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Design and synthesis of fluorescent glycolipid photoaffinity probes and their photoreactivity

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ABSTRACT

Glycolipid–protein interactions at the cell surface are implicated in various biological processes. Toward the investigation of glycolipid binding proteins, we designed and synthesized trifunctional photoaffinity probes, which present a sugar head group with a triazole linkage to the lipid tail unit containing a photoreactive group and a fluorescent tag. The glycolipid photoaffinity probes bearing benzophenone group or diazirine group were evaluated for their photocrosslinking reactivity toward a carbohydrate head group specific protein. The diazirine based glycolipid photoaffinity probe was found to be more effective than the benzophenone-based probe in a comparative analysis involving a competitive ligand to distinguish a specific binding protein.

Glycolipids represent a major component of cell surface carbohydrates, which are implicated in various biological processes such as cell–cell interaction, vesicle trafficking and cellular signaling through carbohydrate–protein interactions.[1] Despite their ubiquity and biological significance, much of the glycolipid–protein interactions remain unknown. Discovery of protein receptors for major glycolipids should facilitate our understanding on their biological roles at the molecular level. Since glycolipids are important as biomarkers, characterization of glycolipid binding proteins will also be useful for the development of new chemotherapeutics and diagnostic agents.

Photoaffinity labeling (PAL) has been pursued as a promising approach toward detection and isolation of glycolipid-binding proteins by covalent crosslinking.[2] For example, Sonnino's group has demonstrated that the glycolipid probes derivatized with photoreactive groups and radiolabels enabled the discovery of TAG1 and VIP21-caveolin as GM1 binding proteins and c-Src and Lyn as GD1b binding proteins.[3] However, such successful examples are still limited in the literature and the investigation of novel glycolipid-binding proteins is still a significant challenge. This is because studying glycolipids is difficult at several levels. The preparation of glycolipids is not easy either by synthesis or by isolation from the natural sources. Their amphiphilic property makes it difficult to handle. Having two distinct biologically relevant binding sites, a sugar head group and a lipid tail group, they can interact with various proteins in multimodal fashions. They could bind proteins at the sugar head group, or at the tail group or at both moieties. There are a number of proteins, which nonspecifically bind to the lipid moiety. These proteins may be readily crosslinked by PAL and complicate the subsequent analysis to determine specific binding proteins. Moreover, carbohydrate–protein interactions are often associated with weak binding affinity, making it difficult to isolate and identify glycolipid binding proteins.[4] Therefore new design of glycolipid photoaffinity probes and methods are desired to explore novel glycolipid binding proteins. Here we designed and synthesized trifunctional photoaffinity probes, which present the sugar head group linked via a triazole linkage to the lipid tails with photoreactive group and a fluorescent tag for investigating glycolipid binding proteins. Based on our interest in the biological role of the carbohydrate moiety in the glycolipid function, the probes were intended to capture glycolipid binding proteins, which specifically recognize the sugar head group. Photoaffinity labeling studies demonstrated that our glycolipid probes are capable of selectively capturing a glycolipid binding protein in the presence of a carbohydrate-binding protein with different specificity.

Glycolipid photoaffinity probes **1** and **2** were designed as shown in Figure 1 to enable covalent crosslinking, subsequent fluorescence detection and isolation of binding proteins. We hypothesized that glycolipid probes with a sugar head group presented on unnatural lipid tail groups can be recognized by glycolipid binding proteins by virtue of a carbohydrate–protein interaction. Based on our previous study, diazirine group would facilitate highly selective photocrosslinking of low affinity carbohydrate-binding

