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Paper

An enzymatic Finkelstein reaction: Fluorinase catalyses direct halogen exchange

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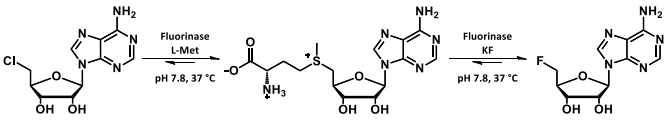
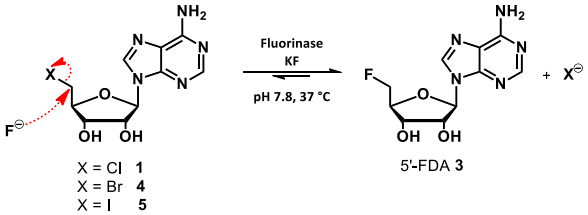
The fluorinase enzyme from *Streptomyces cattleya* is shown to catalyse a direct displacement of bromide and iodide by fluoride ion from 5'-bromodeoxyadenosine (5'-BrDA) and 5'-iododeoxyadenosine (5'-IDA) respectively, to form 5'-fluorodeoxyadenosine (5'-FDA) in the absence of L-methionine (L-Met) or 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 materials[5] 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 fluoroacetate 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 (FLA,[6,7] FLA1,[8] FLA3,[8] FLA4[9,10] and NobA[8,11]), 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 nucleophilic displacement of chloride from 5'-chloro-5'-deoxy adenosine (5'-ClDA) **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'-ClDA **1** to 5'-FDA **3**. The substrate flexibility of this 'two-step' fluorination process extends to 2'-deoxy analogues[16] of 5'-ClDA **1** as well as C-2 decoration of the adenosine base of 5'-ClDA **1** with an acetylene, or terminally functionalised acetylene moieties.[17-23] In this manner, fluorinase-mediated trans-halogenation 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 ^{18}F-radiolabelling of cancer relevant targeting peptides[17-21] and A_2A adenosine receptor agonists[23] 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'-ClDA **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'-ClDA **1** to 5'-FDA **3** *via* SAM **2**.



Scheme 2 Fluorinase-catalysed direct fluorination of **1**, **4** and **5** halogenated-5'-deoxy-adenosine substrates.

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Results and Discussion

The halogenated nucleosides: 5'-ClDA **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'-ClDA **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'-ClDA **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'-ClDA **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** (K_m = 9.7 ± 2.6 µM, k_{cat} = 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'-ClDA **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

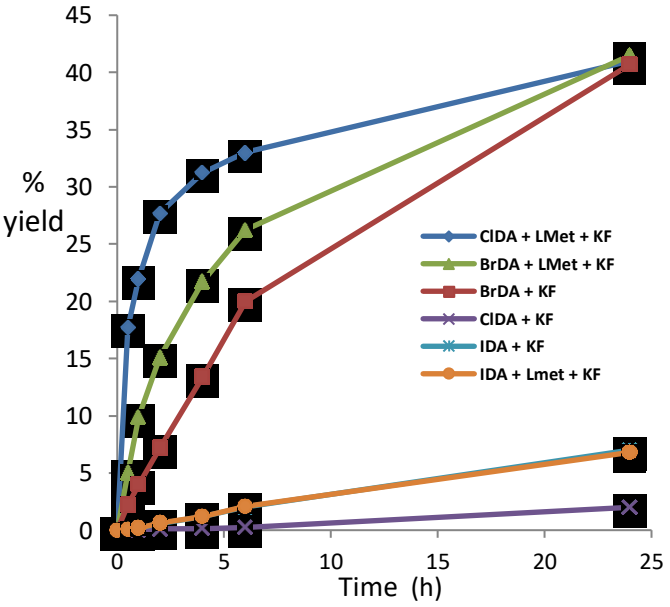


Fig. 1 Reactions rates for the fluorinase with substrates 5'-ClDA (**1**), 5'-BrDA (**2**) and 5'-IDA (**5**) at 37 °C over 24 h. Both conversions to 5'-FDA *via* transhalogenation (with L-Met + KF) and direct fluorination (with KF, without L-Met) are shown. Reaction conditions: substrates **1**, **4**, & **5** (0.2 mM), L-Met (0.1 mM, excluded for direct fluorination experiments), KF (80 mM), and fluorinase (125 μM). Yield = (concentration of 5'-FDA generated/concentration of substrate) x 100%, data are representative of three different experiments.

Table 1 Comparative kinetic data of fluorinase substrates 5'-BrDA **4** and SAM.

