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Enzyme-catalyzed C–F bond formation and cleavage

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Abstract

Organofluorines are widely used in a variety of applications, ranging from pharmaceuticals to pesticides and advanced materials. The widespread use of organofluorines also leads to its accumulation in the environment, and two major questions arise: how to synthesize and how to degrade this type of compound effectively? In contrast to a considerable number of easy-access chemical methods, milder and more effective enzymatic methods remain to be developed. In this review, we present recent progress on enzyme-catalyzed C–F bond formation and cleavage, focused on describing C–F bond formation enabled by fluorinase and C–F bond cleavage catalyzed by oxidase, reductase, deaminase, and dehalogenase.

Keywords: Organofluorines, C–F bonds, Enzyme-catalyzed, Degradation, Formation

Introduction

Incorporation of fluorine into organic compounds usually endows organofluorines with unique chemical and physical properties, a strategy that has been successfully applied in agrochemicals, materials science, and pharmaceutical chemistry (Phelps 2004; Müller et al. 2007; Shah and Westwell 2007; Hagmann 2008; Nenajdenko et al. 2015; Zhang et al. 2016; Lowe et al. 2017). Especially in medicinal chemistry, the unique elemental properties of fluorine have been proved to enhance metabolic stability and alter pharmacokinetic characteristics without increasing the apparent spatial volume; thus, more than 20% of drugs are organofluorines (Zhou et al. 2016; Gillis et al. 2015; Spooner et al. 2019). The wide application of organofluorines has motivated fast methodology development for fluorine incorporation (Purser et al. 2008; Berger et al. 2011). In contrast to their synthesis, their degradation has also attracted significant attention due to their increased use and the cumulative pollution resulting from their high stabilities.

Chemists have developed versatile methods for the formation and cleavage of C–F bonds, but these methods usually require harsh conditions and are not environment friendly (Dillert et al. 2007; Lin et al. 2012; Sulbaek Andersen et al. 2005). To solve these problems, development of mild and green methods is urgently needed. Biocatalysis has been playing an increasingly more important role in modern chemistry due to its high efficiency, specific selectivity, and more environmentally friendly characteristics compared to chemical catalysis. Thus, introducing biocatalytic methods into organic fluorine chemistry is a good choice to counter the deficiencies of chemical catalysis (Kim et al. 2000; Liu and Avendaño 2013; Murphy 2016; Rotander et al. 2012). Although biocatalysis has achieved significant progress in recent years, the field of biocatalytic C–F bond formation and cleavage is almost at an open stage.

Since fluorine atoms are very small and strongly electro-negative, when in an aqueous system fluoride ions are always tightly wrapped by the water molecules, and thus, it is very difficult to form C–F bonds in an aqueous system (O'Hagan 2008; Ni and Hu 2016). Therefore, fluorine-containing natural products are very rare despite the fact that elemental fluorine is the most fecund halogen in the Earth's crust (O'Hagan and Deng 2014). To the best of our knowledge, only two different examples of enzyme-catalyzed C–F bond formation have been reported: one is catalyzed by a mutant of glycosyltransferase, which catalyzes α-fluoroglycosides as transient intermediates

