An enzymatic Finkelstein reaction: Fluorinase catalyses direct halogen exchange

Phillip T. Lowe,a Steven L. Cobb and David O’Hagana,b

The fluorinase enzyme from Streptomyces cattleya is shown to catalyse a direct displacement of bromide and iodide by fluoride ion from 5'-bromodeoxyadenosine (5'-BrDA) and 5'-iododeoxyadenosine (5'-IDA) respectively, to form 5'-fluorodeoxyadenosine (5'-FDA) in the absence of L-methionine (L-Met) or S-adenosyl-L-methionine (SAM). 5'-BrDA is the most efficient substrate for this enzyme catalysed Finkelstein reaction.

Introduction

Fluorine containing compounds have a remarkable record in tuning the properties of medicinal,1,2 agricultural,3,4 and materials5 chemistry products. As such, in recent years the development of methodology to incorporate fluorine into biologically and commercially relevant compounds under mild conditions has intensified. The naturally occurring fluorinase enzyme (5’-fluoro-5’-deoxy-adenosine synthase), originally isolated from Streptomyces cattleya where it is involved in the C-F bond forming step in fluororacetate and 4-fluorothreonine biosynthesis, offers a rare opportunity to introduce fluorine enzymatically. Since its discovery, subsequent investigation has yielded 5 enzyme homologues from actinomycete organisms (FLA1,6,7 FLA1,8 FLA3,9 FLA410 and NobA8,11), along with significant insight into its structure and mechanism of action.12,13 In nature the fluorinase catalyses a C-F bond forming reaction between S-adenosyl-L-methionine (SAM) 2 and fluoride ion to generate 5’-fluoro-5’-deoxy-adenosine (5’-FDA) 3 and L-methionine (L-Met).12,14 The enzyme also catalyses the reverse reaction, for example the nuclophilic displacement of chloride from 5’-chloro-5’-deoxy adenosine (5’-CIDA) 1 by L-Met to generate SAM 2.15 When this reaction is conducted in the presence of fluoride ion, the resultant SAM 2 is then converted to 5’-FDA 3, to achieve a two-step transhalogenation from 5’-CIDA 1 to 5’-FDA 3. The substrate flexibility of this ‘two-step’ fluorination process extends to 2’-deoxy analogues16 of 5’-CIDA 1 as well as C-2 decoration of the adenine base of 5’-CIDA 1 with an acetylene, or terminally functionalised acetylene moieties.17-23 In this manner, fluorinase-mediated transhalogenation reactions have become established as a strategy for the synthesis of fluorinated bioactive compounds under experimentally benign conditions (ambient temperatures in buffer at pH 7.8). In particular, the method has been utilised for the late-stage 18F-radiolabelling of cancer relevant targeting peptides17-21 and A2A adenosine receptor agonists23 for use in positron emission tomography (PET). Recently, in an effort to diversify the substrate scope and optimise the ‘two-step’ fluorination reaction, the fluorinase was subject to directed evolution, and modest rate improvements were achieved for the first step only, involving displacement of chloride from 5’-CIDA 3 by L-Met.24,25 In this paper we report that the fluorinase is able to mediate a direct fluorination reaction of 5’-halogenated-5’-deoxy-adenosines, in a reaction which does not require its natural substrates SAM or L-Met.

Scheme 1 Fluorinase-catalysed transhalogenation of 5’-CIDA 1 to 5’-FDA 3 via SAM 2.

Scheme 2 Fluorinase-catalysed direct fluorination of 1, 4 and 5 halogenated-5’-deoxy-adenosine substrates.
Results and Discussion

The halogenated nucleosides: 5’-CIDA 1, 5’-BrDA 4 and 5’-IDA 5 (Scheme 2) were assayed in initial time course experiments to determine their ability to undergo a direct fluorination. Each substrate, 5’-CIDA 1, 5’-BrDA 4 and 5’-IDA 5, was converted to 5’-FDA 3 in the presence of the fluorinase and inorganic fluoride only (Fig S2), although the rates differed significantly depending on the 5’-halogen (Br >> I > Cl). The bromo substrate 5’-BrDA 4 was the most efficient, reaching conversions above 35% under the conditions, whereas 5’-IDA 5 and 5’-CIDA 1 displayed significantly lower conversions of 8% and 5% respectively.

