Fluorinase mediated C–^{18}F bond formation, an enzymatic tool for PET labelling†

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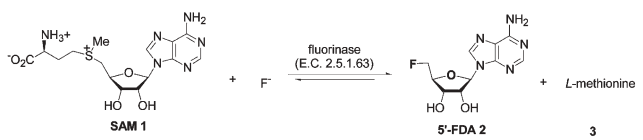
The fluorinase enzyme from *S. cattleya* is applied as a catalyst for the efficient incorporation of [^{18}F]-fluoride into [^{18}F]-5′-fluoro-5′-deoxyadenosine, [^{18}F]-5′-fluoro-5′-deoxyinosine and [^{18}F]-5-fluoro-5-deoxyribose for positron emission tomography (PET) applications.

Enzymatic C–F bond formation is an unexploited area in biotransformation technology due to the virtual absence of appropriate biocatalysts. Nature has hardly evolved a biochemistry of fluoride ion and thus enzymes able to form C–F bonds have been elusive.[1–3] The recent isolation of a fluorination enzyme from the bacterium *Streptomyces cattleya*[4–6] has opened up some prospects for such biocatalysis in this area. The fluorinase (5′-fluoro-5′-deoxyadenosine synthase, 5′-FDAS, E.C. 2.5.1.63) catalyses the conversion of (*S*)-adenosyl-L-methionine (SAM **1**) and fluoride ion to 5′-fluoro-5′-deoxyadenosine (5′-FDA **2**) and L-methionine **3** (Scheme 1). Recent stereochemical and X-ray studies indicate that the enzyme catalyses an S_N2 type reaction mechanism[7–9] and over-expression of this enzyme has made it available in milligramme quantities.

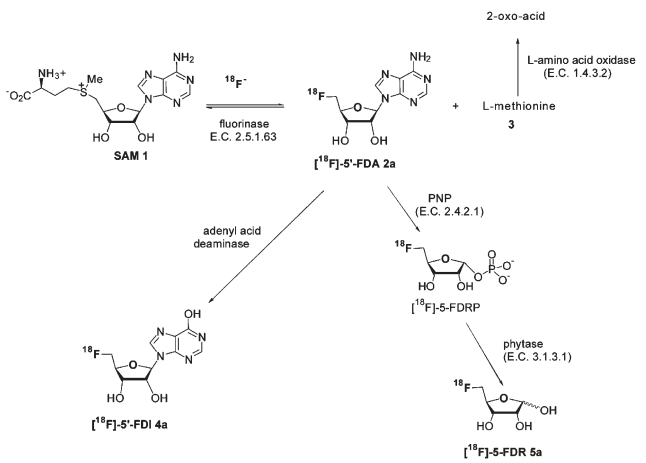
Positron emission tomography (PET) is a powerful diagnostic technique which has the ability of imaging tumors, monitoring the distribution of drugs and identifying cell and receptor degeneration in the brain.[10,11] The majority of PET studies to date utilise the F-18 isotope because the half-life (110 min) is relatively long compared to those for ^{11}C (20 min), ^{13}N (10 min), ^{15}O (2 min) respectively, the other isotopes used for PET applications. The current challenge for F-18-labelled radiopharmaceuticals is to develop rapid and clean synthesis methods with straightforward purification protocols for new *in vivo* imaging probes. Enzymatic methods are in principle attractive because they are chemospecific and they generate few side products. However there are few examples in PET synthesis where enzymes have been used to introduce isotopically labelled atoms, largely because suitable enzymes are not available.



Scheme 1 Biotransformation of (*S*)-adenosyl-L-methionine (SAM) and F^- to 5′-fluoro-5′-deoxyadenosine (5′-FDA **2**) in *S. cattleya*.

In 2003 we reported the synthesis of [^{18}F]-5′-FDA **2a** using wild type fluorinase and found that the process, although successful, was inefficient with a radiochemical yield of ~ 1%.[12] We report now that with over-expressed fluorinase and under optimal conditions the radiochemical yields (RCYs) for [^{18}F]-5′-FDA production from [^{18}F]-fluoride have improved dramatically and up to 95%. Additionally, in a coupled enzyme system the syntheses of [^{18}F]-5′-fluoro-5′-deoxyinosine ([^{18}F]-5′-FDI) **4a** and [^{18}F]-5-fluoro-5-deoxy-D-ribose ([^{18}F]-5-FDR) **5a** were accomplished also in good radiochemical yield.

