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Sulfonium alkylation followed by 'click' chemistry for facile surface modification of proteins and tobacco mosaic virus

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ABSTRACT

Alkylsulfonium salts (ASS) have been shown to act as powerful alkylating agents. However, few studies have addressed the application of sulfonium salts to the modification of biomolecules such as nucleic acids and proteins. Since these large biomolecules play important roles in biological processes, a convenient and fast method for their modification is greatly needed. In this work, for the first time, we used a tandem method of sulfonium alkylation and click chemistry (CuAAC) for modification of biomolecules. Fluorescent labeling of proteins and tobacco mosaic virus were successfully performed after simple incubation of biomolecules with sulfonium salts followed by azido-containing compound at room temperature. This facile bioconjugation assay based on ASS-CuAAC reactions should be useful in protein chemistry and bionanoscience.

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The transfer of an intact methyl group from a donor to a suitable acceptor is a vitally important biological process. The universal donor of intact methyl group is AdoMet,[1] which is an important cofactor for enzymes that methylate a diverse array of nucleophile acceptors within the cell (i.e., DNA, RNA, proteins, biogenic amines, etc.). In order to have a chemical basis for understanding the alkyl transferation, a series of methylsulfonium salts as methylating agents have been investigated.[2] Moreover, since higher alklysulfonium salts have recently become readily available,[3] increasing studies of their alkylating ability were undertaken. It is now clear that under mild conditions employed, alkylsulfonium salts (ASS) indeed act as powerful alkylating agents.[4,5] However, few studies have addressed the application of sulfonium salts to the modification of biomolecules such as nucleic acids and proteins. Since these large biomolecules play important roles in biological processes, a convenient and fast method for their modification is greatly needed. Therefore, we decided to investigate the chemical modification of biomolecules through alkylation with alkylsufonium salts.

On the other hand, the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC),[6] often referred to as 'click' chemistry, is very chemoselective, only occurring between alkynyl and azido functional groups with high yield. In addition, the resulting 1,2,3-triazoles are stable at aqueous conditions and are biocompatible. Recently, click chemistry has prevailed with applications in organic synthesis, drug discovery, materials science, and biotechnology, which is proved to be an ideal tool for incorporating functionalities onto desired scaffolds.[7–13] The present study intends to develop a tandem ASS-CuAAC for labeling and modification of biomolecules. After labeling alkyne groups onto protein surface through sulfonium alkylation, click chemistry was used to incorporate fluorescent moieties to these biomolecules.

Modification of proteins is often accomplished by using various alkylating agents. Moreover, plant virus such as tobacco mosaic virus (TMV) contains coat proteins, which in principle can be modified via these alkylating agents. A sulfonium salt **1** as an alkyne-containing alkylating agent was designed and synthesized (Scheme 1). Compound **1** has potential ability to react with nucleophiles to selectively transfer alkyl group, which could be employed for surface modification of proteins and virus.

Bovine serium albumin (BSA) contains many nucleophilic residue such as amino, imidazole, thiol, and carboxylate groups on its surface. When we combined **1** at room temperature with BSA in phosphate buffer (pH 8.0), we observed the lowered solubility of the protein. After incubation for 24 h and then treated with azide-contained fluorescent compound **2** (Fig. 1A), the samples

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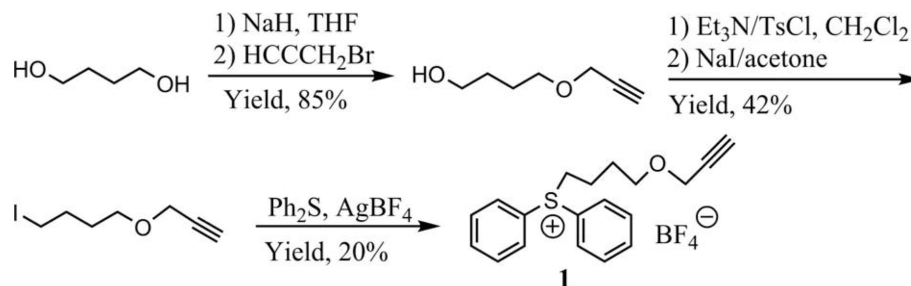

Scheme 1. Synthesis and structure of a sulfonium salt used in this study.