Substrate	K_M (µM)	k_{cat} (min^{-1})	[k_{cat}/K_M] ($mM^{-1}min^{-1}$)
5'-BrDA **4**[a]	9.7 ± 2.6	0.017 ± 0.004	2.1
SAM[b]	6.5 ± 0.3	0.07 ± 0.001	10.8

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

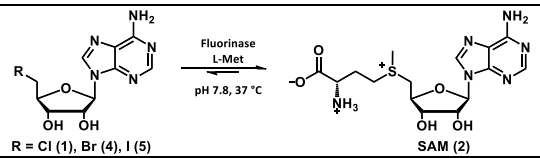
[b] Kinetic data from Zhu *et al*.[13]

substrate in the presence of L-Met but in the absence of KF (Table 2). The observed conversions to SAM after a 4 h incubation (**1** (44%), **4** (27%) and **5** (<0.1%)) were consistent with the rates for the overall two-step fluorination assays favouring 5'-ClDA **1** and then 5'-BrDA **4**. 5'-IDA **5** was not a substrate for this reaction.

5'-BrDA **4** undergoes both direct and 'two-step' transhalogenation reactions with similar efficiencies in the presence of fluoride ion to generate 5'-FDA, whereas 5'-ClDA **1** requires L-Met for efficient conversion and progresses most efficiently *via* the two-step transhalogenation process. When 5'-BrDA **4** was assayed with other halides, e.g. by adding KCl or KI as halide sources, there were no productive reactions to generate 5'-IDA **5** or 5'-ClDA **1** (Scheme S4). Also the reverse reaction to produce 5'-BrDA **4** could not be observed when 5'-FDA **3** was incubated with the fluorinase and KBr (Scheme S5).

Previous X-ray crystallographic analysis of 5'-ClDA **1** bound into the active site of the fluorinase revealed that it adopts two poses (Fig 2).[15] One orientates the 5'-chlorine atom into the halide binding pocket which would otherwise bind fluoride ion (Fig 2a). This orientation is essential for step one of the two-step reaction to proceed as it accommodates the correct trajectory for nucleophilic attack by the sulphur atom of L-Met to the 5'-carbon of 5'-ClDA, for the synthesis of SAM. The second pose orientates the 5'-chlorine towards the empty binding pocket for the L-Met sulfonium sulfur (Fig 2b). This orientation is required

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



R = Cl (1), Br (4), I (5) SAM (2)

Substrate	% Conversion to SAM after 4h[a]
ClDA (**1**)	44 % ± 1.5
BrDA (**4**)	27 % ± 3.2
IDA (**5**)	<0.1 % ± 0

[a] Assays contain: substrate (0.2 mM), L-Met (0.2 mM), and fluorinase (125 mM).

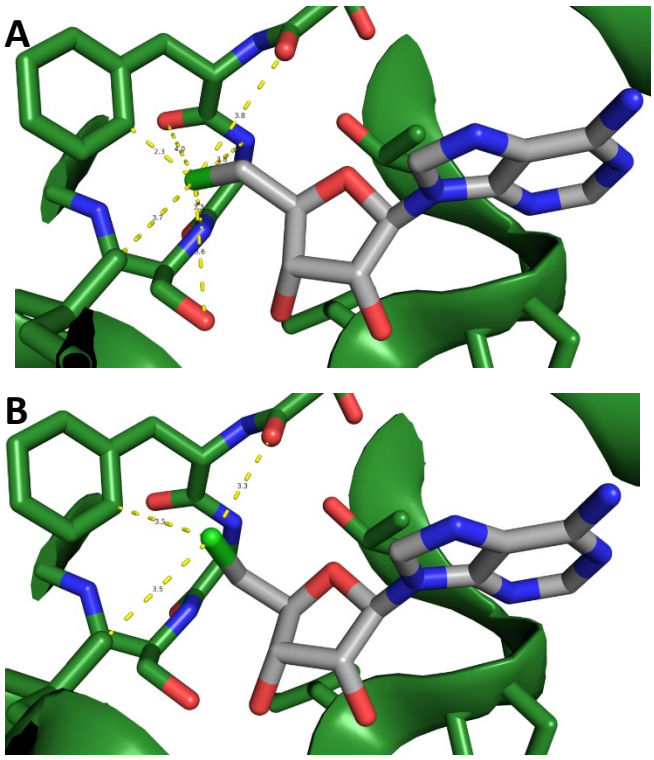


Fig. 2 Structure of the fluorinase enzyme bound to 5′-ClDA **1** accommodating two distinct poses within the active site: **A** - with its 5′-chlorine atom positioned within the halide binding pocket (orientation for two-step fluorination) and **B** - with its 5′-chlorine atom positioned within the L-Methionine binding pocket (orientation for direct fluorination).[15]

to allow direct fluorination of 5′-ClDA **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′-ClDA **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.[24,25] Furthermore there is potential for a simplified one step protocol for the preparation of [^{18}F]-fluoride labelled radioligands for PET imaging. To date [^{18}F]-Fluoride incorporation has largely focussed on two-step enzymatic reactions using 5′-ClDA analogues and L-Met as a cofactor.[17-23]

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

Acknowledgements

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