave a chromatographically pure syrup, (R_f 0.76), which was dried overnight in the presence of P_2O_5 in a vacuum desiccator. The product **6** was obtained as a thick colourless, hygroscopic syrup. ^{13}C-n.m.r. data (0.8M in D_2O, 75 MHz): δ (proton-decoupled) 30.06, 34.55, 66.12, 72.48, and 183.85; δ (proton-coupled) 30.01 (t, C-3), 34.50 (t, C-2), 66.07 (t, C-5), 72.42 (d, C-4), and 183.83 (s, C-1).

Culture conditions and organism. — *Pseudomonas putida*, biotype A (ATCC 12633) was routinely maintained at 30° on a glucose–mineral salts agar medium and harvested after 15 h as previously reported[15]. Protein determinations were by the method of Lowry *et al.*[16] using bovine serum albumin as a standard.

Radiolabeling and fractionation of whole cells. — (*i*) *Supernatant fraction.* Glucose-grown cells (600 mg protein) were incubated in the presence of 1mM D-[U-^{14}C]-4FG in a 0.1M potassium phosphate buffer (total volume, 100 mL, pH 7.1) for 24 h at 30° on a rotary shaker (Lab-Line Instruments, Inc., Melrose Park, ILL.). Centrifugation at 4000g for 25 min at 25° gave a supernatant in which 95 ±5% fluoride release was detected with a fluoride-sensitive electrode (Orion Research, Cambridge, MA). This supernatant was stored at −20° for radioactivity measurement and metabolite isolation. (*ii*) *Cell-envelope fraction.* The remaining cell pellet was suspended in 0.1M potassium phosphate buffer (10 mL, pH 7.1) and placed in a dialysis bag (molecular weight cut-off, 12 000 daltons) and then dialysed against the same buffer, (1.25 L containing 0.1% sodium azide), for 3 × 12 h periods at 4°. The dialysed cells were collected by centrifugation at 4000g at 4° for 25 min. The cell pellet, was suspended in 0.1M sodium phosphate buffer (5 mL, pH 7.1) and ultrasonicated as previously reported[17] to obtain an extract. After centrifugation of this extract at 17 000g at 4° for 10 min, the resulting supernatant was centrifuged at 100 000g for 90 min at 4° to give a cell-free extract and a pellet which, after washing and suspending in 0.1M sodium phosphate buffer, is referred to as the cell-envelope fraction.

Liquid scintillation counting. — Radioactivity in the various samples was measured with a Beckman LS7500 counter (Beckman Instruments, Inc., Fullerton, CA) using a cocktail (10 mL) consisting of a mixture of 2,5-diphenyloxazole (10 g), 1,4-bis(5-phenyloxazol-2-yl)-benzene (0.2 g), and Triton X-100 (333 mL) in scintillation-grade toluene (667 mL). In all cases enough water was added to the samples to ensure that a clear solution was obtained after vigorous mixing with the scintillation cocktail.

Measurement of $^{14}CO_2$. — $^{14}CO_2$ was measured with a Gilson differential respirometer (Gilson Medical Electronics, Middleton, WI). The flask contents [centre well: 20% aq. KOH (400 μL); outer well: *P. putida.* (6.5 mg protein·mL^{-1}) in 0.1M potassium phosphate buffer (pH 7.1) and 1mM D-[U-^{14}C]-4FG in a total volume of 4.0 mL] were incubated for 24 h at 30°. Aliquots (200 μL) of the centre well contents were added to the scintillation cocktail (10 mL) plus water (3 mL). After allowing the cocktail mixtures to stand overnight (to allow chemiluminescence to subside) trapped $^{14}CO_2$ was counted.

Separation and isolation of the radiolabeled metabolite. — This separation was performed on a borosilicate glass column of Dowex1-X8 [Cl^-] anion-exchange resin (a 108 × 1-cm bed) by first converting it to the hydroxide form and then neutralizing the

