Available online at www.sciencedirect.com
ScienceDirect
Analytical Biochemistry 375 (2008) 90–96
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

The investigation of recognition interaction between phenylboronate monolayer and glycated hemoglobin using surface plasmon resonance

Jen-Tsai Liu [a], Liang-Yu Chen [b], Mu-Chin Shih [c], Yung Chang [d], Wen-Yih Chen [a,*]

[a] *Department of Chemical and Materials Engineering, National Central University No. 300, Jhongli City, Taoyuan County 32001, Taiwan*
[b] *Department of Biotechnology, Ming Chuan University, Taipei 111, Taiwan*
[c] *Department of Medical Laboratory Science and Biotechnology, China Medical University No. 91, Taichung 40402, Taiwan*
[d] *R&D Center for Membrane Technology and Department of Chemical Engineering, Chung Yuan Christian University, Chung-Li, Taoyuan County 32023, Taiwan*

Received 23 October 2007
Available online 9 January 2008

Abstract

Glycated hemoglobin (HbA1c) is formed by a nonenzymatic reaction of glucose with the N-terminal valine of adult hemoglobin's β-chain. The amount of HbA1c reflects the average concentration of glucose variation level over the preceding 2 to 3 months. Because the boronate has antibody mimicking for HbA1c, often it is used to detect HbA1c. However, factors such as the ratio of the phenylboronic acid derivatives and diol composition, the pH of the solution, and the stereostructure of phenylboronic acid derivatives could influence the interactions between phenylboronic acid derivatives and diol composition. In this study, the factors were evaluated using surface plasmon resonance (SPR). The results show that pH value is an important factor affecting HbA1c and phenylboronic acid to form the complex and Lewis bases. This could change the stereostructure of phenylboronic acid to form $B(OH)_3$ for binding with saccharine easily. In addition, linear response appeared in HbA1c in the range of 0.43 to 3.49 μg/ml, and the detection limit was 0.01 μg/ml. The results also demonstrated that an SPR biosensor can be used as a sensitive technique for improving the accuracy and correctness of HbA1c measurement.

Keywords: Glycated hemoglobin (HbA1c); Phenylboronic acid; Surface plasmon resonance (SPR)

Glycated hemoglobin (HbA1c)[1] is a stable glycated protein formed by a nonenzymatic reaction of glucose with the N-terminal valine of adult hemoglobin's β-chain [1–3] in the human body. Red blood cells are made up of 80% hemoglobin. The amount of HbA1c reflects the average concentration of glucose variation level over the preceding 2 to 3 months that is attributable to the red cell's life span of 100 to 120 days. In practice, 4 to 7% of HbA1c is considered normal. A person having more than 7% could be diabetic. Therefore, the ratio of HbA1c becomes an important clinical index in evaluation of long-term glycemic control for diabetes and related diseases.

Currently, HbA1c usually is measured as the percentage of total hemoglobin by fluorescent sensing, cation exchange chromatography, electrophoresis, and boronate affinity chromatography [4,5]. The fluorescent sensing method has the advantage of high sensitivity, but the disadvantage is that it requires labeling to detect HbA1c by competitive reaction and, indirectly, to evaluate the amount of sugar and kinetic parameters [6–10]. The cation exchange chromatography [11] and electrophoresis [12] methods are based on charge difference between hemoglobin fractions. Indeed, some factors such as alcohol consumption and

* Corresponding author. Fax: +886 3 422 5258.
E-mail address: wychen@cc.ncu.edu.tw (W.-Y. Chen).

[1] *Abbreviations used:* HbA1c, glycated hemoglobin; HbS, hemoglobin S; SPR, surface plasmon resonance; DTBA, 4,4-dithiodibutyric acid; PBA, 3-aminophenylboronic acid hemisulfate; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride; BSA, bovine serum albumin; PB, phosphate buffer; SAM, self-assembled monolayer; THF, tetrahydrofuran; ATR–FTIR, attenuated total reflection Fourier transform infrared; ESCA, electron spectroscopy for chemical analysis.

0003-2697/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2008.01.004

aspirin [13] have been reported to make falsely elevated HbA1c results in the cation exchange chromatography and electrophoresis methods.

The boronate affinity chromatography method is based on the specific interaction between phenylboronic acid derivatives and diol composition. But this method yields inaccurate results with variation of red blood cells such as hemoglobin S (HbS) [14]. In a recent technical report [15], the boronate affinity HPLC method overcame this interference, but the normal detected range was too wide to see errors by boronate affinity chromatography. Thus, to promote the accuracy in HbA1c estimation by boronate affinity chromatography, it is important to understand the molecule interaction mechanism between boronic acid and HbA1c.

