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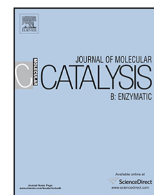

Polymer-immobilized fluorinase: Recyclable catalyst for fluorination reactions

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ABSTRACT

Polymer-immobilized fluorinase for the synthesis of 5′-fluoro-5′-deoxyadenosine (FDA) from S-adenosyl-L-methionine (SAM) and fluoride ion in aqueous media is described. The optimal composition of the poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) and the heterogeneous catalytic reaction conditions were developed to yield FDA in 49% within 150 min. In PET radiochemistry, using [^{18}F]fluoride ion in [^{18}O]H_2O obtained from the cyclotron, [^{18}F]FDA was synthesized with 68% fluorination efficiency. The immobilized fluorinase was recycled for up to four runs with 80% of catalytic activity in the final cycle.

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1. Introduction

1.1. Statement of significance

Research on a simple, mild and selective fluorination methodology of complex molecules is critical in facilitating the development of pharmaceuticals and in accelerating the field of molecular diagnostics. Fluorinated organic molecules are known to enhance lipophilicity, metabolic stability, bioavailability and selectivity through their inductive effects [1a–c]. Additionally, the less stable fluorine-18 isotope counterpart is an ideal nuclide for molecular diagnostic using positron emission tomography (PET) due to the moderate half-life and the high image resolution in PET imaging in comparison to other radioisotopes [2]. To date, fluorine-18 labeling reactions require time consuming multistep synthesis in organic solvents due to the incompatibility of fluoride ions with other functional groups, the use of harsh reagents and the need of high reaction temperatures to produce injectable doses of PET probes in less than 6 h [3,4]. More recently various fluorination methodologies utilizing transition-metal catalysts and bulky protic solvents are emerging to simplify fluorination reactions [5]. Notably, the enzyme catalyzed fluorination reaction using fluoride ion in aqueous media is particularly attractive due to its high chemoselectivity and high reaction yield under extremely mild conditions [6–8]. In the field of enzyme catalysis for organic syntheses, solid supported enzymes enable simpler separation of the enzymes from the product, and the immobilized enzymes could be recycled over a finite number of cycles. These features of immobilized enzymes have the potential to reduce the overall cost and synthesis time [9,10]. However, there is no evidence of successful applications of recyclable biocatalysts for fluorination of organic molecules. Specifically in PET radiochemistry, the short-lived [^{18}F]fluorine isotope ($t_{1/2}$ = 109 min) necessitates the development of a rapid, simple and efficient methodology for the preparation of [^{18}F]-labeled compounds to obtain optimal PET images. Therefore, it is critical to streamline the radiolabeling process with the shortest time for reaction, purification and reformulation. Specifically for homogeneous enzymatic reactions, the enzymes need to be first separated from the reaction product prior to subjecting the crude mixture through a standard purification process. Thus, the combined features of solid phase enzymatic fluorination catalysis and the mild and selective enzymatic reactions have significant potential toward the development of a more efficient, simplified and rapid preparation of fluorine containing molecules.

1.2. Known procedures

O'Hagan and co-workers discovered and isolated a specific halogenase, called fluorinase (also known as 5′-deoxy-5′-fluoroadenosine synthase, EC 2.5.1.63) to catalyze the reaction of S-adenosyl-L-methionine (SAM) with fluoride ion [11,12]. In comparison to conventional synthetic methodologies, which require multistep reactions, high temperature, long synthesis time and necessitate complicated purification processes, enzymatic reactions are performed in a single step under mild reaction

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temperature and produce only a single compound in high yield. It was demonstrated that the fluorination of the S-adenosyl-L-methionine (SAM) substrate in the presence of fluorinase and L-amino acid oxidase produces 5′-deoxy-5′-fluoroadenosine with over 90% yield. The enzymatic fluorination reaction proceeds at 38 °C and the reaction time was as short as 1 h [13,14]. While the fluorination methodology is attractive, the disadvantages of working with enzymes are the need to separate the enzymes from the reaction product prior to the standard purification process and the high cost of the enzyme. To the best to our knowledge, there is only one report on the investigation of immobilized fluorinase preparation and its recyclability. Kitazume and co-workers investigated the recyclability of fluorinase immobilized on the BL-100 polymer (butyl rubber latex) [15], using ionic liquid as the reaction solvent. However, the yield of the desired FDA product is low in the first cycle (19.8%) and dramatically decreased to 2.6% and 0.4% in the second and third cycles, respectively.

Herein, we developed a new immobilization methodology of fluorinase on a functional polymer monolith to catalyze the formation of C-F bond in aqueous media in a higher yield than the previous report using KF. We also investigated various enzymatic processes, recyclability and demonstrated the applicability of this methodology for the preparation of fluorine-18 labeled FDA.

2. Materials and methods

2.1. Enzyme and chemicals

2.1.1. Fluorinase isolation

Fluorinase enzyme was isolated by Dr. Mark Arbing and Sum Chan, at the Protein Expression Technology Center, UCLA-DOE Institute for Genomics and Proteomics, University of California, Los Angeles.

