Sensing Capabilities of Colloidal Gold Monolayer Modified with a Phenylboronic Acid-Carrying Polymer Brush

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A dithiolated random copolymer with pendent phenylboronic acid residues [Cys-poly(3-acrylamidophenylboronic acid-*co*-*N*,*N*-dimethylaminopropyl methacrylamide), Cys-poly(APBA-*co*-DMAPMA)] that shows the abilities of initiation, transfer, and termination (iniferter) was obtained by using a benzyl *N*,*N*-diethyldithiocarbamoyl (BDC) derivative. The obtained disulfide-carrying copolymer was accumulated on a colloidal gold-immobilized glass substrate, and the usefulness of the polymer brush as a sensing element for glycoproteins such as ovalbumin (OVA) was examined by UV−visible spectrophotometry with the help of localized surface plasmon resonance (LSPR). The sensor showed a concentration-dependent binding of OVA with a detection limit of 100 nM, and it had a very high stability at high ionic strength. The sensor chip could be used for a detection of another glycoprotein, avidin, as well. Furthermore, the binding of biotin-modified human serum albumin (biotinylated HSA) to the avidin−phenylboronic acid- (PBA-) carrying polymer brush complex and further specific binding of anti-HSA immunoglobulin G to the biotinylated HSA−avidin−PBA-carrying polymer brush ternary complex could clearly be observed. The polymer-brush-coated device examined here not only was useful as a simple sensor chip, but also is expected to open a new perspective on interfacial phenomena performed by various functional polymer brushes fixed to colloidal gold on glass substrates.

Introduction

Self-assembled monolayers (SAMs) are close-packed and well-ordered monolayers of alkyl or aromatic disulfides and thiols on gold or silver surfaces.[1−5] Because SAMs of disulfides and thiols are analogous to the structure of biomembranes[6,7] and are largely stable,[8−12] they have been widely used as cell mimetic membranes in recent years. To investigate the interactions between biomembranes and peripheral membrane proteins, for example, SAMs of ω-mercaptoalkanoic acids were constructed on silver colloids.[13] In addition, by taking account of the molecular recognition abilities of cyclodextrin (CD) and calixarene (CX), SAMs of thiolated CD and CX derivatives were constructed on a gold electrode as sensing devices.[14−19] Furthermore, SAMs of specific substrates and inhibitors for α-chymotrypsin and aspartyl proteases (pepsin and HIV-1 protease), respectively, have been constructed on a gold electrode for the detection of the enzymes.[20]

In addition to sulfides and disulfides of low molecular weight, various kinds of polymer chains carrying a thiol or disulfide group have been accumulated on gold or silver surfaces. The surface-confined polymer chains are not as closely packed and well-ordered as SAMs of aliphatic disulfides or sulfides. Therefore, they are usually called "polymer brushes", although they can also be included in the category of SAMs in a broad sense. ω-Mercapto polymer chains with many pendent glucose residues, for example, have been accumulated onto silver colloids as a polymer brush.[21]

Recently, brushes of well-defined polymers have been constructed on solid supports using various polymerization techniques. "Iniferter", which has the three functions of initiation, transfer, and termination, has often been used in controlled radical polymerization because of its simplicity and easy of use.[22−27] Benzyl *N*,*N*-diethyldithiocarbamoyl (BDC) derivatives have most extensively been studied as photoiniferters.[22−25,27] For example, a brush of the block telomer was constructed on Au or Ag surfaces using a disulfide-carrying iniferter (Cys-BDC, Scheme 1), and the block telomer-modified metal surface showed a response to external stimuli (pH and lectin).[28] Furthermore, a phospholipid carrying the BDC group was synthesized, and novel block telomer-carrying phospholipids were obtained by the successive photoirradiation of various vinyl monomers in the presence of the iniferter.[29]

Meanwhile, a surface plasmon resonance (SPR), which can only be excited by evanescent waves, is a collective electron

Scheme 1. Preparation of Polymer with Many Pendent Phenylboronic Acid Residues Using the Disulfide-Carrying Iniferter (Cys-BDC)

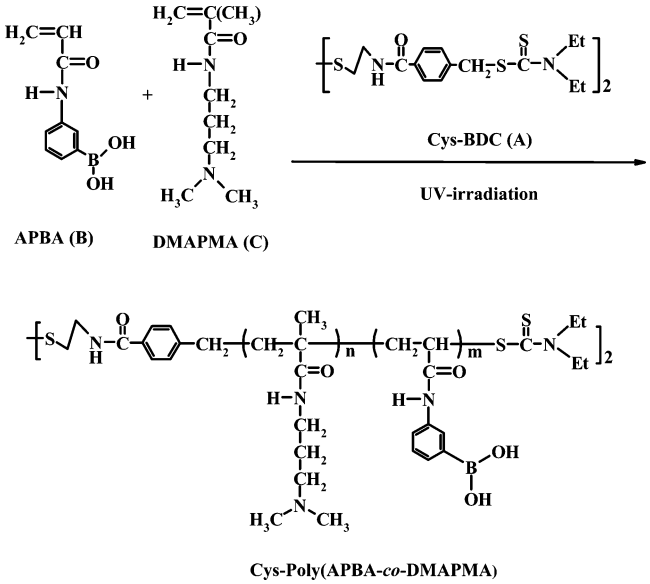

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Table 1. Characteristics of Various Prepared Polymers