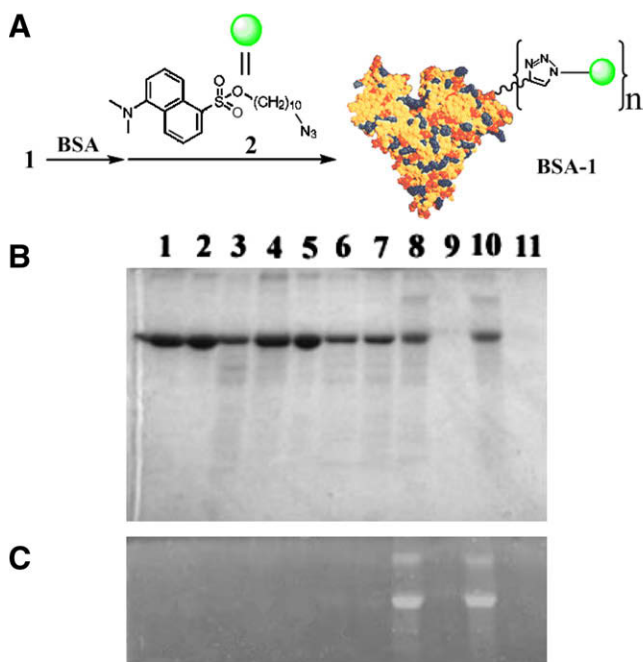

Figure 1. (A) Schematic depiction of the generation of fluorescent labeling BSA by tandem ASS-CuAAC reactions; BSA represents as space-filling model.[16] (B) Lanes 1 and 2, BSA with or without DMSO; lanes 3 and 4, BSA + Cu(II)/ascorbate + ligand N^1,N^1-diisopropylethane-1,2-diamine; lane 5, BSA + **2**; lanes 6 and 7, BSA + Cu(II)/ascorbate; lanes 8–11, BSA treated with ASS-CuAAC reactions; lanes 8 and 10, the reaction precipitation; lanes 9 and 11, the reaction solution; lanes 10 and 11, reaction without the ligand. (C) The same gel of (B) under ultraviolet illumination. See Supplementary data for detailed experimental process.

were analyzed by denaturing gel electrophoresis, as shown in Figure 1B and C.[14] Compared with the gel results under ultraviolet illumination and Coomassie blue staining, at least three points can be concluded: (I) the fluorescent labeling of proteins can only be obtained after sulfonium alkylation followed by click (ASS-CuAAC) reactions; (II) the dye-labeled proteins have decreased solubility in aqueous buffer; (III) the click reaction seems to proceed smoothly even without the ligand used in normal click reaction. The ASS-CuAAC reaction should be a general method for biolabeling.

In order to optimize the protocol of ASS-CuAAC reactions, nonfluorescent **3** was employed as the azido carrying agent (Fig. 2A). Compound **3** is nonfluorescent because of a photo-induced electron transfer (PET) effect from azido group to coumarin group.[15] When click reaction occurs between azido and alkynyl groups, 'turn-on' fluorescence could be observed for the coumarin moiety, which could be used to monitor the reaction in real-time. We found that sulfonium alkylation of proteins can be finished in 4 h by the treatment with ASS in $NaHCO_3$–Na_2CO_3 buffer (pH 9.0). The alkyne-labeled BSA was dialyzed with Tris–HCl buffer (0.1 M, pH 7.5) to remove **1**, and then used for CuAAC reaction. As shown in Figure 2B, fluorescent labeling of BSA was accomplished in 7 h by the appearance of a maximum fluorescence emission band at approximately 480 nm, characteristic to the emission band of coumarin. Control experiments without either **1** or **3** gave no such band. These results showed successful selective fluorescent labeling of BSA.