Escherichia coli BL21-Gold (DE3) pLysS were used for fluorinase production. The fluorinase gene (flA) was amplified from the S. cattleya NBRC14057 genome using primers reported previously [16–18]. Only a single, silent mutation in flA was detected by DNA analysis. The flA was cloned in pET28b+ (GenScript, USA) and consequently fused to the His-tag-containing peptide in the N-terminal of the enzyme, termed pETflA. The His-tagged flA protein was purified by affinity chromatography on nickel-NTA (Sigma) affinity column followed by gel filtration chromatography using a HiPrep 16/60 Sephacryl S-100 High Resolution column (GE Healthcare).

2.1.2. Reagents and buffers

All commercially available reagents and buffers were purchased from Sigma-Aldrich and Fisher Scientific and used without further purification.

2.2. Analytical methods

2.2.1. RP-HPLC analysis

The fluorination reaction was monitored by RP-HPLC analysis carried out using the Knauer Analytical System (Column: Phenomenex® Luna C18 5 μm, 250 mm ×4.60 mm; UV–vis detection at λ = 254 nm). Isocratic elution with water:ethanol (80:20, v/v) mixture was applied and a flow-rate of 0.6 ml/min was used.

2.2.2. Enzyme quantity determination

Fluorinase concentration in solutions was assayed according to the Bradford method [19] with bovine serum albumin as a standard and Coomassie® Brilliant Blue G dye (Fluka Analytical). UV/vis experiments were performed using Thermo Scientific NanoDrop 2000 spectrophotometer at λ = 600 nm.

2.2.3. IR analysis

ATR-IR spectra of polymers as solid powders were analyzed using Thermo Scientific Nicolet iS5.

2.2.4. UV-polymerization conditions

Polymerization reactions were performed using the Electrocure 500 UV flood curing chamber equipped UV lamp at 365 nm and an output power of 30 mW/cm^2.

2.2.5. Radio-TLC conditions

Radio-TLC measurements were performed using a Raytest mini-Gita TLC-station equipped with Beta detector GMC. Silica TLC plates were developed in a water:ethanol (80:20, v/v) mixture.

2.3. Enzyme-catalyzed fluorination with immobilized fluorinase

2.3.1. Preparation of polymers

The polymer monoliths were prepared as described by Yu et al. [20].

Preparation of GMA:EDMA (2:1) polymer monolith:

AIBN 12 mg, 0.073 mmol was placed into a 20 ml scintillation vial containing glycidyl methacrylate monomer (665 μl, 5 mmol) and the ethylene dimethacrylate crosslinker (455 μl, 2.42 mmol). 1820 μl of methanol and 455 μl of ethanol were added as porogens. The reaction mixture was degassed by sonication for 10 min. The mixture was then divided into 1 ml-aliquots, which were placed into three separate 20 ml scintillation vials and were polymerized under the UV light (λ = 365 nm) for 94 min. The polymer was finally washed with methanol through Soxhlet extraction for 12 h and dried in a vacuum oven at 50 °C overnight to obtain a white crystalline powder. ATR: 755, 847, 905, 990, 1145, 1254, 1456, 1635, 1653, 1684, 1718 cm^{-1}.

2.3.2. Preparation of the immobilized fluorinase

Fluorinase solution in sodium phosphate buffer (pH 8.0, 100 μl, C = 2.79 mg/ml) was placed into a scintillation vial containing 20 mg of polymer and 400 μl of sodium phosphate buffer (pH 8.0). The vial was capped and shaken intensively with an orbital shaker at 600 rpm for 1 h at room temperature. The top supernatant was discarded and analyzed for residual protein concentration via the Bradford method and the effective concentration of the immobilized fluorinase was calculated as a difference between the starting concentration (C_{st}) and residual enzyme concentration (C_{res}) in solution ($C_{eff} = C_{st} - C_{res}$). The polymer was washed with phosphate buffer (pH 8.0, 2 × 200 μl) to remove residual protein solution. Then the fluorinase immobilized on the polymer monolith was stabilized by washing with 4 × 300 μl of phosphate buffer, shaking the vial for 1 h at RT during each wash cycle. Finally, the immobilized fluorinase (0.7 mg flA/20 mg polymer) was dried in vacuum over $CaSO_4$.

2.3.3. Determination of enzyme leakage

A portion of the polymer-immobilized fluorinase (20 mg of polymer and 0.7 mg of enzyme) was placed in a scintillation vial containing 600 μl of phosphate buffer (pH 8.0). The heterogeneous mixture was shaken with the orbital shaker at 600 rpm for 1 h at room temperature. The top supernatant was removed and analyzed for the total enzyme concentration that was leached out using the Bradford method. The percentage of leached enzyme was calculated using this formula: $\%\text{leakage} = \{C_{leak}/C_{immob}\} \times 100$. The concentration of the remaining enzyme was calculated as $C_{eff} = C_{immob} - C_{leak}$. The procedure was repeated for several times to obtain the graph of enzyme leakage from the carrier surface (Fig. 1).

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