Because it is well known that phenylboronic acid derivatives and diol composition could form a complex, phenylboronic acid derivatives often have been used to separate and purify the carbohydrates. The phenylboronic acid and diol composition interaction theory states that boronic acid is a good binding affinity receptor for sugar aqueous solution due to its 1,2- or 1,3-diols [16–19]. Also, the interaction between boronic acid and the *cis*-diol moiety of the saccharides appears in the order of 2,3-*cis*-diol < 3,4-*cis*-diol < 1,2-*cis*-diol [20]. However, there are many factors that could influence the interaction between phenylboronic acid derivatives and diol composition. Such factors include the ratio of phenylboronic acid derivatives to diol composition, the pH of the solution, and the stereostructure of phenylboronic acid derivatives.

The goal of the current study was to directly detect HbA1c without any labeling so as to understand the effect of Lewis bases and salt concentration inclusive of different pH values. This is with the use of surface plasmon resonance (SPR), which has high sensitivity and directly detects HbA1c without any labeling. In addition, using high-sensitivity SPR, the study sought to determine the optimum condition needed to detect HbA1c and to arrive at a proper diagnosis in a clinical system.

Materials and methods

Chemicals and reagents

4,4-Dithiodibutyric acid (DTBA) was purchased from Aldrich (USA). 3-Aminophenylboronic acid hemisulfate (PBA), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), and bovine serum albumin (BSA) were obtained from Sigma (USA). HbA1c calibrators were obtained from Primus (USA). Four kinds of buffer were used in this study, as follows: (1) 50 mM phosphate buffer (PB) with sodium chloride (0.3 M) at pH 9. (2) 50 mM PB without the sodium chloride at pH 9. (3) 50 mM PB without the sodium chloride at pH 5. (4) 50 mM sodium chloride solution (dissolved in DI water) at pH 5. Other reagents and solvents were purchased commercially and used without purification.

Synthesis of DTBA–PBA

Fig. 1 shows the synthetic scheme of phenylboronic acid derivatives. PBA (930 mg) was dissolved in 20 ml of deionized water, and the pH was adjusted to 4.8 using 0.1 N NaOH and then cooled to 4 °C in an ice bath. DTBA (447 mg) was dissolved in 30 ml of deionized water, and the pH was adjusted to 4.8 using 0.1 N NaOH and then cooled to 4 °C in an ice bath. EDC (90 mg) was added slowly to the PBA solution with stirring and was cooled to 0 °C for 20 min. Then DTBA solution was put slowly into the PBA solution while stirring. The reaction mixture was stirred for 2 h in an ice bath and stored at 4 °C overnight. Crude powder was collected by filtration and dried at 0 °C under vacuum. The crude product was recrystallized three times from methanol/water (1:1) at 80 °C under vacuum.

Immobilization and characterization of DTBA–PBA monolayer

In this work, BK7 optical glass substrates were used as sensor chips and coated with a 2-nm adhesion-promoting chromium layer and a 50-nm surface plasmon active gold layer by thermal evaporation under vacuum. DTBA–PBA self-assembled monolayers (SAMs) were formed by soaking UV-cleaned, gold-coated substrates in a 1.0-mM tetrahydrofuran (THF)/methanol (9:1) solution of DTBA–PBA for 12 h overnight. The modified chip was washed with methanol several times and dried under nitrogen. The DTBA–PBA monolayer was characterized by attenuated total reflection Fourier transform infrared (ATR–FTIR) spectroscopy (FTS-40, Bio-Rad, USA), contact angle, and electron spectroscopy for chemical analysis (ESCA, Sigma Probe, Thermo VG Scientific, USA) analyses. ATR–FTIR is a useful surface analytical technique and can be applied to understand the structure or orientation of organic molecules confined to metal surface as thin films such as monolayer. Contact angle is an invaluable metric for understanding material surface properties and the effects of surface treatments. ESCA is a quantitative spectroscopic technique that measures the elemental composition.

SPR spectroscopic measurement

A home-built SPR biosensor [21] based on wavelength interrogation with a four-channel Teflon flow cell was used to monitor recognition interaction between phenylboronic acid monolayer and HbA1c. The sensor chip was attached to the base of the prism, and optical contact was established using refractive index matching fluid (Cargille, USA). To obtain interaction measurements, the SPR was first stabilized in carry buffer for 15 min. When the system reached a steady state, the sample was injected for 150 s (flow rate = 100 μl/min). Afterward, the carry buffer was used for washing. Finally, the sample was diluted by carry