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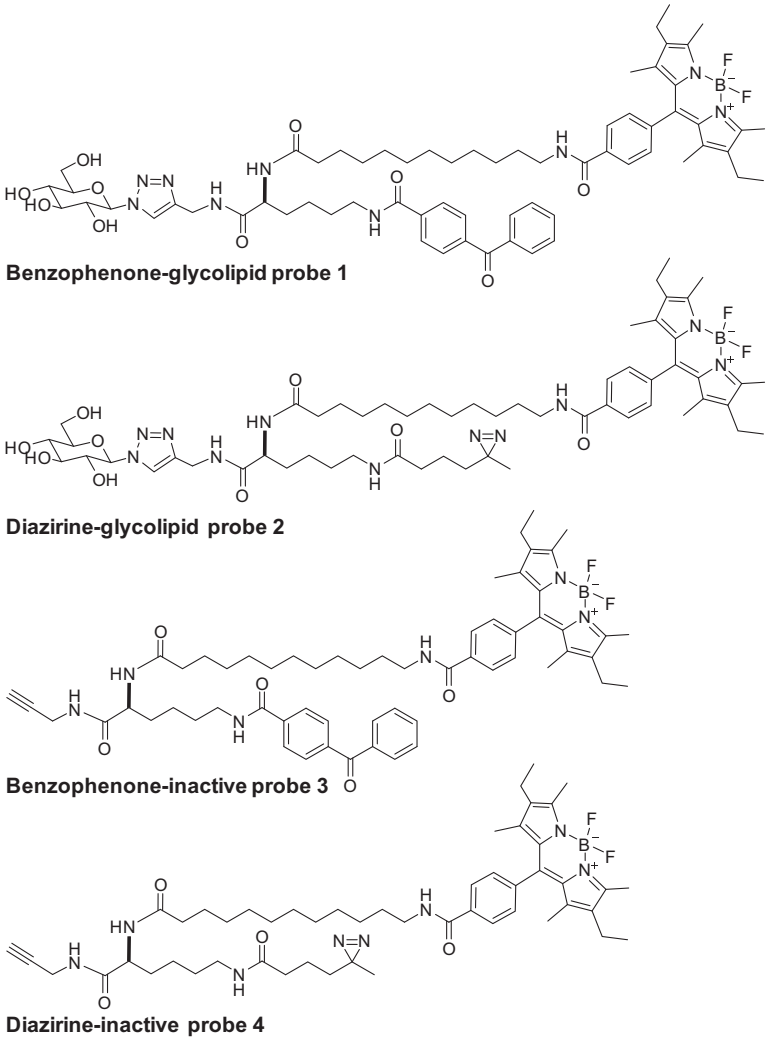

Figure 1. Structures of glycolipid photoaffinity probes **1**–**2** and the corresponding control probes (inactive probes) **3**–**4**.

proteins in complex protein mixtures.[5] Alternatively, benzophenone group may be useful for photocrosslinking of membrane associated proteins due to its tendency to react with hydrophobic amino acid residues via C–H insertion.[6] BODIPY group was employed to serve both as a sensitive fluorescent reporter group as well as a membrane anchoring unit being a part of a lipid tail. It would also be useful for characterization of their cellular localization. The sugar head group is attached to the fluorescent photoreactive lipid tail unit via a triazole linker in place of an *O*-glycosidic bond found in natural glycolipids such as glycosphingolipids and glycosylated glycerophospholipids. We envisaged that use of click chemistry[7] would ensure efficient and reliable assembly of a glycosyl azide and an alkyne-conjugated lipid tail unit without the need for protecting group manipulation. This coupling strategy should be generally applicable to a variety of sugar head groups including complex oligosaccharide structures. Additionally, triazole linked glycosides has been shown as suitable sugar head group mimics, being stable toward glycosidases.[8] For our proof of principle study, we employed glucose as a sugar head group to prepare glycolipid photoaffinity probes modeled after β-glucosyl ceramide, which is one of the simplest glycosphingolipids and a key precursor for the biosynthesis of complex glycosphingolipids.[1a,9]

The syntheses of **1** and **2** were achieved as shown in Scheme 1. Compound **5** and **6** were synthesized from *N*-Boc-lysine as reported previously.[5] Briefly, *N*-Boc-lysine was first acylated using an NHS ester derived from either benzoyl benzoic acid or diazirine carboxylic acid, which was subsequently amidated using propargyl amine. After deprotecting Boc group, BODIPY-conjugated lauric acid **9** was introduced to amine **7** and **8** by amide coupling to yield alkyne-conjugated lipid tail unit **3** and **4**. The sugar head group was introduced to the alkyne-conjugated lipid tail unit under the copper-promoted alkyne–azide cycloaddition (CuAAC) condition[7] using glucosyl azide **10** to provide benzophenone-based glycolipid probe **1** and diazirine-based glycolipid probe **2** in high yield.

The glycolipid photoaffinity probes **1**–**2** were first evaluated for their ability to bind and photocrosslink almond β-glucosidase, which specifically recognizes β-glucosyl ceramide (K_m = 51 μM)[10] and hydrolyzes the sugar head group. The glycolipid photoaffinity probe and β-glucosidase were first allowed to bind at 4 °C for 1 h then were reacted by irradiating 365 nm light at 0 °C. Unreacted probes were removed by acetone precipitation or chloroform/methanol precipitation and the resultant mixture was separated by SDS–PAGE. The amounts of the crosslinked β-glucosidase were quantitated by fluorescence intensity of a corresponding band in the SDS–PAGE gel. The crosslinking yields were calculated as per cent crosslinked based on the molar amounts of β-glucosidase used for a given reaction.

Both of the probes **1** and **2** reacted with β-glucosidase only under the UV irradiation condition (Fig. 2) and in the absence of