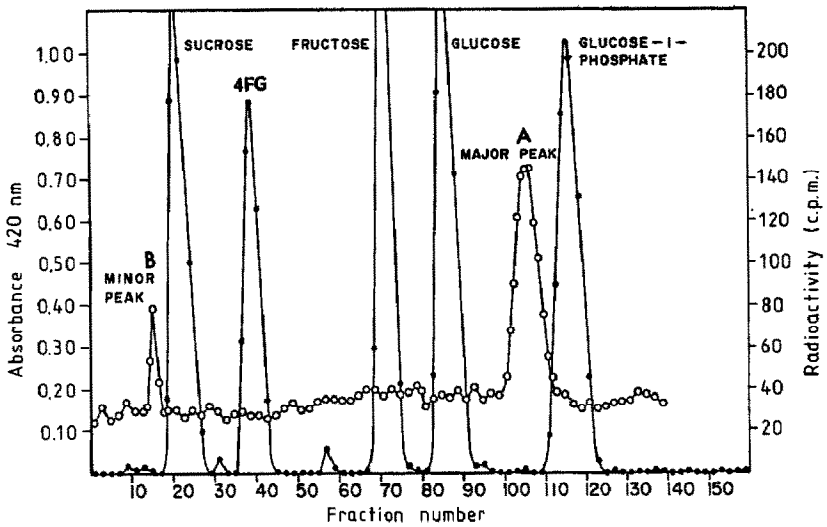

Fig. 1. Dowex1-X8 [borate^{2-}] column chromatographic analysis of the supernatant fraction after incubation of *P. putida* in 1mM D-[U-^{14}C]-4FG: –o–o–o–, radioactivity; –●–●–●–, non-radiolabeled standards. All compounds were eluted with a linear gradient of ammonium tetraborate (see Materials and Methods).

resin with 0.5M boric acid as described by Floridi[18]. All analyses were performed at room temperature. The column of resin was equilibrated with 0.029M ammonium tetraborate–0.057M boric acid buffer (pH 8.5–8.8) prior to every analysis. The supernatant (2.0 mL), obtained after a 24-h incubation of 1mM D-[U-^{14}C]-4FG with *P. putida* as described above, was applied to the column. A linear gradient of ammonium tetraborate [0.029M ammonium tetraborate–0.057M boric acid (600 mL, pH 8.5–8.8) diluted linearly with 0.5M ammonium tetraborate (600 mL, pH 8.9–9.2)] was used for elution. Various sugar standards (Fig. 1) were added to a sample of the supernatant prior to its application on the column. Fractions (4.2 mL) were collected and analysed at 420 nm for carbohydrate by orcinol–sulphuric acid colorimetry[18] and for radioactivity by liquid scintillation counting (1 mL from each fraction). For preparative isolation of the metabolite, the supernatant (30 mL) was concentrated to 3 mL *in vacuo*, prior to application to a slightly larger column (112 × 1.5 cm bed of resin), and no internal standards were added. Fractions containing the major radioactive peak (Fig. 1) were pooled, and ammonium tetraborate was removed by repeated distillation and concentration to dryness with methanol *in vacuo*. The resulting residue was solubilised in D_2O (1 mL) and used directly for subsequent n.m.r. and f.a.b. mass spectroscopic analysis.

Fractionation of the cell envelope. — After incubation of whole cells with 1mM D-[U-^{14}C]-4FG and fractionation as described above, a suspension of the cell-envelope fraction was divided into two equal portions. Each portion was incubated for 1 h at 30° in 0.1M sodium phosphate buffer (pH, 7.1) in the absence or presence of lysozyme (final concentration 1 mg·mL^{-1}). The two fractions were then solubilised with 1% SDS in the same buffer for 30 min at 65°, and each was submitted to gel filtration on a bed of Bio-Gel A-1.5 m (180 mL). The columns were eluted with the same buffer containing

0.1% SDS and 0.05% sodium azide. Fractions (1.4 mL) were collected and assayed for absorbance at 280 nm and radioactivity (Figs. 2 and 3).

N.m.r. spectroscopy and f.a.b. mass spectrometry. — ^{13}C- and ^{1}H-n.m.r. spectra were determined with a General Electric QE-300 n.m.r. spectrometer in the Fourier-transform mode, operating at 75 and 300 MHz, respectively. Spectra were obtained on samples in 5-mm n.m.r. tubes at 23° with deuterium oxide as the solvent. All chemical shifts (δ) are reported in p.p.m. downfield from external tetramethylsilane. Negative-ion

