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Noninvasive Sialic Acid Detection at Cell Membrane by Using Phenylboronic Acid Modified Self-Assembled Monolayer Gold Electrode

Akira Matsumoto,[†] Naoko Sato,[†] Kazunori Kataoka,[†] and Yuji Miyahara*[,†,‡]

Center for NanoBio Integration, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8656, Japan, and Biomaterials Center & International Center for Materials Nanoarchitectonics, National Institute for Materials Science, Namiki 1-1, Tsukuba, Ibaraki 305-0044, Japan

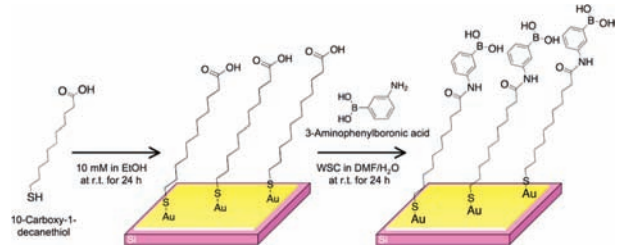
Received April 20, 2009; E-mail: miyahara.yuji@nims.go.jp

Glycosylations, or the alternations of cell surface glycan structures, are dynamic and stage-specific processes during numerous normal and pathological processes including developments and differentiations.[1] Most tumor-associated carbohydrate antigens including those clinically approved as tumor markers involve sialic acid (SA), an anionic monosaccharide that frequently occurs at the termini of the glycan chains. Indeed, overexpression of SA on the cell surface has been implicated in the malignant and metastatic phenotypes for many different types of cancers,[2] while decreased SA expression has also been identified in erythrocytes of diabetic mellitus.[3] Monitoring of the cell surface SA expression therefore provides rational indexes of the dynamic changes in tumor malignancy, metastatic potential, diabetic symptoms, and other SA-associated biological events. Ordinarily, cell surface SA content can be assessed by the use of commercial SA quantification reagents, which, however, involve multiple enzymatic and labeling procedures that are costly, time-consuming and also often require lethal pretreatments. Furthermore, SA residues must be either enzymatically or acid-catalytically cleaved from the glycan chains and then disintegrated into the free forms that can finally be subjected to the quantification. Such methods are very unlikely to prevail as standards for clinical practice.

We demonstrate here a potentiometric method for specific SA detection enabling a label-free, living cell operative, and real-time manner cytology. The principle of specific SA detection capitalizes on a reversible and covalent interaction between phenylboronic acid (PBA) and SA.[4] PBA is a synthetic molecule capable of reversibly binding with 1,2- or 1.3-diols, hallmark structures for a majority of glycan constituent saccharides.[5] In general, PBA can form stable complexes with sugars only in its dissociated form. Since most PBA derivatives are weak acids possessing a pK_a of *ca.* 8−9, many research efforts have so far been devoted to achieve the derivatives with an increased ionization constant or decreased pK_a.[6] In contrast to such historical challenges, some recent works have revealed that SA, exceptionally, can form stable binding with undissociated PBA.[4] This anomalous binding behavior has been correlated to the distinct binding modalities taken by the PBA−SA complexes from those of other saccharide involved cases.[4] An important indication by the finding is that a PBA molecule with properly modulated pK_a could provide a molecular basis for selective recognition of SA among other glycan constituent saccharides.

Scheme 1 summarizes the preparation of a PBA surface-modified gold electrode. A self-assembled monolayer (SAM) of 10-carboxy-1-decanethiol was first formed on a gold electrode followed by a condensation reaction with an amino group functionalized PBA (3-aminophenylboronic acid) resulting in the introduction of meta-amide substituted PBA onto the SAM terminal (SI Figure 1). The PBA-modified gold electrode was then electrically lined to a field effect transistor (FET) gate for real-time monitoring of the charge density changes taking place on the electrode. In this configuration, anionic charges of SA (due to carboxyl groups) bound to the electrode could be detected as positive direction shifts of threshold voltage (V_T) of the FET. Figure 1a shows plots of equilibrium V_T of the PBA-modified FET as a function of pH for the presence of various monosaccharides including SA. In the presence of 0.2 mM SA, V_T markedly increases with a decrease of the pH due to binding of anionically charged SA onto the electrode surface PBA. The pK_a of the utilized meta-amide group substituted PBA can be estimated to be ca. 9 and, therefore, with a decrease of the pH in the range shown in Figure 1a, the fraction of undissociated PBA progressively increases leading to more stable PBA−SA complexation and thus an increase of V_T. With the addition of 2 mM SA, the PBA−SA complexation is saturated showing a constant magnitude of the positive V_T shift over the pH analyzed. In contrast, no V_T shift is observable for any other saccharides or for the case of non-PBA modified FET. These observations thus provide evidence that, at around the neutral pH, most PBA moieties on the electrode surface are undissociated (otherwise positive shift of V_T due to boronate anions should be manifested in the absence of sugars (SI Figure 2)) leading to a predominant binding with SA. When the environmental pH was higher than the pK_a value of the PBA where a large fraction of the moiety is anionically charged, V_T dramatically increased in a manner independent of sugar type except for the case of a non-PBA modified FET (SI Figure 2). As a result, the PBA-modified electrode was no longer specific to SA under such an alkaline condition. Figure 1b shows time courses of the V_T for stepwise changes in various monosaccharide concentrations under the physiological pH (also see SI Figures 3 and 4), demonstrating its remarkably SA specific detection in accordance with Figure 1a. Due to the reversible nature of the PBA−SA binding, the surface-bound SA could be easily removed by rinsing with sugar-free water. Such reversibility could be judged from recovered levels of V_T after each washing, which was continuously observed at the initial ±1 mV. Furthermore, the electrode could

Scheme 1. Preparation of PBA Modified Gold Electrode



[†] The University of Tokyo.
[‡] National Institute for Materials Science.