polymer	Cys-BDC (mg)	TD (mg)	solvent (mL)	APBA (mg)	comonomer (mg)	reaction time (h)	temp (°C)	yield (mg)	M_w[a]	DP[a]
1	52.0	0	3.0[b]	60.0	5.3[d]	6	35	4.6	6100	19.5:0.87
2	33.0	33.0	3.0[b]	200.3	18.0[d]	6	35	8.3	6000	18.5:0.65
3	33.0	32.8	3.0[c]	21.5	20.7[d]	4	25	3.5	5800	18.8:0.42
4	33.1	29.6	3.0[c]	20.8	182.5[d]	4	25	92.3	5170	16.0:0.31
5	30.0	29.6	3.0[c]	48.8	250.8[e]	4	25	125.4	2500	16.01:0.74

[a] Molecular weight (M_w) and degree of polymerization (DP) of each polymer chain were determined by ^{1}H NMR spectroscopy. The total DP of the prepared polymer is twice the tabulated value. [b] Solvent = 1:1 THF/MeOH. [c] Solvent = THF. [d] Comonomer = DMAPMA. [e] Comonomer = DMAA.

oscillation in thin metal layers. Both the permittivity and thickness of the metal and that of any dielectric layer on top of it affect the propagation constant of such a wave.

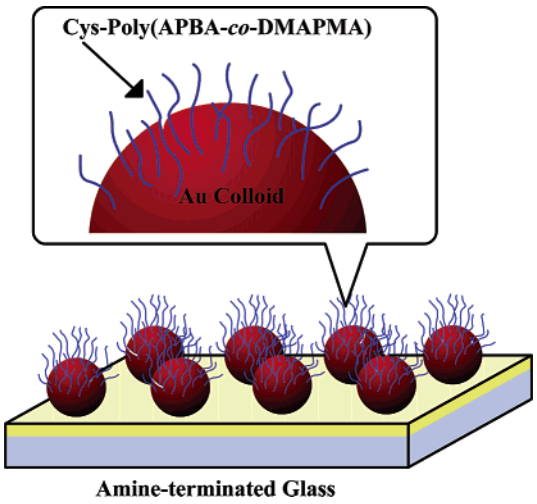
Similarly to metal thin layers, the optical properties of noble metal colloidal particles such as Au or Ag have been studied extensively, because these particles are quite useful as functional materials for optical devices,[30,31] surface-enhanced spectroscopies,[32−37] chemical and biological sensors,[38−41] etc. Previously, it was shown that Au colloids can be self-assembled from solution onto a functionalized glass surface to give a monolayer,[35,42,43] where the assembly is stabilized by both repulsive colloid−colloid electrostatic interactions and strong attractive colloid−surface interactions. More recently, it was shown that the absorbance of the immobilized monolayer of Au colloids is sensitive to the refractive index of the surrounding medium[38] and that Au colloids can be functionalized with biotin.[39] The new label-free optical sensor formed in this way could observe interactions between biotin and streptavidin in situ at the surface. This technique, the so-called "localized surface plasmon resonance" (LSPR) method,[44] has various advantages over the conventional SPR method using a commercial apparatus: (1) An ordinary UV−visible spectrophotometer without any special attachment can be used, (2) a sensor chip can be prepared from a cover glass quite easily and quickly without any special apparatus, and (3) kinetic analysis of the surface recognition phenomena in the stationary liquid phase can very easily be pursued.

In this research, optical biosensors have been prepared with a help of LSPR. Specifically, using the Cys-BDC iniferter, a brush of phenylboronic acid- (PBA-) carrying copolymers has been constructed on a colloidal Au-immobilized glass substrate as a sensor chip for glycoproteins. The boronic acid group has extensively been studied because of its ability to bind various diol derivatives including sugars at alkaline pH.[45−54] Previously, both water-soluble and surface-confined copolymers of 3-acrylamidophenylboronic acid (APBA) and *N*,*N*-dimethyl acrylamide (DMAA) were found to show responsiveness to both temperature and sugars.[55] The copolymer-coated device prepared here not only was useful as a very simple and highly sensitive sensor chip, but also can be expected to expand our knowledge of interfacial phenomena using various functional polymer brushes on colloidal Au.