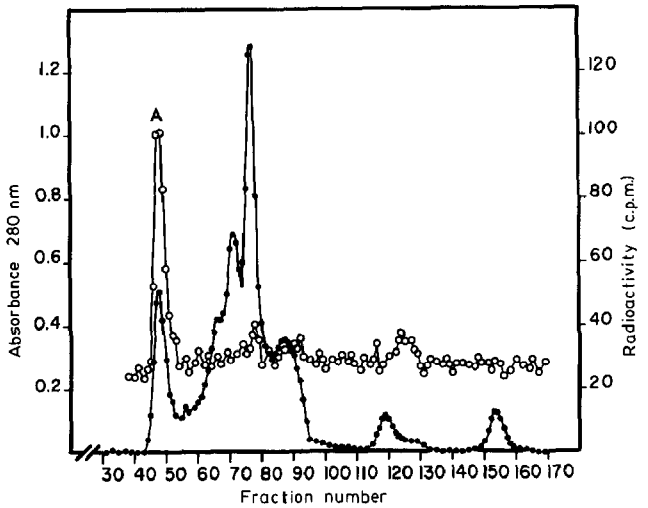

Fig. 2. Gel filtration of the cell envelope fraction obtained from *P. putida* after incubation with 1 mM D-[U-^{14}C]-4FG: –o–o–o–, radioactivity; –●–●–●–, absorption at 280 nm (see Materials and Methods).

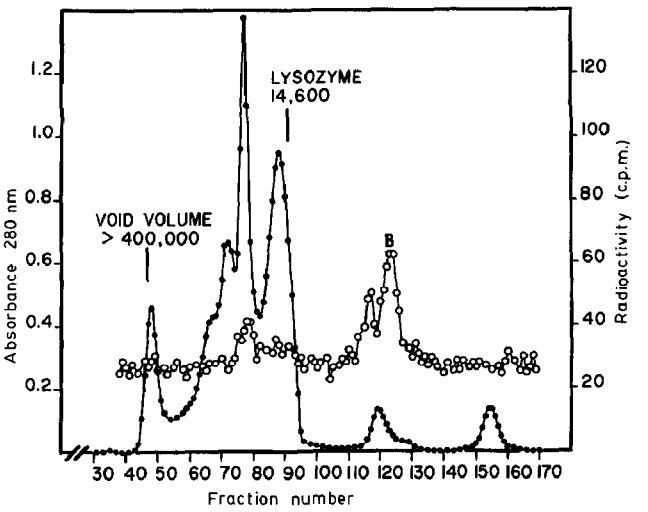

Fig. 3. Gel filtration of the lysozyme-treated cell envelope fraction from *P. putida* after incubation with D-[U-^{14}C]-4FG: –o–o–o–, radioactivity; –●–●–●–, absorption at 280 nm in the presence of lysozyme. The vertical bars correspond to the molecular weight range (see Materials and Methods).

f.a.b. mass spectroscopic analysis was performed on a Finnigan-M.A.T. CH5-DF mass spectrometer. The f.a.b. gun was set at 4 watts (4 kV × 1 mA), and glycerol was used as the sample matrix. Samples were run in deuterium oxide.