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be stored completely dried for at least 3 months with no damage to the sensitivity.

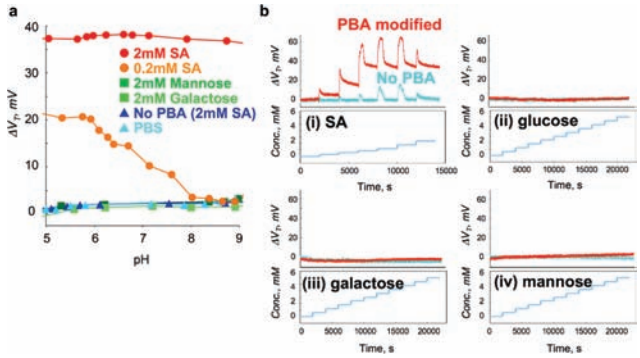

Figure 1. (a) Changes in equilibrium threshold voltage (V_T) of the PBA-modified FET as a function of pH in the presence of various monosaccharides. (b) Time courses of the V_T for stepwise changes in various monosaccharide concentrations investigated at pH 7.4. Light blue colored lines displayed beneath each graph indicate patterns of addition of each monosaccharide.

The PBA-modified FET was then tested for its ability to directly capture the glycan component SA present on the cell surface. Of note, the glycan terminal SA moieties are linked to the neighboring sugar unit at the expense of the C2 hydroxy group, which indeed is a candidate for the most favorable binding site involved in the PBA−SA complexation at pH 7.4.[4b] Therefore, it might be the case that the interaction between PBA and the glycan terminal SA are allowed only via metastable modalities. Even so, a number of works have demonstrated still appreciably specific binding of the system, where the SA side-chain glycerin hydroxyls at C7−C9 positions are likely involved as alternative binding sites.[4]

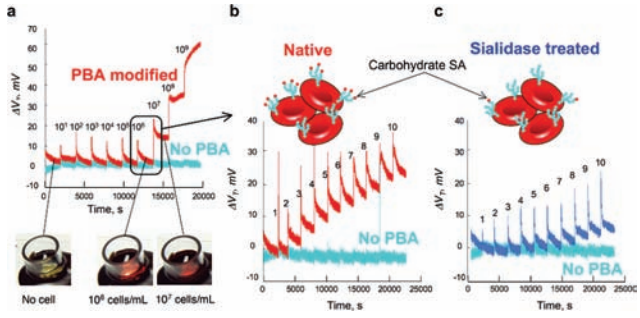

Figure 2. (a) V_T responses on adding rabbit erythrocytes to PBA-modified FET. The concentrations are indicated in numbers (cells/mL) at time points of each addition. (b, c) V_T responses on increasing erythrocyte concentration with steps of 10^6 cells/mL investigated for those native and enzymatically treated, respectively. Numbers denoted in the graph indicate concentrations of the cell suspensions (million cells/mL) and time points of each addition.

As for proof-of-principle, erythrocyte was investigated, for which alternations of SA content (frequently "decline"[3] while conflicting reports[7] also exist) has been reported in diabetes mellitus. Figure 2 shows V_T responses on adding an increased number of rabbit erythrocytes onto the electrode (also see SI Figures 5). Both (b) native erythrocytes and (c) those with enzymatically decreased surface SA (20% remains) were investigated. Comparison between the two systems reveals remarkably distinct V_T profiles with correspondence to the altered SA content per cell. This suggests that once a cell number−V_T calibration is determined for a healthy phenotype, the altered SA expressions can directly be monitored in a real-time manner, simply by placing the known-count living cell suspensions onto the electrode. Haldankar et al. have reported that erythrocyte SA content of Insulin Dependent Diabetes Mellitus (IDDM) decreased by 38% compared to healthy controls,[3c] which apparently is the range of feasible differentiation using the PBA-modified FET.

In summary, we described here a potentiometric detection of free and cell membrane surface SA using a PBA-modified gold electrode without any enzymatic and labeling procedures. The technique may have relevance to diagnoses of diabetes and also could provide a quantitative adjunct to histological evaluation for tumor malignancy and metastasis during intra- or postoperative diagnoses.

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Supporting Information Available: Experimental details, electrode surface characterizations, pH-dependent sugar selectivity, SA detection in competitive environment, sensitivities to charged and polymeric sugars, accompanied by supplementary discussion. The information is available free of charge via the Internet at http://pubs.acs.org.

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