Experimental Section

Materials. The photoiniferter (Cys-BDC, Scheme 1A) was prepared as described earlier.[28] 3-Acrylamidophenylboronic acid (APBA, Scheme 1B) was prepared as previously reported.[55a] *N*,*N*-dimethyl acrylamide (DMAA; Kohjin, Tokyo, Japan) was distilled in vacuo. *N*,*N*-Dimethylaminopropyl methacrylamide (DMAPMA, Scheme 1C) was obtained from Aldrich. 3-Aminopropyl triethoxysilane (APTES) was purchased from ShinEtsu Chemicals, Tokyo, Japan. Human serum albumin (HSA, Green Cross Corporation, Osaka, Japan) was labeled with biotin (biotinylated HSA) as described in detail in the Supporting Information. Anti-human serum albumin (HSA) immunoglobulin G (goat) (anti-HSA-IgG) was obtained from MBL, Nagoya, Japan. Other reagents were also obtained from commercial sources. All aqueous solutions and sensor chips were prepared with ultrapure water (18 MΩ·cm^{-1}, Millipore System).

Chart 1. Probable Structure of the Prepared Biofunctionalized Sensor Chips



Preparation of Brush-Forming Polymer (Scheme 1). A mixture of Cys-BDC, APBA, DMAPMA, and *N*,*N*,*N*′,*N*′-tetraethylthiuram disulfide (TD, capping reagent) dissolved in anhydrous tetrahydrofuran was photoirradiated in a quartz cell with a high-pressure mercury lamp. The phenylboronic acid-carrying copolymer purified by ultrafiltration was lyophilized in the dark [poly(APBA-*co*-DMAPMA), Table 1]. The preparation and characterization of the polymers obtained are described in detail in the Supporting Information.

Preparation of Gold Colloids. The preparation and characterization of the gold colloids are described in the Supporting Information.

Fixation of Colloidal Gold Monolayer on a Glass Substrate. The preparation of the colloidal gold monolayer on a glass surface is described in the Supporting Information.

Functionalization of Colloidal Gold-Fixed Glass Substrate (Chart 1). The colloidal Au-fixed glass substrates were modified with various disulfide-carrying polymers by immersion in a polymer solution as described in the Supporting Information.

Absorption Measurements. The absorbance of the immobilized Au colloids on a glass substrate placed in a quartz cell was followed with a UV−visible−near-infrared spectrophotometer as described in the Supporting Information.

AFM Images. The immobilized Au colloids on the glass substrate were imaged with an atomic force microscope as described in the Supporting Information.

Results and Discussion

A. Construction of Polymer Brushes on a Gold Colloid. It has very often been reported that proteins do not bind to

ligand-carrying amphiphiles with short spacer groups between the ligand and a hydrophobic anchor in membrane systems (liposome, lipid monolayer, etc.).[56−58] This is due to a large steric hindrance preventing the huge protein from approaching the ligand on the membrane surface. Therefore, poly(APBA-*co*-DMAPMA), which has a long polymer chain with pendent phenylboronic acid groups, was adopted as a "fly" to fish for glycoproteins in this study. The tertiary-amino-group-carrying monomer (DMAPMA) was adopted as a comonomer, because it was previously observed that the binding of boronic acid to sugars was largely promoted by the neighboring tertiary amines.[54,59]

Previously, we found that polymer brushes do not form on the Au surface after photoirradiation of the Cys-BDC SAM in the presence of a vinyl monomer,[56] showing that the iniferter SAM is mostly decomposed or forced to migrate from the gold surface during UV irradiation. On the basis of this result, in this work, we modified the Au surface with a polymer-carrying disulfide that had been prepared in the solution phase beforehand (Table 1).

The diameter of the Au colloids determined by dynamic light scattering (DLS) was ca. 20 nm, and with this value, the surface coverage of colloidal gold was estimated to be 28.1% using AFM. The reported surface coverages are average values of five measurements at five points for each colloidal Au−fixed glass substrate. The gold colloids were isolated from each other in the AFM image (see Supporting Information, Figure S1). The image, however, might receive an impression different from the estimated surface coverage, because the image was obtained in tapping mode, which makes the apparent size of the Au colloids larger than their actual size.

The arrangement of the colloidal gold on the amine-terminated glass surface can be controlled by the effective use of various interactions such as colloid−colloid and colloid−amine interactions.[42,60] Therefore, the effect of the conditions used for incubation of the glass with APTES solution on the arrangement of colloidal gold particles was examined. However, the surface coverage of the colloidal gold did not change significantly with the solvent (water or ethanol) or incubation time (20 or 60 min) (25.7−28.1%). The optimum conditions for obtaining the most condensed surface were incubation in a 10% aqueous APTES solution for 60 min at this moment. Furthermore, by adjusting the size of the gold colloids prior to contact with the amine-terminated glass substrate, the density of colloidal gold on the glass surface can be more precisely controlled.