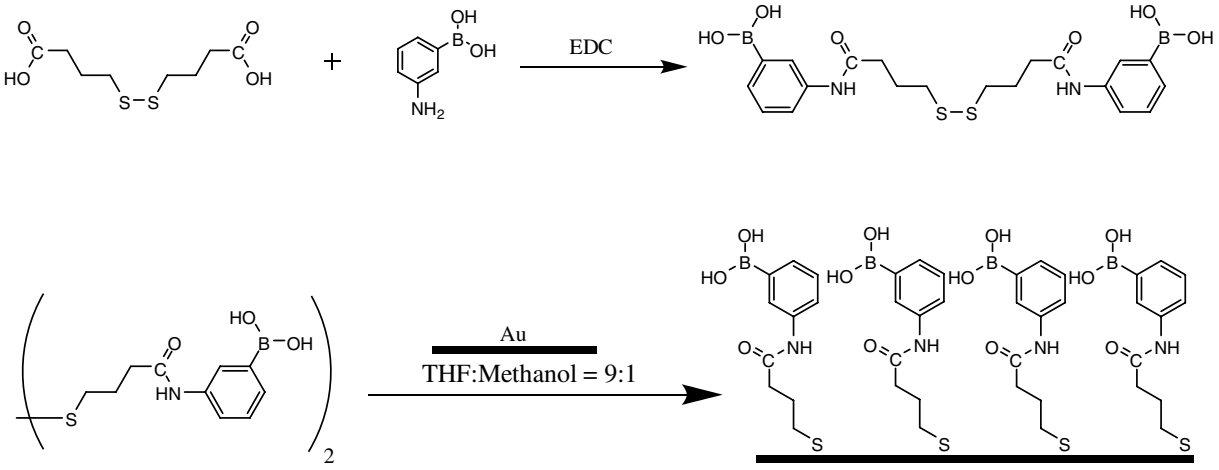

Fig. 1. Synthetic scheme of phenylboronic acid derivatives. THF, tetrahydrofuran.

buffer. Four kinds of carry buffer (pH 9/50 mM PB, pH 5/50 mM PB, pH 9/50 mM PB + 300 mM NaCl, and pH 5/50 mM NaCl) were used in the experiment.

Kinetic calculation

The affinity interactions between immobilized boronic acid and HbA1c in the solution were characterized by the association rate constant (k_a), dissociation rate constant (k_d), and equilibrium association constant (K_{eq}). The data were fitted using a simple 1:1 interaction model, $A + B = AB$, where A is the injected analyte, B is the immobilized ligand, and AB is the analyte–ligand complex formed during the reaction. In the SPR system, the signal R is proportional to the amount of $[AB]$ and the R_{max} is proportional to the initial $[B]$. A set of differential equations is applied to the data to find a solution for the three parameters: k_a, k_d, and K_{eq} The experimental curves were recorded during the association, equilibrium association, and dissociation phases. The k_d was obtained from the dissociation phase by Eq. (1) below. Then k_d was substituted in Eq. (2) below for obtaining k_a from the association phase. Finally, K_{eq} was calculated as the ratio of association and dissociation rate constants [22]:

$$\frac{dR}{dt} = -K_d R \tag{1}$$

$$R = \frac{K_a[A]R_{max}}{([A]K_a + K_d)}\left[1 - e^{-(([A]K_a + K_d)t)}\right] \tag{2}$$

Results

Characterization of DTBA–PBA monolayer

The ATR–FTIR spectra (Fig. 2) have clear and strong peaks that reflect the characteristic of DTBA–PBA powder. The peaks of 2922, 1663, 1431, and 1342 cm^{-1} represent asymmetric CH_2 stretching mode, C=O stretching mode, C–N stretching mode, and B–O stretching mode,

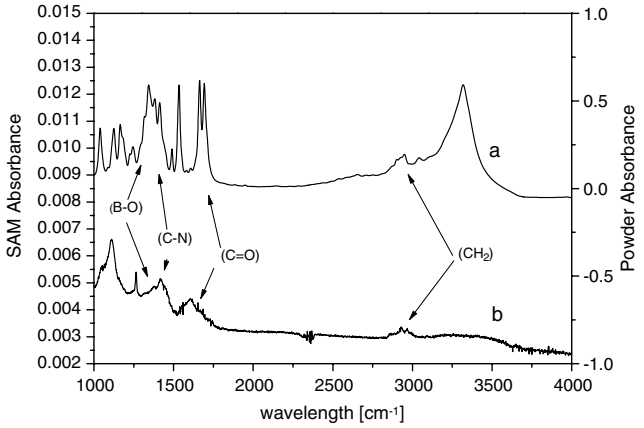

Fig. 2. IR spectra of DTBA–PBA powder in KBr solid state and chip by FTIR (a) and DTBA–PBA monolayer on Au surface by ATR–FTIR (b).

respectively. The peak of asymmetric stretching modes of the CH_2 group is very small on the Au surface. The results show that the DTBA–PBA monolayer stands nearly perpendicular to the substrate surface [23] and has well-packed SAMs [24]. To understand the surface characterization of the DTBA–PBA monolayer, we used the contact angle and ESCA to verify the DTBA–PBA monolayer on the Au surface (Table 1). The contact angle of bare Au was 84.5 ± 1.97°. After forming the DTBA–PBA monolayer, the contact angle was changed from 84.5 ± 1.97 to 63.3 ±1.03°. This change was attributed to the functional group of phenylboronic acid, which is more hydrophilic