To investigate the comparative rates of fluorination of 1, 4 and 5, time course experiments were conducted with the fluorinase over a period of 24 h (Fig. 1). Assays were performed with KF both either with or without added L-Met. As expected, the fluorinase showed significantly greater initial rates for direct fluorination (without L-Met) with 5’-BrDA 4 than for 5’-IDA 5 and 5’-CIDA 1, yielding 15%, 1.2% and 0.2% conversions respectively after 4 h (Fig. 1). Direct fluorination kinetic parameters were evaluated for 5’-BrDA 4 (Km = 9.7 ± 2.6 μM, kcat = 0.017 ± 0.004 min⁻¹). The Km was similar in magnitude to SAM indicating similar affinities for the enzyme. When transhalogenation assays to generate 5’-FDA were performed in the presence of L-Met, 5’-CIDA 1 was the most efficient substrate. 5’-BrDA 4 presented a slightly improved rate over direct fluorination, indicating that it can also participate in the two-step process. 5’-IDA 5 did not demonstrate any improvement in the presence L-Met and both regimes gave low conversions with 5’-IDA 5.

In order to compare the relative ability of the fluorinase to perform the initial step of the two step process, to generate SAM from 1, 4 and 5, the enzyme was incubated with each fluorinase (125 μM). Yield = (concentration of 5’-FDA generated/concentration of substrate) x 100%, data are representative of three different experiments.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Km (μM)</th>
<th>kcat (min⁻¹)</th>
<th>[kcat/Km] (mM⁻¹min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’-BrDA 4</td>
<td>9.7 ± 2.6</td>
<td>0.017 ± 0.004</td>
<td>2.1</td>
</tr>
<tr>
<td>SAM⁵</td>
<td>6.5 ± 0.3</td>
<td>0.07 ± 0.001</td>
<td>10.8</td>
</tr>
</tbody>
</table>

a Assays contain: 4 (10 – 500 μM), KF (200 mM), and fluorinase (8 μM).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>% Conversion to SAM after 4h⁵</th>
</tr>
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<tbody>
<tr>
<td>CIDA (1)</td>
<td>44% ± 1.5</td>
</tr>
<tr>
<td>BrDA (4)</td>
<td>27% ± 3.2</td>
</tr>
<tr>
<td>IDA (5)</td>
<td>&lt;0.1% ± 0</td>
</tr>
</tbody>
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Substrate, % Conversion to SAM after 4h⁵

Table 2 Comparative % SAM product yields of the fluorinase using 5’-CIDA 1, 5’-BrDA 4 and 5’-IDA 5.

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to allow direct fluorination of 5'-CIDA 1 by attack of fluoride from the fluoride ion binding site, however the process is not efficient presumably due to the relative strength of the C-Cl bond and a poor catalytic prowess of the fluorinase to achieve chloride displacement. It follows that the bromine in 5'-BrDA 4 must reasonably locate in the methylsulfonium binding site, and the weaker C-Br bond becomes displaced by fluoride ion (Fig 2b). The inability 5'-IDA 5 to undergo either the two-step transhalogenation reaction or direct fluorination in an efficient manner, suggests the iodine is too large to be accommodated with an appropriate geometry for efficient nucleophilic displacement in either site.

Conclusions

We have extended the substrate scope of the fluorinase enzyme, revealing its capacity to mediate a direct fluorination of 5'-BrDA 4 by fluoride ion and to a lesser extent using 5'-IDA 5 as a substrate. 5'-CIDA 1, though capable of this transformation, is a very poor substrate. This is an enzymatic Finkelstein reaction, a transformation not previously reported in enzymology. The one step process offers an advantage over the previous two-step approach for accelerated evolution. Furthermore there is potential for a simplified one step protocol for the preparation of [18F]-fluoride labelled radioligands for PET imaging. To date [18F]-fluoride incorporation has largely focussed on two-step enzymatic reactions using 5'-CIDA analogues and L-Met as a cofactor.

Experimental

See Supporting Information for experimental detail on: Compound synthesis and characterisation, assay conditions and fluorinase overexpression and purification.

Conflicts of interest

There are no conflicts to declare

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References