Characterization of self-assembled colloidal gold monolayer on the glass and polymer-1-modified sensor chip was pursued spectrophotometrically. The previous study showed that the aggregation of colloidal gold would result in the coupling of plasmons of individual particles and a significant increase in the peak absorbance between 600 and 800 nm in the visible spectrum.[42] Because such a feature did not appear in the visible spectra of the glass covered with colloidal gold and the subsequently prepared polymer-1-modified sensor chip (Figure 1), the gold colloids seemed to be immobilized as a monolayer on the glass surface and isolated from each other, which is consistent with the AFM image.

Furthermore, we examined the ability of the polymer-brush-modified gold colloids on the glass substrate to induce changes in the refractive index of the surrounding medium appearing in the absorbance spectrum.[61] The absorbance spectrum of a colloidal-gold-covered glass substrate exhibited a red shift in the peak wavelength (λ_{max}) in proportion to the refractive index (n) of the medium in the range of 1.00−1.46 and also implied a linear relationship between the n value and the absorbance at 550 nm (see Supporting Information, Figure S2).[39] These results are consistent with the Mie theory predicting a red shift in the position of λ_{max} and an increase in the absorbance maximum with an increase in the n value of the medium.[62] The linear relationship might certainly collapse when the refractive index increases greatly. However, there seemed to be no problem because the measurements were carried out in the low-concentration region of proteins in this study, which induces only a small change in the refractive index in the vicinity of colloidal gold. Therefore, we assumed here a linear relationship between the absorption change and the amount of adsorbed proteins.

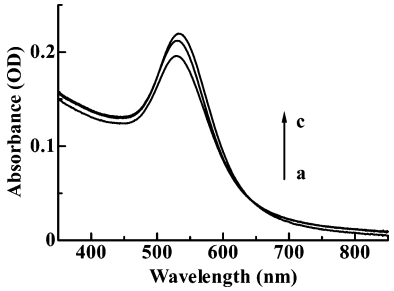


Figure 1. Absorption spectra of a colloidal Au dispersion and poly(APBA-*co*-DMAPMA)- (polymer-1-) modified Au colloids on a glass surface: (a) colloidal Au dispersion, (b) colloidal Au−fixed glass substrate, (c) polymer-1-immobilized Au colloid on a glass surface.

Moreover, in contrast to ordinary colloids, which show electrolyte-induced coagulation [critical flocculation concentration (CFC) of Au colloid = 40 mM (NaCl); see Supporting Information, Figure S3],[63] there was no significant change in the absorption spectrum for the gold colloid−fixed glass surface after immersion in aqueous electrolyte solutions (even in a 5 M sodium chloride), showing the enormous stability of the gold colloids. The high density of amino groups on the glass surface might lead to strong binding between the gold colloids and glass surface, because many amino groups could bind to each gold colloid particle. The sensor chip modified with the phenylboronic acid-carrying polymer brush gave the same results. The brush-modified sensor chip might be more stable at high electrolyte concentration than a brush-free gold colloid−fixed glass substrate, because of the enormous steric stabilization effect of the polymer layer stretching outward from the surface of the colloids.[21]

B. Binding of Sugar Residues on Glycoproteins by PBA Groups on Colloids. Because the copolymer-modified colloidal Au on glass surface can be used in a buffer solution, we examined the binding of sugar residues in a glycoprotein, ovalbumin (OVA), by the PBA-carrying polymer brush using a UV−visible spectrophotometer. After the polymer-1-modified sensor chip was immersed in an OVA solution, a red shift in the peak absorbance and a concomitant increase in the absorption at 550 nm were observed (Figures 2 and 3e). In Figure 2, the spectra are normalized to their absorption maxima to make the red shift of the absorption peak clear. Because sugar groups are well-known to be bound by boronic acid, the absorbance change can be attributed to the binding of the sugar residues in OVA by a boronic acid groups in the copolymer.

To confirm this hypothesis, the effect of small sugars, which have an affinity for phenylboronic acid,[64] on the absorption change was examined. Upon immersion of a chip that had been soaked in the fructose (Fru) solution beforehand, into the OVA solution, the absorption showed only a slight change (Figure

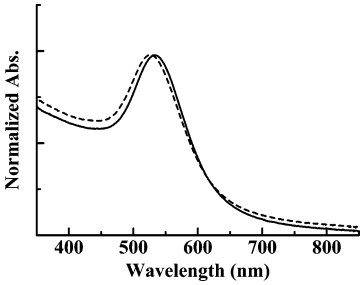


Figure 2. UV spectra of a glass chip before and after immersion in an OVA solution. Spectra were normalized to their absorption maxima. Dashed line, before immersion in OVA solution; solid line, after immersion in OVA solution. [OVA] = 2.2×10^{-5} M in carbonate buffer solution (50 mM, pH 11.2).