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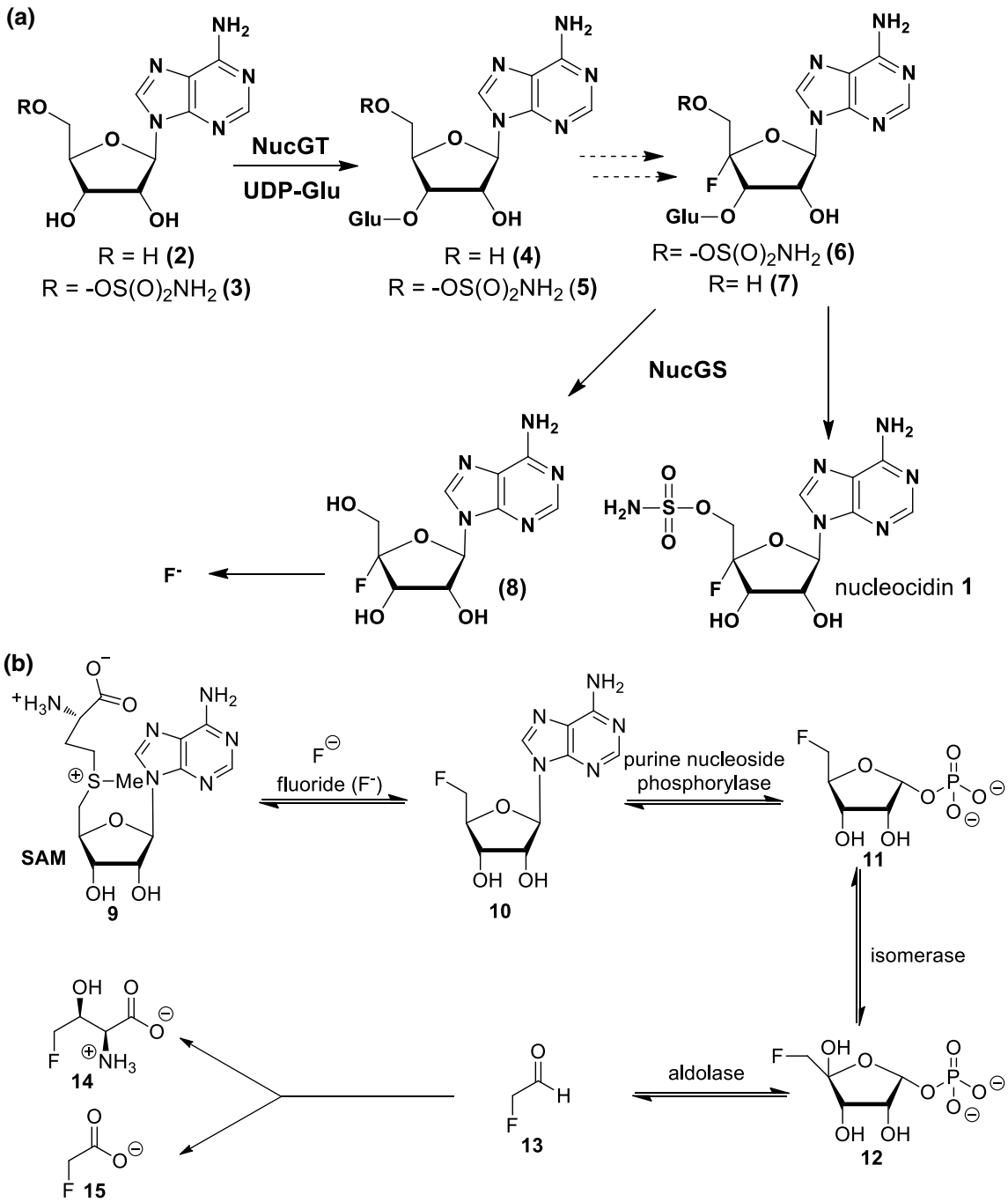
from DNP-activated sugars (Zechel et al. 2001), and the other is the natural fluorinase, prompting the conversion of 5′-fluoro-5′-deoxyribose-1-phosphate (5′-FRP) from *S*-adenosyl-L-methionine (SAM) (Deng et al. 2004). In this review, we focus on summarizing the recent progress in mining and directed evolution of fluorinase and expect to inspire the development of more unnatural fluorinases in the future.