Table 1
ESCA and contact angle for analyzing the bare Au and DTBA–PBA monolayer on the sensor surface

Condition	ESCA (%)						Contact angle (°)
	N	B	C	S	O	Au	
Bare Au	—	—	—	—	—	100	84.5 ± 1.97
DTBA–PBA monolayer	5.43	5.23	53.73	6.39	16.36	12.85	63.3 ± 1.03

than the bare Au surface. The calculations of DTBA–PBA monolayer compositions ($C_8H_9O_3NSB$) are as follows: C, 53.73%; N, 5.43%; S, 6.39%; O, 16.36%; Au, 12.85%. The element ratio of Au/S approached 50% and the element ratio of B/S approached 82% in ESCA data after phenylboronic acid immobilization. Based on these results, we should assume that the DTBA–PBA monolayer has an orderly construction.

Interaction of DTBA–PBA monolayer and HbA1c

To characterize the response of the SPR system, BSA (10 μg/ml) was tested for nonspecific adsorption corresponding to 1.7 μg/ml HbA1c followed by the DTBA–PBA monolayer. The results of these experiments are presented in Fig. 3. The SPR sensorgram obtained for two samples is easily distinguishable, and the DTBA–PBA monolayer was specific to glycated protein.

Fig. 4 presents the dependence on the isotherms of the DTBA–PBA monolayer and the HbA1c spectrum of the four kinds of solutions. The results reveal that if the HbA1c concentration is high in the solution, its corresponding signal intensity also is high. Besides, the condition pH 9/50 mM PB was better than the other conditions and had the maximum signal change of the SPR of approximately 10 nm.

When the condition was pH 9/50 mM PB + 300 mM sodium chloride, the maximum signal change was observed for SPR. This change was approximately 7 nm and had double- or multiple-layer adsorption at concentrations of HbA1c higher than 22 nM. The SPR signal change was smaller than that for the other conditions but also has double- or multiple-layer adsorption at concentrations of HbA1c higher than 50 nM.

The real-time kinetics of SPR (Fig. 5) exhibited a great shift for pH 9/50 mM PB and the extent of shift in the following order: pH 9/50 mM PB + 300 mM NaCl, pH

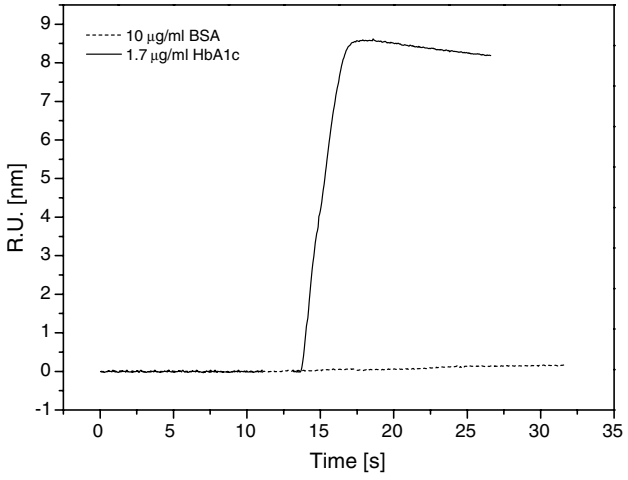

Fig. 3. To characterize the response of the SPR system, BSA (10 μg/ml) was tested for nonspecific adsorption, corresponding to 1.7 μg/ml HbA1c followed by the DTBA–PBA monolayer. R.U. is abbreviated from resonance unit.

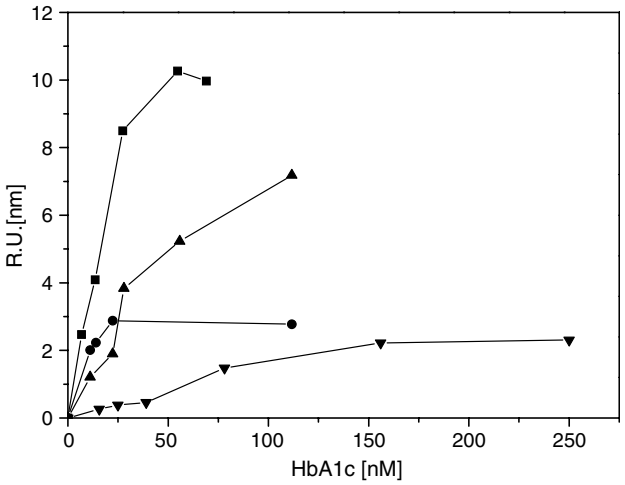

Fig. 4. Association isotherms for different concentrations of HbA1c: ■, pH 9/50 mM PB; ●, pH 5/50 mM PB; ▲, pH 9/50 mM PB + 300 mM NaCl; ▼, pH 5/50 mM NaCl. R.U. is abbreviated from resonance unit.

