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Voltammetric Detection of the Polycation Protamine by the Use of Electrodes Modified with Self-Assembled Monolayers of Thioctic Acid

Victor P. Y. Gadzekpo,[†] Kang Ping Xiao,[‡,§] Hiroshi Aoki, Philippe Bühlmann, and Yoshio Umezawa*

Department of Chemistry, School of Science, The University of Tokyo, 7-3-1 Hongo, Tokyo 113-0033, Japan

The detection of protamine, a polycation, by use of electrodes modified with self-assembled monolayers of thioctic acid is reported. These sensors can detect protamine concentrations as low as 0.5 μg/mL when $[Ru(NH_3)_6]^{3+}$ is used as marker and 2.0 μg/mL when $[Fe(CN)_6]^{3-}$ is used as marker. Binding of protamine to the thioctic acid monolayers controls the reduction rate of $[Ru(NH_3)_6]^{3+}$ and $[Fe(CN)_6]^{3-}$ at the electrode surface due to electrostatic attraction or repulsion between these markers and the monolayer, allowing the indirect detection of protamine. In physiological concentrations, the blood electrolytes sodium, potassium, calcium, and magnesium do not interfere. The sensors respond to protamine in diluted horse serum. They are selective for protamine over Polybrene, another polycation that neutralizes the anticoagulant activity of heparin. Protamine once bound to the electrode surface can be removed by washing with 0.1 M KCl of pH 5.1. An increase in the scan rate of cyclic voltammetry decreased the detection limit for protamine and increased the dynamic range. The sensor was used to detect the end point in heparin−protamine titrations.

Polyions have been finding wide applications in many fields.[1−5] For example, polysaccharides are largely used in medicine and in the food industry; polyphosphate is used in fertilizers; pectin, dermatan sulfate, and ι-carrageenan are used as food additives; heparin is used extensively as an anticoagulant in surgery while protamine is used to neutralize the anticoagulant activity of heparin.[3] Protamine is also used as a titrant within commercially available blood-clotting instrumentation to determine heparin concentrations in blood.[6]

Though it is attractive to sensitively detect these polyions in routine analysis, this kind of analysis is an ongoing challenge. Protamine, for example, is currently determined with conventional protein reagents such as the Folin-phenol reagent and Coomassie Brilliant Blue G-250, as described for example by Lowry[7] or Bradford.[8] However, these methods are not specific for protamine. Heparin, on the other hand, is determined with the activated clotting time method (ACT)[9] or with the colorimetric anti-Xa method, where heparin's ability to inhibit factor Xa activity is measured.[10] Potentiometry with solvent polymeric membrane electrodes has recently been reported for the detection of polyions such as heparin and protamine.[4,5,11−16] Because the charge on these polyions can be high (∼+20 for protamine and ∼−70 for heparin), the Nernstian responses for these ions are very small, i.e., less than 3 mV for a 10-fold increase in protamine concentration and less than −1 mV for a 10-fold increase in heparin concentration. Therefore, Nernstian responses cannot be used for real analytical applications. Instead, non-Nernstian responses of ion-exchanger electrodes were obtained under nonequilibrium steady-state conditions. Despite the fact that they become insensitive to the polyions after 24 h of equilibration with these ions and therefore measurements have to be made within 1−3 min, these electrodes have been successfully used to determine heparin and protamine concentrations.[4,5,13] Van Kerkhof et al. reported on a heparin sensor based on protamine as affinity ligand using an ion-sensitive field effect transistor (ISFET) technique.[17,18] This technique was employed with an indirect ion-step method to detect

[†] On sabbatical leave from the Department of Chemistry, University of Cape Coast, Ghana.

[‡] Research Fellow of the Japan Society for the Promotion of Science (JSPS).

[§] Present address: Department of Chemistry, Michigan State University, East Lansing, MI 48824.

