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# Immobilization of carbohydrate clusters on a quartz crystal microbalance sensor surface

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ABSTRACT

The immobilization of carbohydrates on gold surfaces is a prerequisite technology for carbohydrate-related studies, including those of carbohydrate–biomolecule interactions. Glycolipid domains in cell membranes, such as lipid rafts, are thought to play an important role in cell biology through their carbohydrate portions. To understand the recognition of glycolipid domains such as receptors for bacterial toxins and viruses, we immobilized clusters of carbohydrates on a gold surface by using polyamidoamine (PAMAM) dendrimers as a scaffold. The PAMAM dendrimers were adsorbed on the gold-coated surface of a quartz crystal microbalance (QCM) sensor and were observed by means of QCM with dissipation (QCM-D). After adsorption of the PAMAM dendrimers, lysoganglioside-$GM_1$ and 12-aminododecyl-*N*-acetylglucosaminide (GlcNAc–C12–$NH_2$) were immobilized on the amino groups of PAMAM dendrimers by means of an $NH_2$ cross-linker. Immobilization of the carbohydrates was confirmed by observation of their specific interaction with anti-ganglioside $GM_1$ antibody or wheat germ agglutinin (WGA). Surfaces with different GlcNAc–C12–$NH_2$ cluster sizes and densities were prepared by varying the size of the PAMAM dendrimers or the concentration of GlcNAc–C12–$NH_2$ immobilized on the dendrimers, respectively. Analysis of the binding between the GlcNAc–C12–$NH_2$-immobilized surface and WGA revealed that the size of the PAMAM dendrimers influenced the GlcNAc–C12–$NH_2$–WGA interaction, with larger dendrimers resulting in higher WGA binding constants.



## 1. Introduction

Carbohydrates consist of various kinds of saccharides and multiple linkage structures of saccharide units. Many carbohydrates on cell surface exist as glycolipids or glycoproteins, that is, complexes with lipids or proteins, respectively. Carbohydrates protect cells against harmful environmental factors [1–3], mediate cell attachment [4,5], participate in signal transduction pathways [6,7], and serve as receptors for microorganisms and their toxins [8–10]. Glycolipids frequently aggregate owing to the hydrophobicity of their lipid moieties, forming carbohydrate-enriched microdomains known as "lipid rafts" on cell surfaces. Recently, these lipid rafts have been suggested to serve as platforms for various cellular events, such as those mentioned above [11].

Numerous studies, including studies of the interactions between carbohydrates and proteins, carbohydrates, and other biomolecules, have been carried out to understand the role of carbohydrates in biological events. The immobilization of carbohydrates on a solid substrate, such as gold sensor chips for quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) [12], are essential techniques for the study of carbohydrate–protein interactions. However, because the lipid moieties of glycolipids are unreactive, glycolipids cannot be readily immobilized on solid substrates. To overcome this problem, various immobilization methods utilizing the lipids' hydrophobicity have been reported, such as attachment of a self-assembled monolayer (SAM) of alkanethiols on a gold surface [13], direct attachment of gangliosides to a conventional CM5 carboxymethyl dextran sensor chip [14], and utilization of vesicles composed of glycolipids and alkanethiols onto gold electrode [15]. In addition, ligand-conjugated saccharides, such as SH-saccharides synthesized by amination between an aminophenyl disulfide and a carbohydrate [16], have been utilized for the modification of a gold surface.

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In general, the binding affinity of an individual carbohydrate motif for a protein receptor is lower than that required for biological function. To increase the binding affinity between carbohydrates and protein receptors, carbohydrates often exist in aggregated (i.e., lipid raft) or polymeric forms. Multivalent linker compounds containing one, three, or four aromatic amines and thioctic acid moieties [17] have been developed to immobilize carbohydrates on SPR sensor chips, and the effect of these compounds on the clustered carbohydrates' binding affinity has been evaluated. Although such linkers allow the immobilization of clustered oligosaccharides on SPR sensor chips, the preparation of the linker and subsequent reaction with the ligand-conjugated saccharide require many complicated synthetic steps.

We have developed a comparatively simple method to immobilize clustered carbohydrates on a gold sensor surface, without the need for complex synthetic steps. In this report, we describe a novel strategy for controlling carbohydrate density on sensor surfaces (Fig. 1). Polyamidoamine (PAMAM) dendrimers are adsorbed strongly on the gold surface of a QCM sensor chip. The immobilization of carbohydrates on the PAMAM dendrimers is shown schematically in Fig. 1A. The immobilization is carried out with 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride), which cross-links the amino group of the PAMAM dendrimer with that of a lysoganglioside ($GD_{1a}$ or $GM_1$, Fig. 2) or a 12-aminododecyl carbohydrate derivative (GlcNAc–C12–$NH_2$, Fig. 2). Under a reaction of the chlorine atoms in cyanuric chloride with amine derivatives, sequential substitutions of the first, second, and third chlorine atoms in cyanuric chloride is reported to occur at 0–25, 20–40, and 60–100 °C, respectively [18]. In our immobilization method, firstly, the adsorption of PAMAM dendrimers on the gold sensor surface of QCM-D was carried out. After removing non-adsorbed dendrimers, the reaction mixture of carbohydrates (Fig. 2) and cyanuric chloride was loaded on the PAMAM dendrimers immobilized on the gold sensor surface. In the reaction mixture the cyanuric chloride can bind one or two carbohydrates at room temperature. However, the products bearing two carbohydrates cannot react with any amino residues of PAMAM dendrimer more because the substitution reaction of the third chlorine atom hardly occurs at room temperature. Hence, we suppose that the products bearing one carbohydrate exist on the surface of PAMAM dendrimer (Fig. 1A).