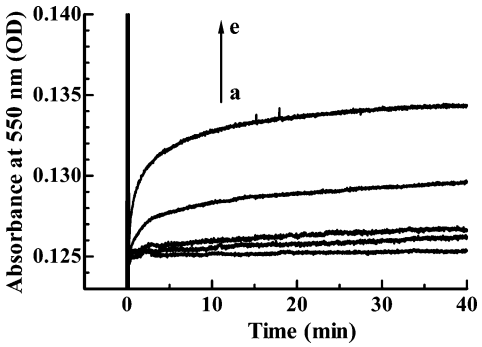


Figure 3. Time evolutions of absorbance for the polymer-1-immobilized sensor chip after immersion in various solutions: (a) Fru solution prior to immersion in OVA solution, (b) mixture of OVA and Fru, (c) mixture of OVA and Man, (d) mixture of OVA and GlcNAc, (e) OVA solution. [OVA] = 4.4×10^{-5} M, [Fru] = [Man] = [GlcNAc] = 10 mM.

3a). In addition, the strong retardation effect of Fru on the increase in absorbance at 550 nm (Figure 3b) showed that the absorbance change was due to the binding of the sugar residues in OVA by the copolymer.

Figure 3 also show that the inhibitory effects of mannose (Man) and *N*-acetyl glucosamine (GlcNAc) on the OVA-induced absorption increase were smaller than those of fructose (Fru > Man ≫ GlcNAc; Figure 3b−d). It was previously reported that the order of affinity of phenylboronic acid group for sugar molecules depends on the position of the OH groups in the sugar as follows: fructose ≫ mannose > glucose.[45] The apparent 1:1 binding constant was 25 times larger for fructose than for mannose. Norrild and Eggert clearly indicated that fructose binds with *p*-tolylboronic acid in the *β*-D-fructofuranose form in a tridentate manner via the 2, 3, and 6 OH groups forming a very stable 1:1 complex.[65] In the SAM system of 3-aminophenylboronic acid-4,4′-dithiobis(*n*-butyric acid) conjugate constructed on gold colloid, the inhibitory effect of mannose on the mannan-mediated aggregation of the colloid was much smaller than that of fructose.[64] The tendency in Figure 3 is not in good agreement with the previous results. Such a behavior has been attributed to the effects of the microenvironment[54,64,66] and the introduction of other functional groups[49] on the recognition of sugars by boronic acid.

Adsorption of four kinds of proteins (OVA, BSA, streptavidin, and lysozyme) onto various brushes [polymer 1 = poly(APBA-*co*-DMAPMA) and polymer 5 = poly(APBA-*co*-DMAA), Table 1] was also examined. Because the background absorbances (at 550 nm) of polymer-brush-modified sensor chips differed from each other, the differences in absorbance before and after contact of proteins with the brushes of poly(APBA-*co*-DMAPMA) and poly(APBA-*co*-DMAA) were compared. Figure 4A shows that,

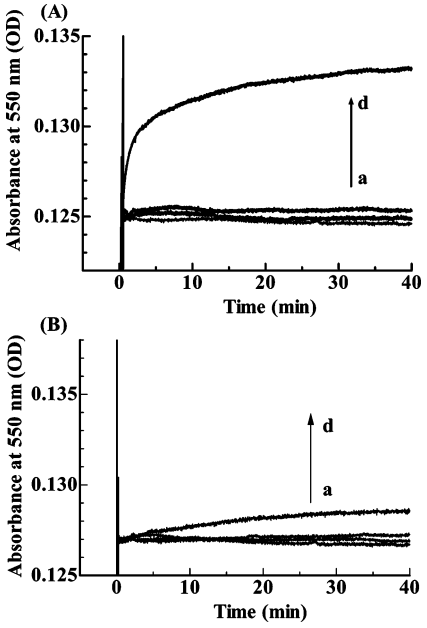


Figure 4. Absorbance changes after contact with various proteins of (A) the polymer-1-immobilized sensor chip and (B) the polymer-5-immobilized sensor chip. The absorbance was monitored at 550 nm. (a) Streptavidin, (b) lysozyme, (c) BSA, and (d) OVA. [protein] = 0.4 mM in a carbonate buffer (pH 11.2, 50 mM).

upon immersion in OVA solution, the absorbance at 550 nm for the polymer-1-modified colloidal gold on the glass chip gradually increased and leveled off after ca. 40 min. In contrast to OVA, bovine serum albumin (BSA), streptavidin, and egg white lysozyme showed only very slight absorption changes, indicating that nonspecific adsorption of the protein had little or no effect on the sensor chips. A similar result was obtained for the polymer-5-modified sensor chip (Figure 4B). The absorbance changes of the polymer-5-modified sensor chip were far smaller than those of the polymer-1-modified chip, indicating that the polymer-1-modified sensor chip is more sensitive because of the cooperative effects of tertiary amine and boronic acid on the binding to sugar groups as described above.