Encouraged by the results in proteins, the bioconjugate reactions were also used to functionalize virus. A TMV virus contains a single stranded RNA of 6395 nucleotides encapsulated in a helical virion by 2130 coat protein (TMV CP) monomers, showing about 300 nm length.[17] TMV particles have recently shown great potential application in bionanoscience.[18–20] As shown in Figure 2A, TMV was first treated by ASS in $NaHCO_3$–Na_2CO_3 buffer (pH 9.0) for 4 h. It is noted that the modified TMV can easily be precipitated during the reaction. Bioconjugate reactions for TMV were performed with careful shaking. After centrifugation and washed by Tris–HCl buffer (0.1 M, pH 7.5) for several times, the alkyne-labeled virus was used for CuAAC reaction. After 7 h, the TMV-1 was isolated by centrifugation and washed by buffer.[21] As shown in Figure 2C, the TMV is non-fluorescent, while the TMV-1 at the same concentration has strong emission at center about 480 nm (Fig. 2C), showing the successful fluorescent labeling on TMV surface.

The modified TMV was further analyzed by denaturing SDS gel electrophoresis and transmission electron microscopy (TEM) (Fig. 3). It is clear that fluorescent dye was attached to viral protein (Fig. 3, lanes 4 and 5), which is consistent with result obtained from emission spectra. The SDS gel electrophoresis of modified TMV coat protein (TMV CP) gave three major bands; all bands showed dye labeling in Figure 3B. TEM showed that TMV particles were not destroyed by the ASS-CuAAC reactions (Fig. 3C), implying that the reaction conditions are mild and biofriendly.

In summary, for the first time, we developed a tandem method of sulfonium alkylation and click chemistry for modification of biomolecules. Fluorescent labeling of proteins and virus were successfully performed after simple incubation of biomolecules with sulfonium salts followed by azido-containing compound at room temperature. We believe that the mild and biocompatible conditions of ASS-CuAAC reactions used here can efficiently bioconjugate a wide range of compounds and/or functional groups to the surface of proteins and virus. This facile bioconjugate assay should be useful in protein chemistry and bionanoscience.