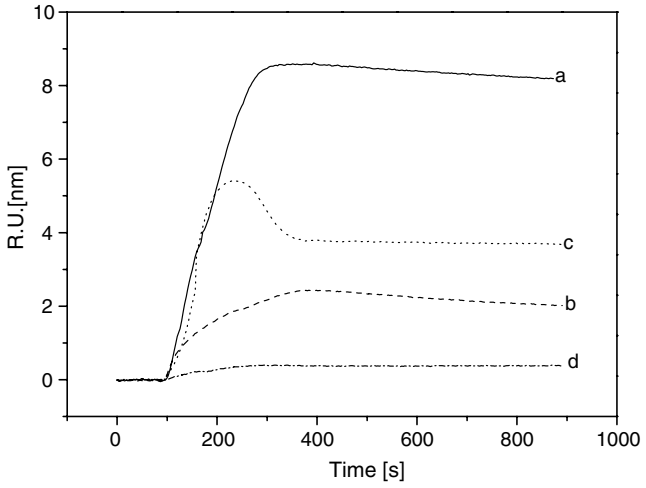

Fig. 5. SPR sensorgrams of phenylboronic acid monolayer for HbA1c (1.7 μg/ml) solution with different carry buffers: (a) pH 9/50 mM PB; (b) pH 5/50 mM PB; (c) pH 9/50 mM PB + 300 mM NaCl; (d) pH 5/50 mM NaCl. R.U. is abbreviated from resonance unit.

5/50 mM PB, pH 5/50 mM NaCl. The results showed that the SPR signal shift at pH 5/50 mM NaCl was smaller than at other conditions. This is because HbA1c was adsorbed on the chip surface by nonspecific interaction. It also interfered with the solute and substrate. Furthermore, the SPR signal shift increased with an increasing pH and decreased with a decreasing pH. Therefore, pH is an important factor that influences recognition between HbA1c and phenylboronic acid.

Fitting analysis of nonspecific adsorptive kinetics

To understand the mechanism of molecule reorganization, data fitting of the interaction was conducted under a condition such as pH 9/50 mM PB + 300 mM NaCl.

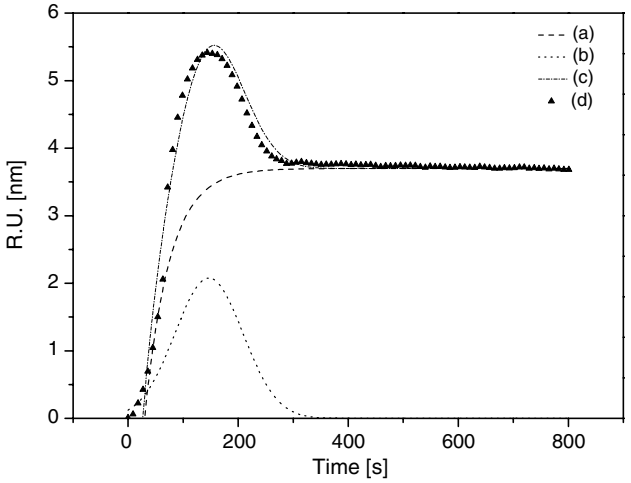

Fig. 6. Peak fitting of SPR shifts of the interaction between HbA1c (1.7 μg/ml) and phenylboronic acid monolayer in the condition pH 9/50 mM PB + 300 mM NaCl: (a) specific interaction between HbA1c and phenylboronic acid; (b) physical adsorption interaction between HbA1c and HbA1c on the surface; (c) resultant curve from adding curve a and curve b; (d) experimental curve. R.U. is abbreviated from resonance unit.

Fig. 6 shows the adsorptive curve (experimental curve) composed of specific and nonspecific adsorptions. The curve of adsorption (dashed line) was fitted by Eq. (1), which represents specific interaction between HbA1c and phenylboronic acid. The dotted line represents the nonspecific interaction between HbA1c and phenylboronic acid. The dashed-dotted line is composed of a green line and a red line. The results show that nonspecific adsorption was enhanced under a high salt concentration. The kinetic data were strongly supported by the isotherm data shown in Fig. 4, where double or multiple layers of adsorption were clearly demonstrated. As compared with the condition pH 9/50 mM PB (Fig. 5), adding a high concentration of salt could enhance nonspecific adsorption and decrease specific adsorption. This was due to hydrophobic interaction force that is apparent under a high salt concentration. Because of conspicuous hydrophobic interaction, the specific interaction of HbA1c and phenylboronic acid decreased.