The C–F bond is the strongest σ bond, and thus, it is difficult to cleave it under mild conditions (Goldman 1969; Lemal 2004). When hydrogen is substituted by fluorine, the metabolic stability of the compounds will be significantly improved; this property benefits the pharmaceutical industry, but leads to the accumulation of organofluorines in the environment (Wang et al. 2016). Enzyme-catalyzed C–F bond cleavage has attracted attention from researchers in environmental protection, C–F bond activation, and enzymology, and several reviews have been published on the subject (Natarajan et al. 2005). However, in recent years, reports in this area have been very rare. Herein, therefore, we present recently published examples of enzyme-catalyzed C–F bond cleavage, dividing them into two types: hydrolytic defluorination, and oxireductive defluorination. Hopefully, this review will attract increasing numbers of workers to this important field.

Enzyme-catalyzed C–F bond formation

The first natural organofluorine compound, identified in 1943, was fluoroacetate, a metabolite of the Southern African plant *Dichapetalum cymosum* (Marais 1943, 1944). The second one was isolated in 1956 from *Streptomyces calvus*; it is a nucleoside product named nucleocidin **1** which belongs to a new form of fluorine metabolites (Scheme 1a). Subsequently, the third structurally novel, fluorine-containing natural product 4-fluorothreonine was isolated from the bacterium *Streptomyces cattleya* in 1986 (Sanada et al. 1986). Despite considerable interest and a variety of speculative suggestions for uncovering the biochemical mechanism of fluorination, no specific details of fluorination's biochemistry in any organism were provided until 2002. This milestone regarding fluorinase was published in 2002 by O'Hagan's group (O'Hagan et al. 2002), who first described an enzymatic reaction occurring in the bacterium *Streptomyces cattleya* that catalyzes the conversion of fluoride ions and S-adenosylmethionine (SAM) to 5′-fluoro-5′-deoxyfluoroadeno-sine (5′-FDA) (O'Hagan et al. 2002; see Scheme 1b). In the following year, O'Hagan's team isolated and characterized fluorinase from *Streptomyces cattleya* (Schaffrath et al. 2003), and the crystal structure of fluorinase was resolved by the same group in 2004 (Dong et al. 2004; Deng et al. 2004). Although the basic enzyme was characterized before 2005, it took approximately 10 years to clarify the biosynthetic pathway of all fluorinated products in *Streptomyces cattleya*, and their work proved that fluorinating enzyme that converts inorganic fluorine into organic fluorine (Deng et al. 2006; Zhu et al. 2007; Winkler et al. 2008; Dall'Angelo et al. 2013; O'Hagan and Deng 2014; Wang et al. 2014; Carvalho and Oliveira 2017). However, attempts to identify enzymes that biosynthesize nucleocidin **1** have failed for decades, because the production of this molecule has been mysteriously silenced in the bacterium *Streptomyces calvus* (Jenkins et al. 1976; Nashiru et al. 2001; Zechel et al. 2003). In 2015, Zechel's group reported that complementation of *S. calvus* ATCC13382 with a functional *bldA*-encoded Leu-tRNA^{UUA} molecule restores the production of nucleocidin **1** and identified the genes encoding the biosynthesis of the 5′-*O*-sulfamate group of the nucleocidin **1** (Zhu et al. 2015). In the next year, O'Hagan's group provided the first biosynthetic data on nucleocidin **1** assembly from isotope labeling studies (Bartholomé et al. 2016; Feng et al. 2017). However, there was still no illumination for the mechanism of fluorination involved in this biosynthetic pathway. In 2019, O'Hagan group disclosed two structures of novel fuorometabolites in *S. calvus*, which belong to 3′-O-glucosylated, 4′-fuoro-riboadenosines (**6** and **7**) (Scheme 1a). They are analogous of nucleocidin **1** and suspect to incorporate fluorine via a same biocatalytic pathway (Feng et al. 2019). The identification of these metabolites highly suggests that there is a new type of fluorinase existing in *S. calvus* which deserves our attention.

