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Identification of 5-fluoro-5-deoxy-D-ribose-1-phosphate as an intermediate in fluorometabolite biosynthesis in *Streptomyces cattleya*

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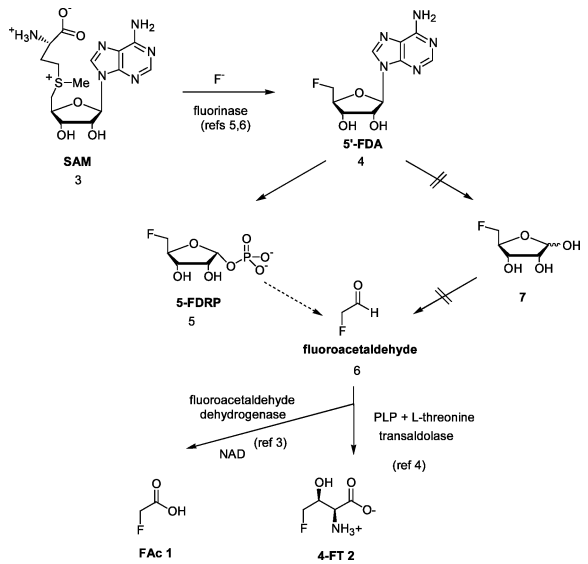
5′-Fluoro-5′-deoxy-D-ribose-1-phosphate (FDRP) is identified as a biosynthetic intermediate during fluorometabolite biosynthesis in *Streptomyces cattleya*.

Streptomyces cattleya is unusual in that it elaborates the organofluorine metabolites, fluoroacetate **1** and 4-fluorothreonine **2**.[1] It is only one of two bacteria identified with a capacity to biosynthesise fluorine containing metabolites.[2] Our studies on the biosynthesis of fluoroacetate (FAc) **1** and 4-fluorothreonine (4-FT) **2** in this microorganism have identified several of the enzymes[3–6] on the fluorometabolite pathway.

Most recently a fluorination enzyme was identified which mediates a reaction between *S*-adenosyl-L-methionine (SAM) **3** and fluoride ion to generate 5′-fluoro-5′-deoxyadenosine (5′-FDA) **4** as the first committed step on the pathway.[5,6] Earlier studies have revealed the later steps on the biosynthetic pathway. An NAD dependent aldehyde dehydrogenase was purified which oxidises fluoroacetaldehyde **6** to FAc **1**[3] and a pyridoxal phosphate (PLP) dependent transaldolase was isolated[4] which mediates a reaction between fluoroacetaldehyde **6** and L-threonine to generate 4-FT **2** and acetaldehyde. These enzymes and a previous isotope labelling study[7] clearly suggested a role for fluoroacetaldehyde **6** as the last common intermediate to FAc **1** and 4-FT **2**. The identity of fluoroacetaldehyde was confirmed unambiguously in an experiment where 5′-FDA was incubated with a crude cell free extract from *S. cattleya.*[8] This experiment also revealed additional ^{19}F-NMR signals of unknown identity and time course experiments suggested that they were metabolic intermediates in fluorometabolite biosynthesis. The known biosynthetic reactions involved in fluorometabolite biosynthesis in *S. cattleya* are summarised in Scheme 1. In this communication we describe results which indicate that 5-fluoro-5-deoxy-D-ribose-1-phosphate (5-FDRP) is the next formed intermediate after 5′-FDA on the biosynthetic pathway to **1** and **2**.



Scheme 1 Summary of the known intermediates in fluorometabolite biosynthesis in *S. cattleya*.

At the outset it appeared appropriate to explore a role for 5-fluoro-5-deoxyribose **7**, a potential hydrolysis product of 5′-FDA **4**. To this end a sample of 5-fluoro-5-deoxyribose **7** was synthesised[9] and incubated with a cell free extract from *S. cattleya* using our established protocol.[8] Ribose **7** was not biotransformed to either FAc **1** and 4-FT **2** nor did it undergo any identifiable metabolism and it remained stable in the extract. The characteristic signals (α and β anomers) in the ^{19}F-NMR spectrum of **7** in various buffers did not coincide with any of the unknown fluorinated intermediates identified in the earlier experiments. Accordingly it is concluded that 5-fluoro-5-deoxyribose **7** is not an intermediate on the biosynthetic pathway for fluorometabolite production by *Streptomyces cattleya*.

Nucleoside phosphorylases act to displace nucleoside bases and generate sugar phosphates.[10] In order to explore the role of a purine nucleoside phosphorylase (PNP) during fluorometabolite biosynthesis it was necessary to prepare a sample of 5-FDRP **5** as a reference compound. This was achieved using a commercially available bacterial PNP which converts inosine and free phosphate to hypoxanthine and ribose-1-phosphate. When this enzyme was incubated with the unnatural substrate 5′-fluoro-5′-deoxyinosine (5′-FDI)[11] it efficiently mediated a phosphorolysis reaction to generate xanthine (detected by UV-HPLC) and 5-FDRP **5**. The identity of **5** was confirmed directly by ^{19}F-NMR,[12] electrospray mass spectrosocopy (ES-MS) and after derivatisation by gas chromatography mass spectroscopy (GC-MS). Indirect conformation was achieved by subsequent treatment of the product with a high activity phosphatase.[13] This resulted in the accumulation of 5′-fluoro-5′-deoxy-D-ribose **7** which had an identical ^{19}F-NMR pattern to synthetic **7** discussed above. Incubation of **5**[14] with a cell free protein extract from *S. cattleya* resulted in the accumulation of FAc **1** as determined by ^{19}F-NMR, indicating *in vitro* biotransformation and supporting its role as an intermediate on the biosynthetic pathway. Further purification[15] of the cell free extract from *S. cattleya* led to a partially purified protein fraction which was able to convert 5′-FDA **4** to 5-FDRP **5**, a reaction that could be monitored both by HPLC and by ^{19}F{^{1}H}-NMR (Fig. 1). The