Kinetics calculation

The interaction process of HbA1c and phenylboronic acid was divided into association and disassociation phases, and the obtained kinetic parameters for the phases are listed in Table 2. In the association phase, the condition pH 9/50 mM PB and the condition pH 5/50 mM PB exhibited the same order association rate constant, but one small difference is that HbA1c and phenylboronic acid charges both were negative in alkalinity (pH 9/50 mM PB). Therefore, the electrostatic force facilitated repulsion between HbA1c and phenylboronic acid, which in turn decreased the association constant to a smaller value as compared with that in the other conditions. On the other hand, the phenylboronic acid charge tended toward zero in acidity (pH 5) and caused the electrostatic force to be nonsignificant.

Table 2
Kinetic parameters for the affinity interaction between HbA1c (1.7 μg/ml) and phenylboronic acid monolayer in different buffers

Condition	$k_a \times 10^5$ (M^{-1} s^{-1})	$k_d \times 10^{-4}$ (s^{-1})	$K_a \times 10^9$ (M^{-1})	R_{max} (nm)
pH 9/50 mM PB	1.99	1.23	1.6	12.8
pH 5/50 mM PB	3.40	3.56	0.9	2.68
pH 5/50 mM NaCl	2.53	0.324	6.6	0.46

In the disassociation phase, the condition pH 5/50 mM NaCl exhibited a slow disassociation rate constant of approximately 0.324×10^{-4} s^{-1}. Also, the condition of pH 9/50 mM PB and the fast disassociation constant rate was approximately 3.56×10^{-4} s^{-1} under the condition of pH 5/50 mM PB. The disassociation rate constant ratio of the condition pH 5/50 mM PB and pH 5/50 mM NaCl was 10. When the HbA1c and phenylboronic acid complex was formed under the condition that the HbA1c would break away from the ester bond, pH value was an important factor affecting the stability of the complex [18,19]. The pH 9 value could stabilize the complex stereostructure, but HbA1c had broken away from the complex because the ester bond easily hydrolyzes in acidity (pH 5). Therefore, the condition pH 5/50 mM PB had a fast disassociation rate constant. It exhibited that different concentrations of salt could have significant effects on the disassociation rate constant. Besides, HbA1c might adsorb tightly onto the chip surface according to nonspecific adsorption with the change of protein structure at pH 5/50 mM NaCl. This lowered the disassociation rate constant much more than the others did.

From the point of binding affinity, the maximum shift of SPR signal represented the stability of the complex that was formed on HbA1c and phenylboronic acid. In alkalinity (pH 9), the R_{max} ratios of pH 5/50 mM PB and pH 5/50 mM NaCl were 5- and 20-fold, respectively. The HbA1c and phenylboronic acid formed a complex in an alkaline condition easily, whereas forming a complex was not easy to do in an acidic condition. The results reveal that pH 9/50 mM PB for detecting HbA1c is the best condition for specificity and stability.

Experiments with HbA1c

From the above results, we can conclude that the best condition to detect HbA1c is pH 9/50 mM PB. To estimate the detectable range for clinical diagnosis application, we designed a concentration range of HbA1c between 0.43 and 3.49 μg/ml, which is 0.001-fold in the physiology range (3–13 mg/ml) [25] for measurement. Fig. 7 shows the standard curve of HbA1c. In the range of the experiments, the concentration of HbA1c and SPR signal corresponds with a good linear fitting ($R = 0.992$, $P < 0.0001$). In our system, the detection limit was 0.01 μg/ml. Compared with traditional methods [12,15], the sample concentration decreased approximately 1000-fold by using the SPR system. The

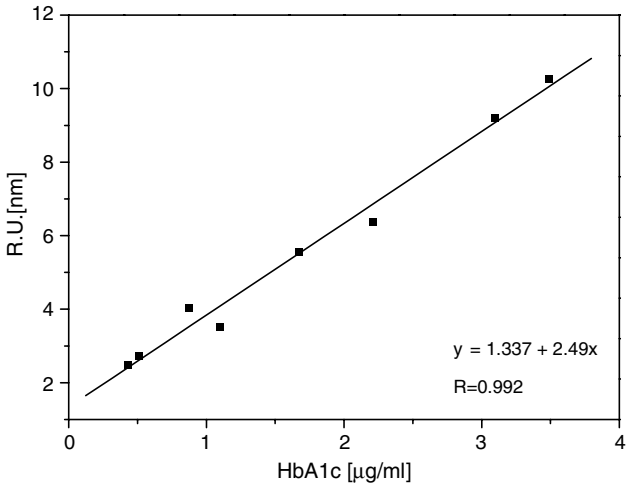

Fig. 7. Linear response to HbA1c in the range from 0.43 to 3.49 μg/ml in the condition pH 9/50 mM PB buffer. R.U. is abbreviated from resonance unit.

blood sample dilution can reduce the substrate interference and enhance the signal/noise ratio to improve the accuracy of detecting HbA1c. In addition, the blood sample quantity used also decreased. Therefore, the results show that the SPR system is enough to investigate changes in HbA1c concentration in a physiological range. Overall, the SPR system has good potential for clinical diagnosis.