Although the specific activity of fluorinase has attracted considerable attention, its application is limited due to the drawbacks of narrow substrate scope and low activity. To explore its utilization, it is necessary to mine new types of fluorinase or improve the activity of known forms of fluorinase by directed evolution. Most research on fluorinase is focused on discovering new forms of fluorinase through gene mining. To date, four new fluorinases have been identified, three of which have been characterized (see Table 1) (Deng et al. 2014; Wang et al. 2014; HimáTong 2014). The first was identified from *Streptomyces* sp. MA37, a strain isolated in 2011 from Ghana. Full genome sequences of the South American hospital pathogens, *Nocardia brasiliensis* (Deng et al. 2014; Wang et al. 2014) and *Actinoplanes* sp. N902-109, were deposited into the public domain in 2012 and 2013, respectively. The draft genome of the marine bacterium *Streptomyces xinghaiensis* NRRL B-24674 was deposited in the public domain in 2011. More recently, a new fluorinase (FIA) gene has been identified in the *Streptomyces xinghaiensis* genome. Full genome sequencing of the organism revealed a gene encoding of a putative



Scheme 1 **a** Putative minimal pathway for nucleocidin 1 biosynthesis. **b** Intermediates and enzymes on the biosynthesis of fluoroacetate and 4-fluorothreonine

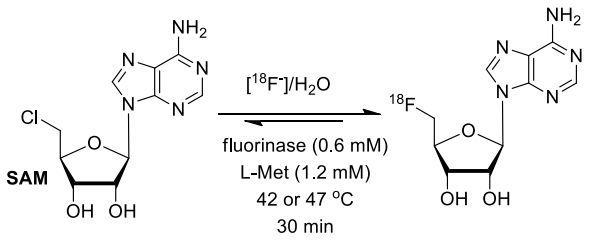
fluorinase with 80% sequence identity to that of *Streptomyces cattleya*. Although the fluorinase in *Streptomyces xinghaiensis* was not isolated, the production of fluoroacetate in culture suggests a functioning fluorinase. This is the first instance of a fluorometabolite isolated from a marine organism (HimáTong 2014).

In addition to mining new fluorinases, directed evolution has achieved new progress. In 2016, Zhao's group first directed evolution of the fluorinase FIA1 for improved conversion of the non-native substrate 5′-chloro-5′-deoxyade-nosine (5′-ClDA) into 5′-fluoro-5′-deoxyadenosine (5′-FDA) (see Table 2; Sun et al.

Table 1 Comparative kinetic data of known fluorinase enzymes

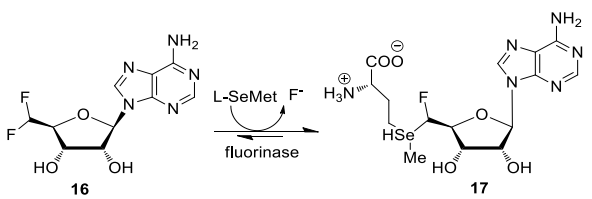
Fluorinase (FIA) source	SAM K_m (μM)	Turnover no. k_{cat} (min^{-1})	Specificity constant k_{cat}/K_m (mM^{-1} min^{-1})	References
S. cattleya	29.2 ± 2.41	0.083	2.84	Schaffrath et al. 2003
Streptomyce sp. MA37	82.4 ± 18.6	0.262	3.18	Deng et al. 2014
N. brasiliensis	27.8 ± 4.23	0.122	4.40	Wang et al. 2014
Actinoplanes sp. N902-109	45.8 ± 7.91	0.204	4.44	Deng et al. 2014

Table 2 Fluorinase variants obtained by directed evolution



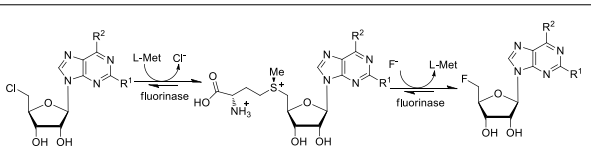
Fluorinase	T (°C)	RCC (analytical) (%)	RCC[a] (overall) (%)
FIA1	42	8 ± 1	7 ± 1
FIA1	47	11 ± 2	8 ± 2
fah2081 (A279Y)	42	32 ± 3	24 ± 2
fah2114 (F213Y, A279L)	47	46 ± 2	34 ± 3