RESULTS AND DISCUSSION

A determination of the distribution of radiolabel in various cell fractions, obtained after incubation of 1mM D-[U-^{14}C]-4FG with *P. putida* for 24 h, showed that most of the initial radioactivity appeared in the supernatant (52.4 ±1.3%), cell dialysate (34.8 ±4.5%), and cell-free extract (5.17 ±0.15%). Radiolabel was also found in trapped $^{14}CO_2$ (4.8 ±0.2%), and a small but significant amount of label was found in the cell-envelope fraction (0.42 ±0.04%). As previously reported[4], fluoride was also detected (95 ±5%) in the supernatant. When the supernatant fraction was submitted to Dowex-1 [borate] column chromatography and eluted with a linear ammonium tetraborate gradient, the labeled material was resolved into a poorly retained minor peak B and a strongly retained major peak A (Fig. 1). Preparative isolation of peak A yielded a single product on t.l.c. [R_f 0.76 (ethyl acetate–acetic acid–water)]. A 75-MHz proton-decoupled ^{13}C-n.m.r. spectrum of this product indicated the presence of five carbon atoms with resonances at δ 29.57, 34.03, 65.65, 72.03, and 183.28. A 75-MHz proton-coupled ^{13}C-n.m.r. spectrum indicated the presence of one –CH (δ 72.03), three –CH_2s (29.57, 34.03, and 65.65), and one carbonyl group (183.28), which were confirmed by DEPT editing. In the 300-MHz ^{1}H-n.m.r. spectrum, four resonances were found. The chemical shift of two of these, δ 3.67 (1H) and 3.53 (2H), correspond to protons on oxygenated carbon and another, δ 2.27 (2H), corresponds to protons adjacent to a carbonyl group. No methyl groups were evident, ensuring that the chain was terminated by an oxygenated carbon. 2D ^{1}H-n.m.r. (COSY, 90°-pulse sequence) allowed complete determination of the structure (data not shown). Cross-peaks (coupling) appeared only between protons located at C-2 and C-3, C-3 and C-4, and C-4 and C-5. The structure of 2,3-dideoxy-D-*glycero*-pentonic acid is the only plausible one which will accommodate these data. The stereochemistry at C-4 is probably of the D-configuration, although this is not unequivocally established. Additional evidence for the structure of 2,3-dideoxy-D-*glycero*-pentonic acid was provided by an f.a.b. mass spectrum which yielded a single intense peak with a m/z = 133. This corresponds to the molecular weight of the carboxylate ion. A final confirmation of the structure was provided when the ^{13}C proton-decoupled and coupled n.m.r. spectrum of the metabolite was shown to be identical to synthetic 2,3-dideoxy-DL-*glycero*-pentonic acid (**6**) (see Materials and Methods). The latter was prepared by titration of the authentic γ-lactone of 2,3-dideoxy-DL-*glycero*-pentonic acid[11] with 0.2N sodium hydroxide. Based on a chromatographic comparison the minor peak B, detected with Dowex-1 [borate] column chromatography (Fig. 1), is considered to be due to the formation of this γ-lactone from the acid.

The non-dialysable C^{14}-labeled material, incorporated into the cell-envelope fraction (0.42 ±0.04%) after solubilisation in SDS, was submitted to gel filtration. As

can be seen (Fig. 2), all the radiolabel is associated with a component (peak A) which elutes with the void volume and has, therefore, a molecular weight >400 000. Treatment of this cell-envelope fraction with lysozyme, prior to SDS solubilisation and identical gel filtration, gave a labeled material (Fig. 3, peak B) with a molecular weight <14 000. Furthermore, the amount of material absorbing at 280 nm was unchanged. The results suggest a covalent attachment of radiolabel to the peptidoglycan in the cell envelope which is degraded to smaller fragments by lysozyme. ^{19}F Fourier-transform n.m.r. analysis of this cell-envelope fraction failed to detect the presence of fluorine. Although the amount of radioactivity found in the peptidoglycan is small, it is important to establish whether or not this is due to the presence of a carbohydrate analogue derived from 4FG. For example, the incorporation of 4-deoxy-*N*-acetylglucosamine (a possible defluorinated metabolite of 4FG) into the peptidoglycan could terminate cell-wall biosynthesis and hence cell growth. Recently, we have incubated *P. putida* with D-[U-^{14}C]-4FG and isolated the peptidoglycan by established procedures[19,20]. H.p.l.c. analysis of the peptidoglycan acid hydrolysates showed that only aspartate, threonine, and glutamate contained significant radioactivity. No radioactivity could be detected in *N*-acetylglucosamine, *N*-acetylmuranic acid, or in any new carbohydrate components. The incorporation of these amino acids into peptidoglycan would be consistent with the formation of $^{14}CO_2$ (4.83 $\pm$0.2%) from 4FG and the operation of known biosynthetic enzymes (*e.g.*, glycine synthase, pyruvate carboxylase, and aspartate transaminase). The results were confirmed[21] by the incubation of *P. putida* with $NaH^{14}CO_3$ which showed a similar radiolabeled peptidoglycan pattern.

Our results demonstrate that over a long incubation period (24 h), 4FG is metabolised in *P. putida* with an extensive release of fluoride ion (>95%) and the formation of CO_2, some of which is incorporated into the amino acids of the cell wall. In addition, a final major end-product metabolite of the defluorinated sugar is shown to be 2,3-dideoxy-D-*glycero*-pentonic acid. The detailed biochemical fate of 4FG remains to be elucidated. However, when D-[6-^3H]-4FG is incubated with *P. putida*, tritium loss at C-6 (as ^3H_2O) occurs subsequent to defluorination[7]. A pathway which may account for this observation and the formation of 4-deoxy-D-*xylo*-hexonic acid 6-phosphate (**11**) is shown (Fig. 4). Interaction of 4FG with an extra-cellular inducible/repressible protein[4], followed by elimination of HF, gives the unsaturated sugar **7**. Tautomeric equilibration of the acyclic isomers of **7** (**8**$\rightleftharpoons$**9**$\rightleftharpoons$**10**) would allow exchange of tritrium at C-6. The transport of **9** into the cell, followed by phosphorylation and action of the appropriate reductase and dehydrogenase, would lead to the formation of **11**. The aldonic acid **11** is an attractive analogue substrate for 6-phosphogluconate dehydratase, one of the Entner–Doudoroff enzymes, known to be active in *P. putida*[22]. Decarboxylation of the product **12**, followed by oxidation and the action of a phosphatase, would account for the observed formation of CO_2 and 2,3-dideoxy-D-*glycero*-pentonic acid (**13**).