The extent of carbohydrate immobilization on the sensor surface can be controlled by two different means. First, the size of the carbohydrate cluster can be controlled by varying the diameter of the PAMAM dendrimers adsorbed to the sensor surface (Fig. 1B). Second, the density of carbohydrates within an immobilized cluster can be regulated by controlling the amount of cyanuric-chloride-linked carbohydrates that are coordinated to the PAMAM dendrimers (Fig. 1C). Using these methods, we obtained immobilized, clustered carbohydrates that were tightly bound to the PAMAM dendrimers, owing to the multivalent interactions of the carbohydrates, and that mimicked the lipid raft structures found on cell surfaces. Because the lipid rafts exist like scattered dots on a cell surface, we aim to fabricate a concentrative immobilization of the carbohydrates on scattered PAMAM dendrimer surface. Therefore, we attempted efficient immobilization with small amount of carbohydrates rather than the high loading capacity of

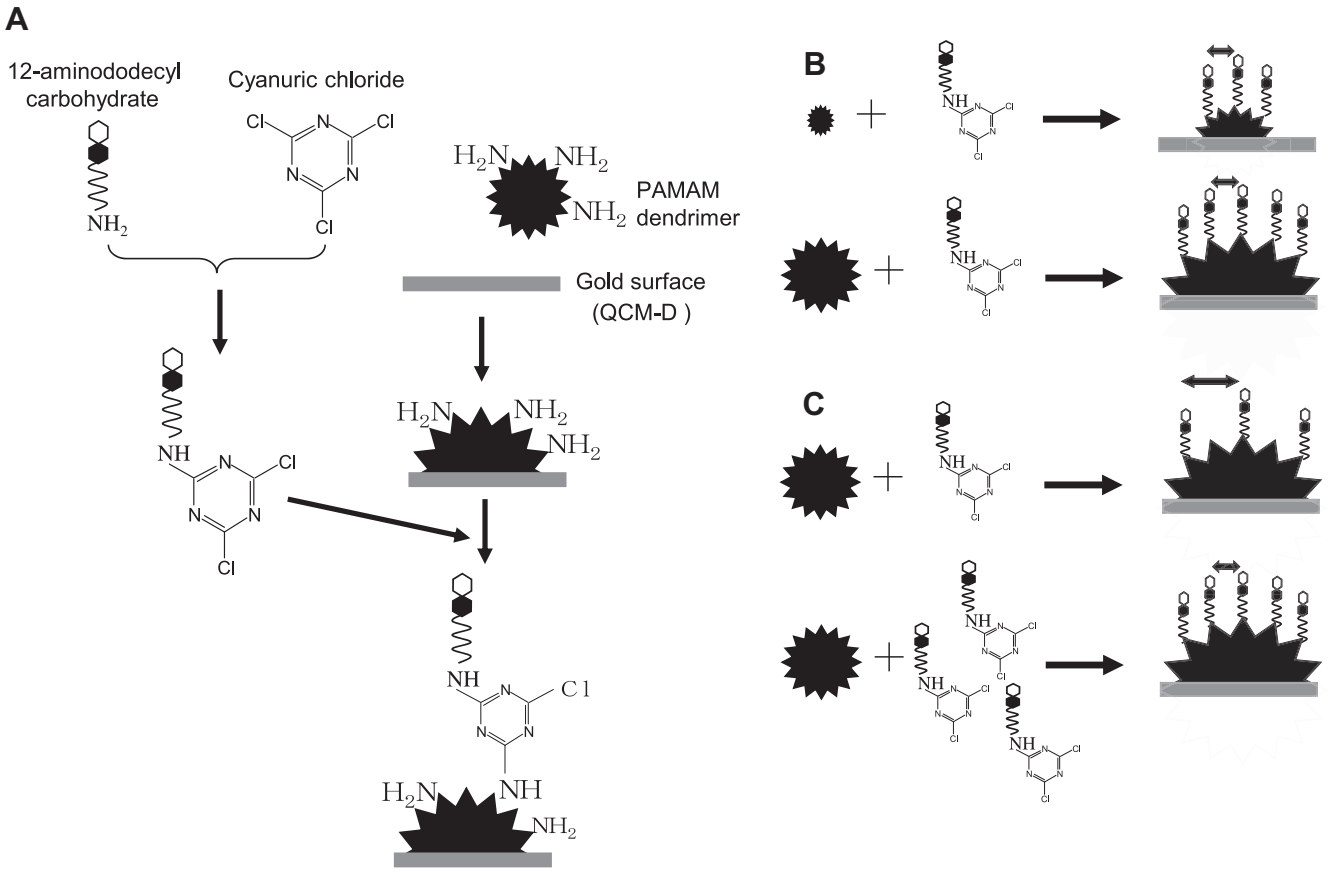


**Fig. 1.** (A) Schematic depicting the immobilization of a carbohydrate molecule on a PAMAM-adsorbed sensor surface for QCM-D studies. The cluster sizes and densities of carbohydrates immobilized on the sensor surface can be controlled by adjusting (B) the size of the PAMAM dendrimers or (C) the density of carbohydrates immobilized on the dendrimers.