Discussion

Effect of pH

The experimental results exhibit that the adsorption behavior at low HbA1c concentration resembles the Langmuir isotherm. In addition, the alkaline condition (pH 9) has greater adsorption than the acidity condition (pH 5). This means that phenylboronic acid transitioned from binary form, $B(OH)_2$, to ternary form, $B(OH)_3$, in the alkaline solution. It also created the stereostructure for binding the HbA1 c to form a stable complex. Therefore, the SPR signal has a greater shift in the alkaline condition than in the acid condition. However, in the latter, the environment's pH value was smaller than the pK_a of phenylboronic acid and the pI of HbA1c. Thus, the electric charges of HbA1c and phenylboronic acid are positive and zero, respectively. This resulted in a decreasing electrostatic force, and phenylboronic acid existed in a binding form that decreases the specific interaction, thereby decreasing the SPR signal shift.

Effect of salt concentration

Electrostatic interaction is shielded by the presence of ions and the effects of salt. According to protein composition, the polar and nonpolar regions are separated. The charge and polar region of the protein are dependent on the protein's pI and the environment's pH value. Fig. 4 shows high ion strength that could shield the electrostatic interaction between HbA1cs and phenylboronic acid. It also enhanced hydrophobic interaction. On the other hand, it has a double-layer adsorption between HbA1c at concentrations of HbA1c higher than 22 nM, and the adsorption behavior is toward the Freundlich type of adsorption.

In a low salt concentration, the interaction behavior is similar under alkalinity (pH 9, 50 mM PB) and acidity (pH 5, 50 mM PB.). This proves that the molecule interaction is not significant in a low salt concentration. However, the adsorption behavior was different in a high salt concentration. This proves that the interaction process is not facilitated by only one adsorptive mechanism.

Effect of Lewis bases

In experimental conditions, adding Lewis bases in carry buffer influences the phenylboronic acid stereostructure. When the concentrations of HbA1c were lower than 50 nM, the addition of Lewis bases could help to enhance the specific interaction between HbA1c and phenylboronic acid (Fig. 4). The SPR signal improved five times better due to Lewis bases that could change the stereostructure of phenylboronic acid for binding with saccharine.

From the adsorption process, the signal of interaction between HbA1c and phenylboronic acid was not significant without the Lewis bases. A little shift was due to nonspecific interaction. It also demonstrated that the Lewis bases could change the stereostructure of phenylboronic acid to form $B(OH)_3$ for easy binding with saccharine.

Conclusion

The pH value is one of the important factors that affect the formation of a stable complex between HbA1c and phenylboronic acid. When the pH value is higher than the pK_a of phenylboronic acid, the phenylboronic acid exists as an anion. This anion is easy to bind with diol compositions. The addition of Lewis bases can help phenylboronic acid to reduce the sensitivity to pH and change the structure. Furthermore, nonspecific interaction is enhanced and the specific interaction is decreased in a high salt concentration.

This work has demonstrated that SPR can successfully detect and quantify the binding of HbA1c to phenylboronic acid monolayer by the specific interaction. This methodology may prove to be useful in clinical diagnosis and serve as an assay for HbA1c binding ability.

Acknowledgment

The authors thank financial support from National Science Council, Taiwan (NSC 95-2218-E-008-022) and Veter-

ans General Hospitals University System of Taiwan Joint Research Program, (VGHUST97-P3-07), Tsou's Foundation for this study.

References

[1] H.F. Bunn, D.N. Haney, S. Kamin, K.H. Gabbay, P.M. Gallop, The biosynthesis of human hemoglobin A1c: Slow glycosylation of hemoglobin in vivo, J. Clin. Invest. 57 (1976) 1652–1659.

[2] H.B. Chandalia, P.R. Krishnaswamy, Glycated hemoglobin, Curr. Sci. 83 (2002) 1522–1532.

[3] S. Liu, U. Wollenberger, M. Katterle, F.W. Scheller, Ferroceneboronic acid-based amperometric biosensor for glycated hemoglobin, Sens. Actuat. B 113 (2006) 623–629.

[4] M.R. Buchmeiser, New synthetic ways for the preparation of high-performance liquid chromatography supports, J. Chromatogr. A 918 (2001) 233–266.

[5] Y.C. Li, U. Pfuller, E.L. Larsson, H. Jungvid, I.Y. Galaev, B. Mattiasson, Separation of mistletoe lectins based on the degree of glycosylation using boronate affinity chromatography, J. Chromatogr. A 925 (2001) 115–121.

