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Short communication

Direct introduction of R-SO2F moieties into proteins and protein-polymer conjugation using SuFEx chemistry

Shaohua Li [a], Devora Cohen-Karni [a], Laura T. Beringer [a], Changgong Wu [b], Ethan Kallick [a, c], Howard Edington [c], Michael J. Passineau [b], Saadyah Averick [a, *]

[a] Laboratory for Biomolecular Medicine, Allegheny Health Network Research Institute, Allegheny General Hospital, 320 E. North Ave., Pittsburgh, PA, 15212, USA
[b] Gene Therapy Program, Allegheny Health Network Research Institute, Allegheny Health Network, Pittsburgh, PA, 15212, USA
[c] Division of Surgery, Allegheny Health Network, Pittsburgh, PA, 15212, USA

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ABSTRACT

The modification of proteins with reactive handles has facilitated the use of these biomolecules in diverse fields including drug delivery, diagnostics, environmental remediation, and cell culture matrices. Recently, a new "click" type reaction, sulfur(VI) fluoride exchange (SuFEx), was reported between sulfuryl fluoride and various organic moieties (e.g. hydroxyl, amine) to yield a sulfamoyl fluoride ($-NSO_2F$) or fluorosulfate ($-OSO_2F$) moiety under biphasic conditions. The model protein bovine serum albumin (BSA) was reacted with SO_2F_2 gas under biphasic conditions to yield $-SO_2F$ functionalized protein. The resultant BSA-SO_2F was characterized using gel electrophoresis, mass spectroscopy and Fourier transmission infrared spectroscopy to confirm the addition of $-SO_2F$ functional group. SuFEx modification of BSA caused a marked change in the pH dependent size and zeta potential of the protein as well as increased the protein's denaturation temperature. Crucially, BSA-SO_2F was demonstrated to be biocompatible after 72 h incubation with A549 lung endothelial cells. Due to the unique and elective reactivity of the $-SO_2F$ group with amines, BSA-SO_2F could be self-condensed to form a biocompatible hydrogel that was used in co-culture with HEK 293 cells. In addition, polyethylene glycol (PEG) with reactive $-OSO_2F$ groups at chain end was conjugated with BSA under various pH conditions through SuFEx chemistry. This communication, to our knowledge, is the first report of the application of the SuFEx in the field of bioconjugates.

1. Introduction

Through the modification of native amino acid residues, proteins have been utilized in a wide range of applications including therapeutics [1], diagnostics [2] and biomaterials [3] for tissue engineering. Surface modification of materials, especially for biomedical applications is integral to cell attachment and proliferation [4]. Lysine residues are attractive groups for bioconjugation due to their ubiquity across all protein classes [5]. The lysine residue has been used to prepare a wide range of materials including modification with acrylate groups to form protein nanogels [6], immobilization with atom transfer radical polymerization initiators [7], or reversible addition fragmentation chain transfer agents [8] to graft-from protein polymer hybrids [9]. Grafting-to strategies employ activated esters for polymer immobilization onto the lysine residue including the preparation of PEGylated protein pharmaceuticals [10].

"Click" type conjugation chemistries have dramatically expanded the range and types of molecules that can be conjugated to proteins using lysine [11]. In order to expand the class of bioorthogonal lysine modification strategies, we have utilized the newly described Sulfur(VI) Fluoride exchange reaction (SuFEx) [12]. One manifestation of the SuFEx reaction utilizes sulfuryl fluoride gas as a reagent to react with primary amines to yield sulfamoyl fluoride functional groups [12a]. The hallmarks of a "click" reaction are demonstrated in this process, namely the ease of reaction set-up, high yields, and simple purification method. In this

Abbreviations: SuFEx, sulfur(VI)-fluoride exchange; BSA, bovine serum albumin; PBS, phosphate buffered saline; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; GFP, green fluorescent protein.

* Corresponding author.
E-mail address: saverick@wpahs.org (S. Averick).

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communication we report the modification of, a model protein, BSA with sulfuryl fluoride to yield BSA-SO_2F and demonstrate the effects of surface group modification on the physical and chemical nature of this model protein. A model polyethylene glycol (PEG) with reactive $-OSO_2F$ groups at chain end was also synthesized and utilized in conjugation with BSA under various pH conditions. This is the first report to our knowledge that demonstrates the use of the SuFEx reaction to modify a biomolecule (see Scheme 1).

2. Materials and methods

BSA was purchased from EMD Millipore and used as received. SO_2F_2 (gas) was purchased from SynQuest Laboratories. 4-Aminophenol and triethyl amine was purchased from Aldrich. Dulbecco's Phosphate Buffered Saline (10X) was purchased from Corning Life Sciences. mPEG-SPA (NHS ester, M_n = 5000) was purchased from Nektar. All other chemicals were purchased from Aldrich and were used without further purification.