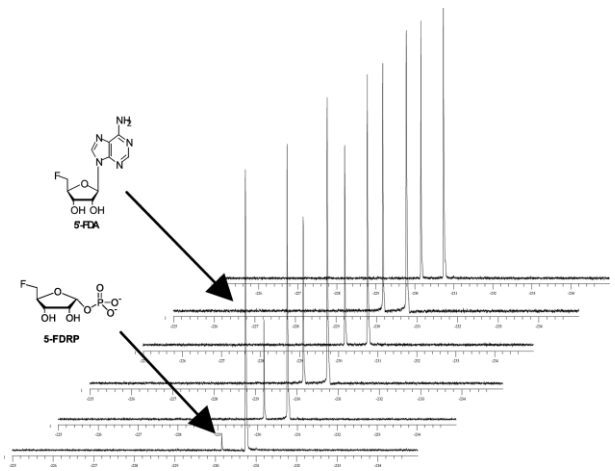


Fig. 1 ^{19}F{^{1}H} NMR (470 Hz) spectra, recorded hourly for 6 h, showing the bioconversion of 5′-FDA **4** to 5-′FDRP **5** using a partially purified protein extract from *S. cattleya.*

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presence of 5-FDRP **5** in the resultant reaction mixture was also confirmed by ES-MS and GC-MS analysis.

In summary 5-FDRP is identified as the next intermediate after 5-FDA in fluorometabolite biosynthesis in *S. cattleya*, a process that is mediated by a purine nucleoside phosphorylase. Purification and characterisation of this enzyme are currently under investigation. The remaining intermediates and enzymes between 5-FDRP **5** and fluoroacetaldehyde **6** on the fluorometabolite pathway in *S. cattleya* remain to be identified.

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11 5′-Fluoro-5′-deoxinosine was prepared from 5′-fluoro-5′-deoxyadenosine **4** (5′-FDA)[8] using the commercially available enzyme, 5′-adenylic acid deaminase from *Aspergillus species* (Sigma Chemical Co., A-1907). Selected spectroscopic data for 5′-fluoro-5′-deoxinosine: ^{1}H NMR (DMSO-d_6, 300 MHz): δ/ppm 8.23 (s, 1H, 2H/8H), 8.09 (s, 1H, 2H/8H), 5.92 (d, 1H, $J_{1',2'}$ = 4.8 Hz, 1′H), 4.64 (dm, 2H, $J_{CH2,F}$ = 47.5, CH_2), 4.51 (t, 1H, $J_{2',1'}$ = 4.8 Hz, 2′H), 4.20 (m, 1H, 3′H), 4.13 (dm, 1H, $J_{4',F}$ = 24.3 Hz, 4′H); ^{13}C NMR (DMSO-d_6, 75 MHz): 156.6, 148.2, 146.0 (C2/C8), 138.5 (C2/C8), 124.4, 87.6 (C1′), 84.3 (d, J = 168.6 Hz, C5′), 82.5 (d, J = 18.2 Hz, C4′), 73.5 (d, J = 1.6Hz, C′2), 69.3 (d, J = 6.1 Hz, C3′); ^{19}F NMR (DMSO-d_6, 282 Hz): δ −227.71 (dt, $J_{F,CH2}$ = 47.5 Hz and $J_{F,4'}$ = 24.2 Hz); ESMS (−ve): 269 (M − H)$^+$.
12 5′-Fluoro-5′-deoxy-D-ribose-1-phosphate (5′-FDRP) **5** was prepared from 5′-fluoro-5′-deoxinosine using a commercially available bacterial PNP enzyme (EC. 2.4.2.1, Sigma Chemical Co., unknown bacterial source, N-8264). Selected spectroscopic data for **5**:^{19}F NMR (10% D_2O, 470 Hz): δ −229.28 (dt, $J_{F,CH2}$ = 47.07 Hz and $J_{F,4'}$ = 28.07 Hz); ES-MS (−ve): 231 (M − H)$^-$. GC-EIMS data for pertrimethylsilyl derivative of **5**: m/z = 299 (100), 73 (96), 315 (53), 300 (24), 140 (24), 147 (20), 211 (21), 505 (16), 353 (14), 382 (11), 369 (10).
13 Dephosphorylation of **5** was achieved using a phytase enzyme from *Aspergillus ficuum* (EC. 3.1.3.8, Sigma Chemical. Co., P-9792).
14 5′-Fluoro-5′-deoxy-D-ribose-1-phosphate **5** (10mM) was incubated in a crude cell free extract from *S. cattleya* inTris-HCl buffer (50 mM, pH 7.8) at 37 °C for 16 h. The presence of FAc **1** and 4-FT **2** was confirmed by ^{19}F NMR analysis.
15 *S. cattleya* purine nucleoside phosphorylase (PNP) was partially purified by ammonium sulfate precipitation (35–50%) (total protein 56.2 mg), phenyl high performance hydrophobic interaction chromatography (4.89 mg), and 15Q anion exchange chromatography (2.97 mg) respectively. Enzyme activity at each stage of the purification was established by incubating the active PNP fraction with 5′-FDA **4** and monitoring by HPLC-UV for adenine release and then ^{19}F NMR and ESMS to confirm the presence of 5′-fluoro-5′-deoxy-D-ribose-1-phosphate **5**.