An important development in improving the efficiency of ^{18}F-labelling was the realisation that the fluorination reaction is reversible[13] and that the equilibrium of the reaction must be pulled over towards [^{18}F]-5′-FDA synthesis for efficient conversion. This could be achieved using different coupled enzyme strategies. The first method involved coupling of the fluorinase to an L-amino acid oxidase (Scheme 2). The resultant L-methionine **3**, after conversion of **1** to **2**, was then removed by the action of the oxidase, and this improved the rate of synthesis and the overall conversion to **2**. The second method involved coupling the fluorinase to an adenylate deaminase to generate [^{18}F]-5′-FDI **4a**. This provided access to another labelled purine nucleoside and demonstrates the versatility of the fluorinase in delivering novel



Scheme 2 Fluorinase coupled enzyme systems for the synthesis of various ^{18}F-labelled compounds.

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C5′-fluorodeoxy nucleoside. Finally, coupling the fluorinase reaction to both a purine nucleotide phosphorylase (PNP) and then a high activity phytase resulted in the sequential depurination and phosphate hydrolysis to produce the free sugar [^{18}F]-5-FDR.

For the synthesis of [^{18}F]-5′-FDA **2a** in the oxidase coupled reaction, typically [^{18}F]-HF (*ca.* 300 MBq) was incubated with SAM **1** (1 mM) and both the recombinant fluorinase (8 mg/ml in 100 μl = 1.85 × 10^{-3} units) and the oxidase (0.15 units) in a one-pot reaction. The bioconversion to [^{18}F]-5′-FDA **2a** was monitored by HPLC/UV and simultaneously using a radioactive detector over a 4 h period at RT. Under these conditions the RCY for **2a** production reached 70% over the 4 h period (Fig. 1). This can be contrasted to the control reaction with recombinant fluorinase but without the oxidase, which resulted in an RCY of *ca.* 26% over the same period. These experiments show a clear improvement from our first experiments with the wild type enzyme (RCY ∼ 1%) (Fig. 1).

A more efficient bio-conversion to [^{18}F]-5′-FDA **2a** (RCY ∼ 86%) was achieved by increasing the reaction temperature to 35 °C (Fig. 1). Thus a modest increase in temperature had a significant influence on the RCY. Also the fluorinase concentration has a significant influence on the biotransformation. A decrease in the enzyme concentration (8 mg/ml to 2 mg/ml) resulted in a significant drop in the RCY over the 2 h period (Fig. 2a). However, a higher concentration of enzyme (24 mg/ml in 100 μL = 5.6 × 10^{-3} units) gave a much higher RCY at both reaction temperatures and also shortened the reaction time from 4 h to 2 h (Fig. 1), a clear advantage for radiochemical labelling protocols.

[^{18}F]-Fluoride is added to these reactions at picomolar (10^{-12} M) concentrations and the enzyme is present at μM concentrations such that there is a 10^6 M excess of enzyme over [^{18}F]-fluoride. Although the fluorinase is a slow enzyme (k_{cat} 0.03 min) this ratio facilitates high conversions. When unlabelled fluoride ion [^{19}F$^-$] carrier was added to these reactions however, the RCY's dropped significantly, consistent with lower overall conversions with concomitant radiochemical dilution (Fig. 2b).

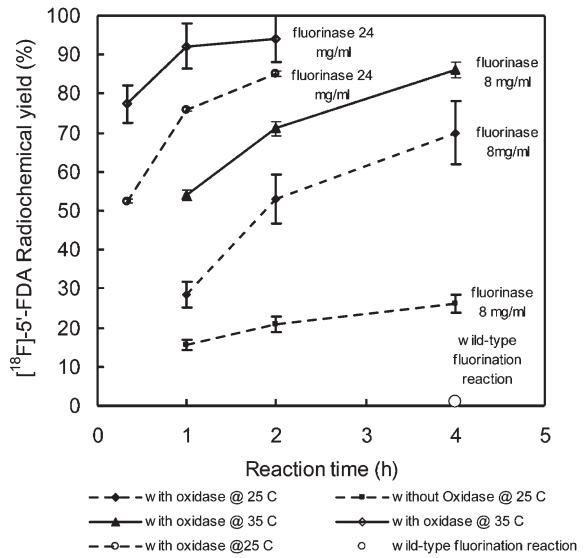

Fig. 1 A time course showing the RCY of [^{18}F]-5′-FDA **2a** production during the fluorinase catalysed biotransformations of SAM and [^{18}F]-HF at different concentrations of the enzyme and at different temperatures, in 100 μL assays, with and without the L-amino acid oxidase.