C. Kinetics of Adsorption of OVA from the SAM-Modified Sensor Chip. We further investigated the kinetics of OVA adsorption by monitoring the absorbance change at 550 nm in real time. First, we determined the association and dissociation rate constants (k_{assoc} and k_{diss}, respectively) from the relaxation process using eqs 1 and 2

$$\Delta\mathrm{Abs} = \mathrm{Abs}_t - \mathrm{Abs}_0 = \Delta\mathrm{Abs}_{t\to\infty}[1 - \exp(-t/\tau)] \quad (1)$$

$$1/\tau = k_{assoc}[\mathrm{OVA}] + k_{diss} \quad (2)$$

where τ is the relaxation time of absorption changes in the association process; ΔAbs is the increase in absorbance; Abs_0 and Abs_t are the absorbances at times 0 and t, respectively; and $\Delta\mathrm{Abs}_{t\to\infty}$ is the ΔAbs value at infinite time.

The equilibrium constant K_{assoc} can also be calculated by another method using the initial slope of the relaxation curve, $(\mathrm{d}[\mathrm{Complex}]/\mathrm{d}t)_{t\to 0}$, and the maximum concentration of the complex ($[\mathrm{Complex}]_{t\to\infty}$) using eqs 3 and 4 (see Supporting Information)

$$1/[\mathrm{Complex}]_{t\to\infty} = (1/[\mathrm{APBA}]) + (1/[\mathrm{OVA}][\mathrm{APBA}])(k_{diss}/k_{assoc}) \quad (3)$$

$$(\mathrm{d}[\mathrm{Complex}]/\mathrm{d}t)_{t\to 0} = k_{assoc}[\mathrm{OVA}][\mathrm{APBA}] \quad (4)$$

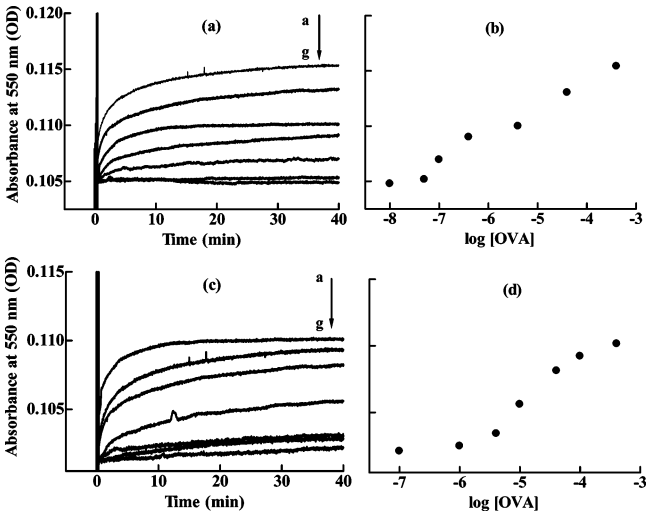


Figure 5. (a,c) Time dependence of surface plasmon absorbance at 550 nm after immersion of a glass plate modified with the phenylboronic acid-carrying colloidal gold in OVA solution and (b,d) saturated absorbance as a function of the logarithm of the OVA concentration. (Top) Polymer brush = polymer 1; for spectra a−g, [OVA] = 4.0×10^{-4}, 4.0×10^{-5}, 4.0×10^{-6}, 4.0×10^{-7}, 1.0×10^{-7}, 5.0×10^{-8}, and 1.0×10^{-8} M. (Bottom) Polymer brush = polymer 5; for spectra a−g, [OVA] = 4.0×10^{-4}, 1.0×10^{-4}, 4.0×10^{-5}, 1.0×10^{-5}, 4.0×10^{-6}, 1.0×10^{-6}, and 1.0×10^{-7} M.

Table 2. Binding Constants and Detection Limits for Sugar-Carrying Proteins with PBA Derivatives

protein	K_{assoc} (M^{-1})[a]	K_{assoc} (M^{-1})[b]	detection limit
	APBA−DMAPMA		
OVA	$(1.30 \pm 0.12) \times 10^4$	$(1.91 \pm 0.20) \times 10^4$	100 nM
avidin	$(5.05 \pm 0.48) \times 10^3$	$(5.59 \pm 0.55) \times 10^3$	1 μM
streptavidin	no binding	no binding	−
BSA	no binding	no binding	−
lysozyme	no binding	no binding	−
	APBA−DMAA		
OVA	$(8.50 \pm 1.04) \times 10^2$	$(9.02 \pm 1.18) \times 10^2$	4 μM
avidin	$(4.23 \pm 0.28) \times 10$	$(3.35 \pm 0.42) \times 10$	55 μM
streptavidin	no binding	no binding	−
BSA	no binding	no binding	−
lysozyme	no binding	no binding	−

[a] Calculated with eqs 1 and 2. [b] Calculated with eqs 3 and 4.

where [APBA] is the surface concentration of APBA residue. Assuming that the surface concentration of the OVA−APBA complex, [Complex], is proportional to the increase in absorbance (ΔAbs) at 550 nm, K_{assoc} can be calculated from Figure 5a and c even if the values of [APBA] and [Complex] are definitively known.