[a] Overall RCC = % radioactivity in supernatant W% RCC based on radioHPLC. Typical % radioactivity in supernatant is in the range 73–80%



Scheme 2 Enzymatic transformation of **16** to its corresponding **17**

2016). NNK-based saturated mutagenesis of the active sites (within 5 Å of substrate) was performed on the FIA1 gene to generate libraries for high-throughput screening. The evolutionary variants fah2081 (A279Y) and fah2114 (F213Y and A279L) have been successfully applied to the radiosynthesis of 5′-[^{18}F] FDA, and their total radiochemical conversion (RCC) is more than threefold higher than wild-type FIA1. In 2016, O'Hagan's group found that fluorinase can be applied to the synthesis of 5′,5′-Difluoro-5′-deoxyadenosine. (see Scheme 2; Thompson et al. 2016). Some research has also been



Scheme 3 Two-step fluorinase reaction and the corresponding products

done on the recognition of substrates by fluorinase (Yeo et al. 2017; Sun et al. 2018). In 2017, Yeo's team explored fluorinase specificity (Yeo et al. 2017). In addition, their evolved FIA1 luciferin variants are active against substrates modified at the C-2 and C-6 positions of the adenine ring, although they are directed against 5′-ClDA 1 and L-Met bottoms and conferred novel activity towards substrates not readily accepted by wild-type FIA1 (see Scheme 3). Regarding the recent research progress on fluorinase, the application of fluorinase is still very limited. We believe that the directed evolution can be used on fluorinase to catalyze more non-natural fluorination reactions.

Enzyme-catalyzed C–F bond cleavage

Large-scale applications of fluorinated compounds have caused increasing environmental concerns due to their toxicity, global warming potential, environmental persistence, and bioaccumulation character (Douvris and Ozerov 2008; Houde et al. 2006). Environmental biotransformation, one of the most promising strategies with the lowest energy consumption, has provided some encouraging results in cleaving the highly stable C–F bond, the dissociation energy of which is the highest among all the natural products. At present, there are two ways of catalyzing the cleavage of C–F bonds by enzymes: hydrolytic defluorination and oxireductive defluorination.

The presence of naturally produced fluoroacetate in the environment has resulted in the evolution of mechanisms to degrade this substrate. Goldman identified the first microbial defluorinases in *Pseudomonas* sp., which catalyzed the hydrolytic cleavage of the C–F bond, yielding glycolate and fluoride ions (Goldman

1965). Subsequently, other defluorinases were isolated from several micro-organisms (Goldman 1965; Kawasaki et al. 1981; Walker and Lien 1981; Liu et al. 1998; Kurihara et al. 2003; Donnelly and Murphy 2009). The mechanism of C–F bond cleavage by defluorinases has been investigated (see Fig. 1; Liu et al. 1998; Chan et al. 2011; Kim et al. 2017; Mehrabi et al. 2019), and a two-step reaction mechanism for fluoroacetic acid dehalogenase was proposed. First, aspartate attacks α-carbon connected with F atom. This leads to the leaving of F atom from the substrate. The covalent intermediate, thus, produced is then hydrolyzed by a histidine-activated water molecule, which is assisted by the second aspartate residue. The catalytic process involves the conservative aspartic acid–histidine–aspartic acid catalytic triad. In 2017, Reetz's group reported a study of substrate range and enantioselectivity of defluorinases via using stereochemical probes (Wang et al. 2017). A favorable (*S*)-configuration was observed in the fast reaction using racemic 2-fluoro-2-phenylacetic acid (see Scheme 4a). Surprisingly, the results show that the enzyme pocket can accept a larger substrate than the natural substrate fluoroacetic acid, and that this non-natural compound is more reactive than fluoroacetic acid. Another hydrolyzed defluorinated enzyme is transaminases (Cuetos et al. 2016); transaminases are valuable enzymes in industrial biocatalysis and enable the preparation of optically pure amines. Lavandera reported a non-natural reaction type of transaminase, and the promiscuous reactivity of transaminases can be applied to obtain a series of enantiopure β-fluoroamines by an unprecedented formal tandem hydrodefluorination or deamination kinetic resolution of racemic β-fluoroamines (see Scheme 4b).