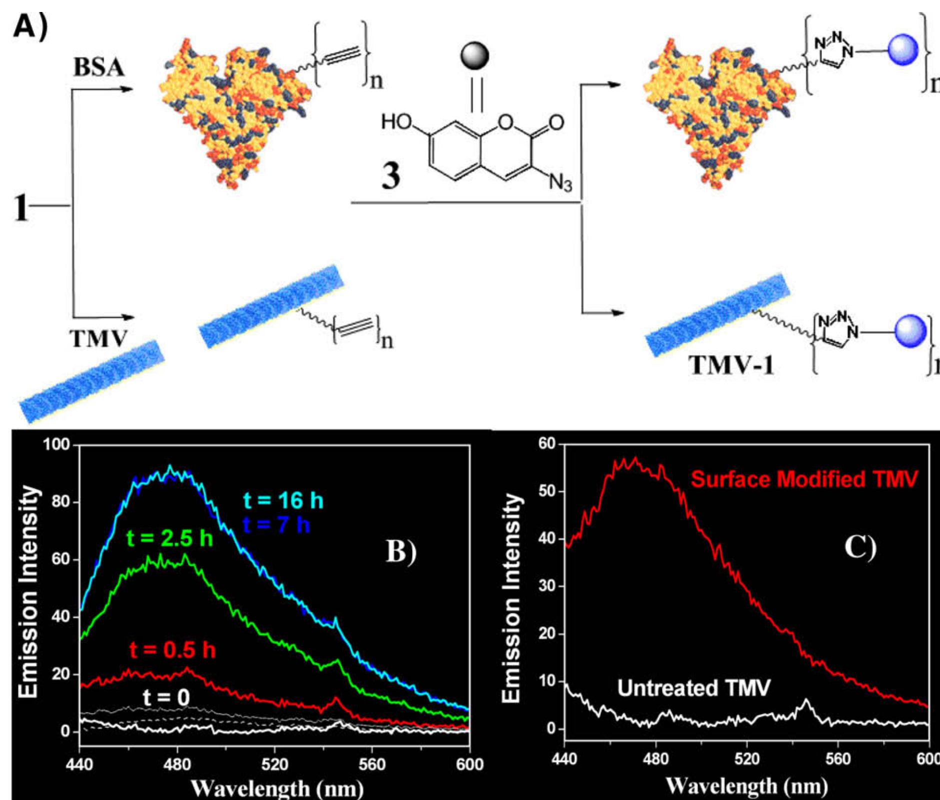

Figure 2. (A) Bioconjugation of BSA and TMV to give fluorescent labeling products. (B) Time-dependent emission spectra of CuAAC reaction between alkylated-modified BSA with **3**. For CuAAC reactions, **3** and the alkyne-modified BSA were mixed in 2 mL Tris-HCl buffer (0.1 M, pH 7.5). After the addition of $CuSO_4$ and ascorbate, emission spectra of the reaction solution excited at 400 nm were monitored; slit width of both excitation and emission are 5 nm. Dot and dash line in (B) represents BSA treated with ASS or CuAAC only. (C) Compare the emission spectra of TMV and TMV-1 at the same concentration.

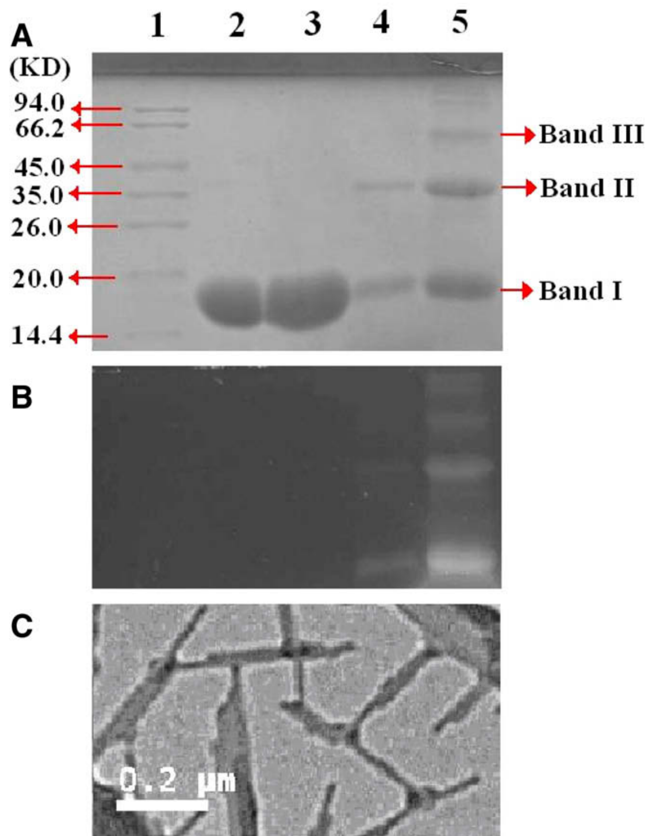

Figure 3. Surface modification of TMV by ASS-CuAAC reactions. (A) Lane 1, marker; lane 2, TMV CP;[22] lane 3, TMV; lane 4, TMV-1 CP, lane 5, TMV-1. (B) The same gel of (A) under ultraviolet illumination. (C) TEM image of TMV-1.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.11.107.

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14. For bioconjugate process: **1** (3 mM) and BSA (3 mg/ml) in 40 μl phosphate buffer (pH 8.0), buffer (0.1 M, pH 9.0) were incubated at room temperature for

24 h. Then **2** (3.75 mM), $CuSO_4$ (0.5 M), and ascorbate (0.5 M) were added into the reaction solution for another 24 h at room temperature.

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21. For bioconjugate process: TMV (200 μl, 1 mg/ml) was first treated by **1** (final concentration 3 mM) in 500 μl $NaHCO_3$–Na_2CO_3 buffer (pH 9.0) for 4 h with careful shaking. After centrifugation at 12,500 rpm and washed by Tris–HCl buffer (0.1 M, pH 7.5) for several times, the alkyne-labeled virus was used for CuAAC reaction. After 7 h, the modified TMV was also isolated by centrifugation and washed.
22. TMV coat protein was prepared by the acetic acid method. See: Fraenkel-Conrat, H. *Virology* **1957**, *4*, 1–4.