The pH dependent size and zeta potential of the BSA-SO_2F was measured using a Nanoplus3 dynamic light scattering (DLS) instrument equipped with an autotitrator. 0.1 mg/mL solutions of BSA and BSA-SO_2F were prepared in ultra-pure water and were used in the size and zeta potential measurements. FT-IR spectra were measured on a Perkin Elmer Frontier Optica FT-IR spectrometer using lipolized protein powders or dried films.

2.1. Mass spectrometry of modified BSA

Modified and unmodified BSA were desalted using C-18 Ziptip and MS was detected on a MALDI-TOF/TOF tandem MS (Bruker UltrafleXtreme MALDI TOF/TOF Mass Spectrometer, Bruker Daltonics Inc. Billerica, MA, USA). The data analysis was performed using FlexAnalysis software (Bruker Daltonics Inc.).

2.2. Preparation of BSA-SO_2F

BSA (1.08 g) was dissolved in 10 mL of ultrapure water in a round bottom flask equipped with a magnetic stir bar. Dichloromethane (10 mL) and triethylamine (2 mL) were added, and the flask was sealed with a rubber septum. The air in the flask was gently removed under vacuum, and sulfuryl fluoride gas was introduced by a balloon with a needle. The reaction was stirred vigorously at room temperature overnight. The dichloromethane was removed under vacuum, and the product was dialyzed against 1X PBS solution. The dialyzed product was then freeze-dried.

2.2.1. Preparation of the condensed BSA-SO_2F hydrogel

The aforementioned BSA solution after reacting with the SO_2F_2 gas was directly used in the preparation of a BSA-SO_2F hydrogel. Briefly, the dichloromethane in the BSA-SO_2F solution mixture was removed under vacuum at 40 °C. 1 mL of the remaining aqueous solution was directly placed in a glass vial with cap and was heated at 80 °C for 24 h. The aliquot of remaining solution on the gel surface was removed, and the as-formed BSA gel was suspended within 1X PBS solution. The suspension was shaken for 24 h while the PBS solution was changed 3 times to ensure all impurities were removed. The BSA hydrogel was then used in characterization studies before being dried into a thin film and then rehydrated for the biocompatibility study.

2.3. Preparation of PEG-Bn-OSO_2F

2.3.1. PEG-Bn-OH

mPEG-SPA (NHS ester, 500 mg, 0.1 mmol, M_n = 5000) and 4-aminophenol (109 mg, 1 mmol) was dissolved in 1 mL dichloromethane. The solution was constantly stirred at room temperature for 24 h. The solvent was evaporated under vacuum, and the product was purified by dialysis against water using 2 K MWCO membrane to afford pure **PEG-Bn-OH**.

2.3.2. PEG-Bn-OSO_2F

PEG-Bn-OH (500 mg, 0.1 mmol) and triethylamine (2 mL) was dissolved in 10 mL dichloromethane in a round bottom flask sealed with a rubber septum. The air in the flask was gently removed under vacuum, and sulfuryl fluoride gas was introduced by a balloon with a needle. The reaction was stirred vigorously at room temperature overnight. The dichloromethane was removed under vacuum, and the product was dialyzed against water using 2 K MWCO membrane.

2.4. Preparation of BSA-PEG using SuFEx chemistry

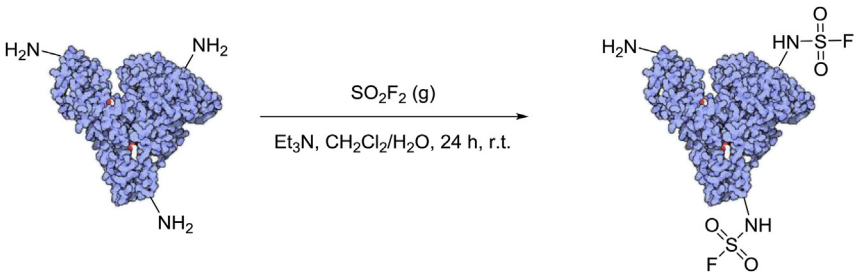
BSA (5 mg) and **PEG-Bn-OSO_2F** (20 mg) was dissolved in 1 mL PBS buffer (PH = 7.4). In two other reaction conditions, buffer with pH = 9, 11.3 were used instead of PBS buffer. The mixture was stirred at room temperature for 2 d. The modified BSA was purified by dialyzed against water using 10 K MWCO membrane.

2.5. Cell culture and experimental setup: A549

The adenocarcinoma line A549 which is frequently used as a lung transport epithelial model [13] was used for the soluble biocompatibility study. Cells were passaged several times and grown in DMEM (Cellgene) supplemented with 10% fetal bovine serum (FBS) prior to the experiment. BSA and BSA-SO_2F were prepared as soluble aqueous solutions in increasing concentrations and added to a confluent layer of cells prior to incubation for 72 h.

2.6. Cell culture and experimental setup: HEK 293 GFP

HEK 293 cells transfected with a green fluorescent protein (GFP) were cultured in DMEM supplemented with 10% FBS for several



Scheme 1. SuFEx modification of BSA with SO_2F_2(g).