Oxireductive dehalogenation is a common pathway for detoxification of organic halide by micro-organisms. Biocatalytic oxidation and reduction defluorination by micro-organisms containing organic fluorine compounds have been reviewed to analyze their ability to handle such specific chemicals and to explore the potential of this knowledge in biotechnology applications (Natarajan et al. 2005). To avoid unnecessary repetition, here we only introduce works published in the last 5 years. In 2016, Bergen's team reported that ATP-dependent BzCoA reductase can promote C–F bond cleavage. An ATP-dependent study of defluorination

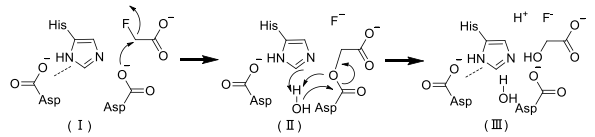
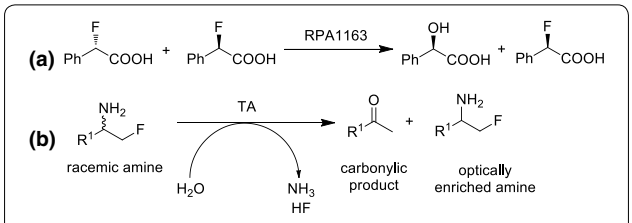


Fig. 1 Proposed two-step reaction mechanism of fluoroacetate



Scheme 4 Enzymatic hydrolysis of defluorination reaction: **a** hydrolytic kinetic resolution of racemate catalyzed by fluoroacetate dehalogenase RPA1163 and **b** novel tandem hydrodefluorination/deamination kinetic resolution of racemic β-fluoroamines

of 4-fluorobenzoacyl-CoA (4-F-BzCoA) with benzoyl-CoA (BzCoA) and HF catalyzed by class I BzCoA reductase was carried out. An unprecedented mechanism for reductive arylic C–F bond cleavage via a Birch reduction-like mechanism resulting in a formal nucleophilic aromatic substitution was proposed (see Fig. 2; Tiedt et al. 2016). In 2018, Liu et al. reported cleavage of a C–F bond by an engineered cysteine dioxygenase (Whittaker 2003; Simmons et al. 2006; Li et al. 2018). Experimental data suggest that protein-bound O_2-dependent carbon–halogen bonds cleave iron centers under mild, physiologically relevant conditions. Although the C–F bond is the strongest covalent single bond in organic chemistry, cysteine dioxygenase is able to cleave a C–F bond to realize co-factor biogenesis (see Scheme 5a). In 2019, Wang's group discovered that a histidine-linked heme enzyme can catalyze the cleavage of C–F by hydroxylation (Wang et al. 2019). LmbB2 has a wide range of catalytic activities toward L-tyrosine analogues, as long as the 4-hydroxyl group is present. The monosubstituted tyrosine analogues represented by 3-fluoro-L-tyrosine presumably bind in two different orientations at the heme