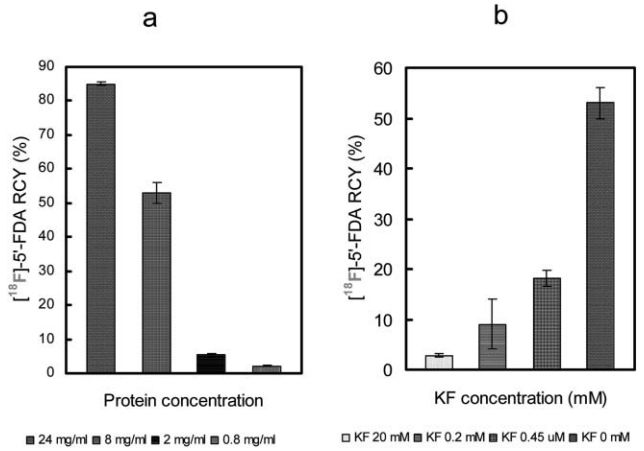

Fig. 2 a: The influence of protein concentration on the RCY of [^{18}F]-5′-FDA **2a** production from SAM and [^{18}F]-fluoride catalysed by the fluorinase at RT over 2 h. b: The effect of cold carrier (^{19}F$^-$ ion) on the RCY of [^{18}F]-5′-FDA **2a** production from SAM and [^{18}F]-fluoride, catalysed by the fluorinase at RT over 2 h.

In a second biotransformation, the fluorinase was coupled with 5′-adenylic acid deaminase (*Aspergillus sp.*). This combination resulted in the bioconversion of SAM to labelled **4a**, *via* **2a**. A RCY of ∼ 60% resulted after a 3 h incubation at 20 °C. However, the bioconversion of **2a** to **4a** was incomplete and the ratio of **4a** : **2a** was 1 : 1 at RT. However the ratio of 5′-FDA to 5′-FDI could be improved significantly (13 : 1) if the reaction was conducted at 35 °C. A time course for the production of [^{18}F]-5′-FDI at 35 °C is shown in Fig. 3a and demonstrates that an RCY of 75% could be achieved over 4 h from [^{18}F]-fluoride.

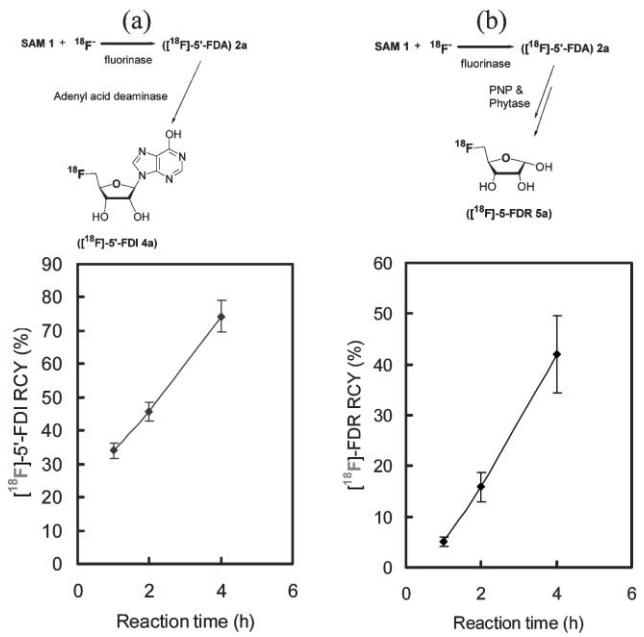

Fig. 3 a: A time course showing the RCY of [^{18}F]-5′-FDI **4a** from SAM and [^{18}F]-fluoride, catalysed by the fluorinase in a coupled reaction with 5′-adenyl acid deaminase at 35 °C. b: Radiochemical yield of [^{18}F]-5-FDR, a time course of the coupled enzyme reaction with fluorinase, immobilized PNP and phytase at 35 °C.

It was attractive to develop a protocol for the synthesis of [^{18}F]-5-FDR **5a** providing access to a radiolabelled monosaccharide derivative. The bioconversion of SAM **1** and [^{18}F]-fluoride to [^{18}F]-5-FDR **5a** was accomplished in a three enzyme system consisting of the fluorinase, an immobilised purine nucleotide phosphorylase (PNP) and a phytase at 35 °C (Scheme 2). A time course evaluating the production of [^{18}F]-5-FDR demonstrates a conversion reaching a RCY of 45% after 4 h (Fig. 3b). The experiments demonstrate for the first time an enzymatic approach to ^{18}F isotope incorporation in PET synthesis and they extend the range and versatility of the fluorinase as a biocatalyst inviting the exploration of these radiolabelled novelties for PET imaging applications. Incubation times of 1 h–4 h, although within a useful range, are relatively long to challenge mainstream ligands in PET synthesis, however these results can only be improved through accelerated evolution techniques[14] to improve the enzyme's efficiency. The fluorinase is a relatively specific enzyme, and can never be a general fluorination reagent, however the enzyme displays some versatility with substrates modified both on the adenine base and on the ribose ring. Our studies on the substrate specificity of the fluorinase will be published in due course and this flexibility will clearly extend the range of substrates for fluorination.

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