[6] G.B. Springsteen, B. Wang, A detailed examination of boronic acid–diol complexation, Tetrahedron 58 (2002) 5291–5300.

[7] W. Yang, J. Yan, G. Springsteen, S. Deeter, B. Wang, A novel type of fluorescent boronic acid that shows large fluorescence intensity changes upon binding with a carbohydrate in aqueous solution at physiological pH, Bioorg. Med. Chem. Lett. 13 (2003) 1019–1022.

[8] G. Shouhai, W. Wei, W. Binghe, Building fluorescent sensors for carbohydrates using template-directed polymerizations, Bioorg. Chem. 29 (2001) 308–320.

[9] W. Yang, S. Gao, X. Gao, V.V.R. Karnati, W. Ni, B. Wang, W.B. Hooks, J. Carson, B. Weston, Diboronic acids as fluorescent probes for cells expressing sialyl Lewis X, Bioorg. Med. Chem. Lett. 12 (2002) 2175–2177.

[10] L.I. Bosch, T.M. Fyles, T.D. James, Binary and ternary phenylboronic acid complexes with saccharides and Lewis bases, Tetrahedron 60 (2004) 11175–11190.

[11] M.B. de la, C. Guntiñas, R. Wissiack, G. Bordin, A.R. Rodríguez, Determination of haemoglobin A_{1c} by liquid chromatography using a new cation-exchange column, J. Chromatogr. B 791 (2003) 73–83.

[12] C.W. Weykamp, T.J. Penders, C.W.M. Siebelder, F.A.J. Muskiet, W.V.D. Slik, Interference of carbamylated and acetylated hemoglobins in assays of glycohemoglobin by HPLC, electrophoresis,affinity chromatography, and enzyme immunoassay, Clin. Chem. 39 (1993) 138–142.

[13] C.F. Shuman, M.D. Hamalainen, U. H Danielson, Kinetic and thermodynamic characterization of HIV-1 protease inhibitors, J. Mol. Recogn. 17 (2004) 106–119.

[14] L.K. Koskinen, Specificity of hemoglobin A_{1c} measurement by cation exchange liquid chromatography: Evaluation of a Mono S column method, Clin. Chim. Acta 253 (1996) 159–169.

[15] R.R. Little, H. Vesper, C.L. Rohlfing, M. Ospina, Validation by a mass spectrometric reference method of use of boronate affinity chromatography to measure glycohemoglobin in the presence of hemoglobin S and C traits, Clin. Chem. 51 (2005) 264–265.

[16] J.P. Lorand, J.O. Edwards, Polyol complexes and structure of the benzeneboronate ion, J. Org. Chem. 24 (1959) 769–774.

[17] R. Pizer, L. Babcock, Mechanism of the complexation of boron acids with catechol and substituted catechols, Inorg. Chem. 16 (1977) 1677–1681.

[18] J.C. Norrild, H. Eggert, Evidence for mono- and bisdentate boronate complexes of glucose in the furanose form: Application of 1JC–C coupling constants as a structural probe, J. Am. Chem. Soc. 117 (1995) 1479–1484.

[19] J.C. Norrild, H. Eggert, Boronic acids as fructose sensors: Structure determination of the complexes involved using J coupling constants, J. Chem. Soc. Perkin Trans. 2 (1996) 2583–2588.

[20] N. Soh, M. Sonezaki, T. Imato, Modification of a thin gold film with boronic acid membrane and its application to a saccharide sensor based on surface plasmon resonance, Electroanalysis 15 (2003) 1281–1290.

[21] L.Y. Chen, M.C. Wu, M.T. Chou, L.A. Kao, S.J. Chen, W.Y. Chen, Effects of solute–matrix interaction on monitoring the conformational changes of immobilized proteins by surface plasmon resonance sensor, Talanta 67 (2005) 862–867.

[22] R. Karlsson, A. Michaelsson, L. Mattsson, Kinetic analysis of monoclonal antibody–antigen interactions with a new biosensor based analytical system, J. Immunol. Methods 145 (1991) 229–240.

[23] S.C.B. Mannsfeld, T.W. Canzler, T. Fritz, H. Proehl, K. Leo, S. Stumpf, G. Goretzki, K. Gloe, The structure of [4-(phenylazo)phenoxy]hexane-1-thiol self-assembled monolayers on Au(111), J. Phys. Chem. B 106 (2002) 2255–2260.

[24] K. Tamada, J. Nagasawa, F. Nakanishi, K. Abe, T. Ishida, M. Hara, W. Knoll, Structure and growth of hexyl azobenzene thiol SAMs on Au(111), Langmuir 14 (1998) 3264–3271.

[25] J. Pribyl, P. Skladal, Quartz crystal biosensor for detection of sugars and glycated hemoglobin, Anal. Chim. Acta 530 (2005) 75–84.