Shorter time-profile incubation and further cell fractionation studies are in progress in order to establish the mechanism of defluorination of 4FG and determine whether the above or alternative pathways operate.

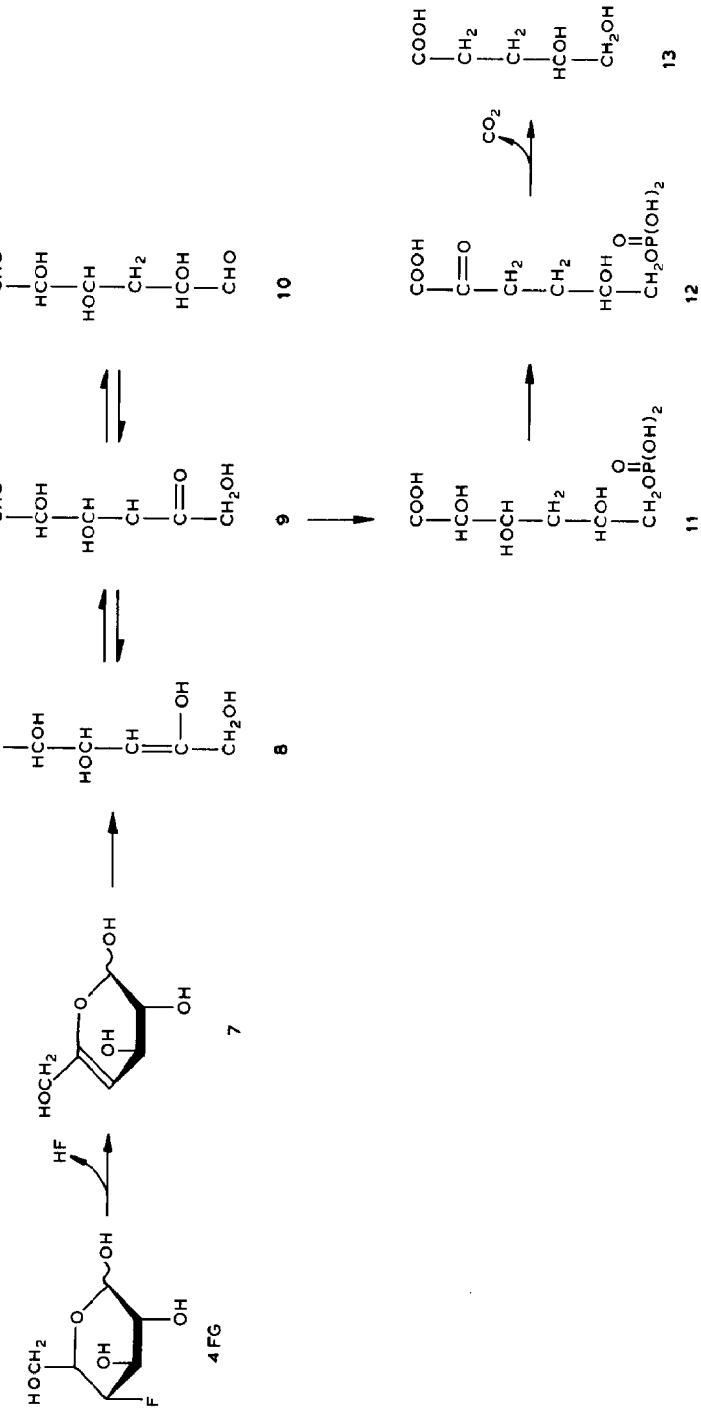

Fig. 4. Possible pathway in *P. putida* for the formation of 2,3-dideoxy-D-*glycero*-pentonic acid (**13**) from 4FG.

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