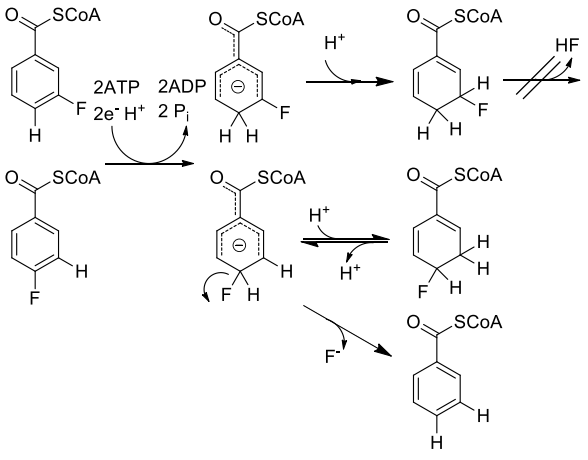
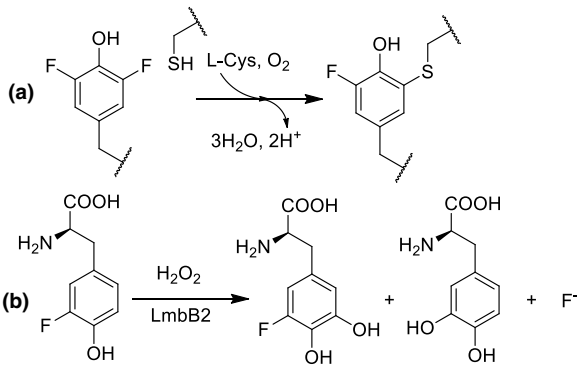


Fig. 2 Possible mechanism for reductive defluorination of BzCoA by ATP-dependent class I BCR



Scheme 5 Enzymatic of defluorination reaction: **a** cleavage of C–F bond by engineered cysteine dioxygenase and **b** summary of LmbB2 reaction for 3-fluoro-L-tyrosine

active site, with the substituents pointing away from or toward the heme center (see Scheme 5b). A mono-substituted tyrosine analog represented by 3-fluoro-L-tyrosine may have two different binding modes at the heme active site, and the substituent is directed to the center of the heme or away from its center. For example, the C–F bond undergoes hydroxylation to generate DOPA. The above describes the enzymatic hydrolysis or oxidation of the C–F bond, broadening the scope of fluorine chemistry and further expanding the potential industrial application of natural or engineered proteins.

Conclusions

Organofluorines play an increasingly important role in the pharmaceutical and agrochemical industries, making the prospect of using enzymatic reactions to form C–F bonds bright. However, the extensive use of organofluorines has also caused environmental pollution, and thus, development of a mild green enzyme to degrade these compounds is a matter of urgency. With its highly catalytic selectivity and environmental friendliness, enzymatic catalysis will play an increasingly more important role in fluorine organic chemistry. In this review, details of the formation of C–F bonds catalyzed by fluorinase and the cleavage of C–F bonds by oxidase, reductase deaminase, and fluoroacetate dehalogenase are demonstrated. These fluorinase and defluorinase all have been isolated and identified for more than a decade, and their catalytic mechanisms illuminated. However, a narrow substrate range or low activity has hindered their application. With the fast development of biotechnology, mining new enzymes or improving their properties by directed evolution holds promise to eliminate these barriers, which will greatly accelerate the development of enzymatic organic fluorine chemistry.

Abbreviations

5′-FRP: 5′-fluoro-5′-deoxyribose-1-phosphate; SAM: *S*-adenosyl-L-methionine; 5′-FDA: 5′-fluoro-5′-deoxyfluoroadeno-sine; FIA: fluorinase; 5′-ClDA: 5′-chloro-5′-deoxyade-nosine; RCC: radiochemical conversion; 4-F-BzCoA: 4-fluorobenzoacyl-CoA; BzCoA: benzoyl-CoA; class I BCR: catalyzed by class I BzCoA reductase; TA: transaminases; LmbB2: tyrosine hydroxylase.

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Authors' contributions

JW and WT conceived and wrote this paper. QH and ML were involved collecting related material and critical reading of this paper. All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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