Using eqs 1 and 2, the k_{assoc} and k_{diss} values obtained from Figure 5a and c were 46.2 M^{-1} s^{-1} and 3.55×10^{-3} s^{-1}, respectively. The apparent association constant (k_{assoc}/k_{diss}) was 1.30×10^4 M^{-1}, which is similar to the K_{assoc} value calculated by eqs 3 and 4 (1.91×10^4 M^{-1}) (Table 2). The K_{assoc} values are larger than those previously reported for the complexation of boronic acid derivative with small sugars (α-D-glucofuranose, 2.5×10^3 M^{-1}),[67] probably because of multipoint (cooperative) fixation of sugar residues in OVA by the boronic acid residues in polymer 1 (the so-called "cluster effect").[68] The difference in the K_{assoc} values for the sensor chips carrying polymer 1 and polymer 5 suggests the contribution of the tertiary amine groups in the polymer chain to the effective binding of the target molecule by the PBA-carrying polymer-modified sensor chips.[54,59]

The same experiments as reported Figure 5 were carried out for another glycoprotein, avidin from egg white. The absorbance of the chip at 550 nm increased upon immersion in the avidin solution, and the K_{assoc} value was calculated to be 5.05×10^3 M^{-1} with eqs 1 and 2. The K_{assoc} value for avidin was slightly smaller than that for OVA (Table 2). This is due to the sugar residues of the two glycoproteins: OVA is in rich mannose [$(Man)_6(GlcNAc)_2$], whereas avidin is rich in *N*-acetyl glucosamine [$(Man)_4(GlcNAc)_6(Gal)$].[69] The PBA group might prefer Man to GlcNAc because of the larger number of OH groups in the Man residue than in GlcNAc (Figure 4c and d), which might result in the larger K_{assoc} value for OVA.

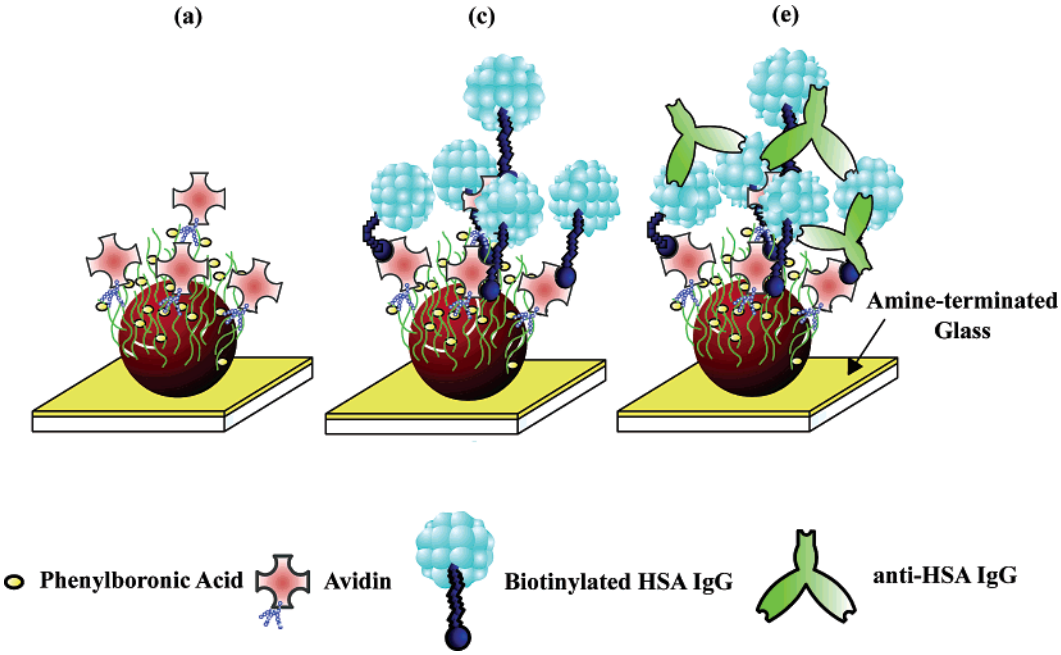
D. Detectability of Glycoprotein by the Sensor Chips. Next, the detectability of the glycoprotein sensor was estimated using various concentrations of OVA. The criterion for the determination of the detection limit is that it is the lowest concentration that the sensor chip can detect. We regarded results with a signal-to-noise ratio of >3 as detectable in this study. Figure 5b shows that the maximum amount of OVA bound to the surface in the steady state is directly related to the solution concentration, and an absorbance change at 550 nm could be observed only when the solution concentration was above 100 nM. The very low detection limit of OVA using the polymer-1-carrying sensor chip indicates a very high relative sensitivity of the chip.

In Figure 5d, absorption changes for another polymer-modified sensor chip are also shown (polymer 5). In the case of the polymer-5-carrying sensor chip, the detection limit increased to 4 μM. The difference in detection limits for the sensor chips carrying polymer 1 and polymer 5 shows the contribution of tertiary amine groups in the polymer chain to the effective detection of target molecules by the polymer-modified sensor chips examined here, which is consistent with the K_{assoc} values for the polymer-1- and polymer-5-modified sensor chips in Table 2 and is in agreement with the previous reports by Shinkai et al.[59] and our group,[54] as well.

Previously, Nath and Chilkoti reported that the detection limit of their biotinylated sensor chip for streptavidin was 16 nM.[39] Taking account of the extremely large association constant for streptavidin−biotin complex (10^{15} M^{-1}),[39] the detection limit of the phenylboronic acid polymer-modified sensor chip for OVA examined here (K_{assoc}, $1.3-1.9 \times 10^4$ M^{-1}; detection limit, 100 nM) was rather satisfactory.

E. Multilayered Sensing. Finally, the "multilayered sensing" of various proteins with the phenylboronic acid- (PBA-) carrying polymer brush was examined. Upon immersion in avidin solution (1.5×10^{-5} M), the absorbance of the sensor chip increased (Figure 6a), whereas immersion in a solution of streptavidin (not glycoprotein) showed only a very slight absorption change (Figure 6b). Furthermore, after the experiment of Figure 6a, the chip was rinsed and immersed in a solution of biotin-modified human serum albumin (biotinylated-HSA). The further increase in absorbance indicated that the HSA molecule was bound to the chip via a biotin−avidin−PBA ternary complex (Figure 6c).[70] In contrast to the biotinylated HSA, nonbiotinylated HSA induced only a very slight absorption change (Figure 6d), indicating that the adsorption of biotinylated HSA to the avidin−PBA complex was specific. Furthermore, after the experiment of Figure 6c, the chip was rinsed again and immersed in a solution of anti-HSA immunoglobiulin G (anti-HSA IgG). The further increase in absorbance indicated that anti-HSA IgG molecule was bound to the chip via an HSA−anti HSA IgG complex (Figure 6e), whereas the immersion in human IgG solution showed only a very slight absorption change

Chart 2. Schematic of the Complexes Formed by the Various Association Steps[a]



[a] Complexes a, c, and ecorrespond to those appearing at the steps indicated in Figure 6.

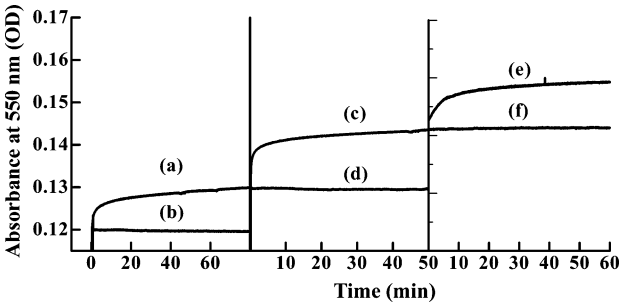


Figure 6. Time dependence of surface plasmon absorbance at 550 nm after immersion of the glass plate modified with the phenylboronic acid-carrying colloidal gold in (a) avidin solution (1.5×10^{-5} M), (b) streptavidin solution (1.5×10^{-5} M), (c) sample a after subsequent immersion in biotinylated HSA solution (1.5×10^{-5} M), (d) sample a after subsequent immersion in nonbiotinylated HSA solution (1.5×10^{-5} M), (e) sample c after subsequent immersion in anti-HSA IgG solution (6.6×10^{-5} M), (f) sample c after subsequent immersion in human IgG solution (6.6×10^{-5} M).

(Figure 6f), indicating that anti-HSA IgG was specifically bound to HSA (Chart 2). These results clearly show that various types of polymer−protein and protein−protein interactions could easily and sequentially be analyzed up to at least 20 nm from the surface of the gold colloid using LSPR (avidin, $8.015 \times 8.015 \times 8.528$ nm^3; HSA, $5.968 \times 9.698 \times 5.972$ nm^3; anti-HSA IgG, $8.84 \times 11.00 \times 18.67$ nm^3).[71−73]

Conclusion

The disulfide-carrying polymer poly(APBA-*co*-DMAPMA) prepared by the iniferter technique could easily be accumulated on colloidal Au attached to a glass substrate. The changes in the absorbance spectrum associated with the selective binding of a glycoprotein to the phenylboronic acid-carrying polymer brush on the gold colloids could easily be measured using an ordinary UV−visible spectrometer, which is a quite efficient way to quantify the interactions. The sensor chip exhibited both high stability and high detectability of glycoprotein ([OVA] = 100 nM).

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Supporting Information Available. (1) Preparation of brush-forming polymers, (2) preparation of gold colloids, (3) fixation of colloidal gold monolayer on a glass substrate, (4) photograph of the colloidal gold−fixed glass substrate taken by AFM, (5) absorbance spectra of polymer-1-modified gold colloids on glass immersed in various solvents, (6) method of calculating the association constant from the kinetic analysis of protein adsorption, and (7) preparation of biotinylated human serum albumin. These materials are available free of charge via the Internet at http://pubs.acs.org.

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