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heparin. The detection range of this sensor is 0.1−2.0 units/mL. Also, a surface plasmon resonance sensor based on polyethyleneimine (PEI) as affinity surface has been reported for heparin measurements in blood plasma with a detection limit of 0.2 units/mL and a linear range of 0.2−2 units/mL using protamine.[19−21]

In this paper, we describe the detection of protamine using ion channel sensors based on electrodes chemically modified with self-assembled monolayers (SAMs). Electrochemical sensors that mimic biological ion channels by using receptor-modified electrodes[22−25] are called ion channel mimetic sensors or ion channel sensors. They were so named because their working principle is similar to that of ion channel proteins in biomembranes.[26−30] Binding of analytes to the receptors at the electrode surface in these sensors controls the reduction or oxidation rate of electroactive ions or molecules, often referred to as markers. This control is due to either physical exclusion[22] or electrostatic attraction or repulsion[22−24] between these receptors and markers. Ion channel sensors have the inherent possibility of signal amplification. This interesting characteristic has led many researchers to investigate their use as sensors for the detection of ions and molecules.[30] For example, electrodes modified with receptors for hydrogen ions,[31−34] metal cations,[22,35−38] nucleotides,[23,24,39,40] and antibodies [40−43] have been reported. Recently, we reported that the highly hydrophilic phosphate ion can be selectively detected with the ion channel mimetic sensing mode by using a neutral receptor that binds phosphate by four hydrogen bonds.[44] These results suggest that phosphate is still substantially hydrated when it is bound to the receptor monolayer. From this, it has been concluded that ion-channel sensing is very promising for the detection of highly hydrophilic, relatively large ions or molecules. Prompted by this result, we wondered whether ion channel sensors can be used to detect polyions, which have high charge numbers and are usually highly hydrophilic and large in size. In fact, compared to ions with one or several charges, polyions with their much higher charges would have the advantage of being much more strongly bound to receptor monolayers. Furthermore, these multiple charges can electrostatically much more influence the access of marker ions to the electrode surface if their binding results in higher charge densities at the electrode surface. As a result, it was expected that ion channel sensors allow the selective detection of polyions in the presence of ions of lower charge number.

We report below the detection of the polycation protamine by use of ion channel sensors based on electrodes chemically modified with self-assembled monolayers of thioctic acid. The response performance of these electrodes is described.

EXPERIMENTAL SECTION

Reagents. Thioctic acid (1,2-dithiolane-3-pentanoic acid) and protamine sulfate (salmine sulfate from salmon sperm with average M_r of 4500) were obtained from Tokyo Chemical Industry (TCI), Tokyo, Japan. Polybrene (hexadimethrine bromide) and $[Ru(NH_3)_6]Cl_3$ were purchased from Aldrich Chemical Co., Milwaukee, WI. Tris(hydroxymethyl)aminomethane (Tris), $K_3[Fe(CN)_6]$, $Na_2B_4O_7$, $HClO_4$, KCl, NaCl, $MgCl_2$, and $CaCl_2$ were obtained from Wako Pure Chemical Industries Ltd., Osaka, Japan. Heparin was a donation from Daiichi Chemical Co., Tokyo, Japan. All chemicals were of the highest grade available and used without further purification. Buffer solutions of pH 1.5, 7.4, and 9.1 were prepared from 3.6% perchloric acid, 10 mM Tris, and 10 mM of sodium borate, respectively. The pH values of the buffer solutions were adjusted with KOH or HCl. All solutions were prepared from deionized and charcoal-treated water (specific resistance >18.0 MΩ cm) prepared by passage through a Milli-Q cartridge filtering system (Millipore Ltd., Bedford, MA).

Self-Assembly of Thioctic Acid Monolayers on Au Electrodes. A gold electrode (Bioanalytical Systems, West Lafayette, IN) was polished with wet 0.3- and 0.05-μm alumina slurry (Alpha Micropolish alumina No. 2 and Gamma Micropolish alumina No. 3, respectively; Buehler, Lake Bluff, IL) on a felt pad for 10 min, rinsed several times with water, and finally cleaned in a sonicator. The polished electrode was then dipped in 0.5 M KOH, and the potential was cycled between 0 and −1.4 V until the cyclic voltammogram indicated a perfectly clean electrode surface. The electrode was then rinsed several times with water and finally with anhydrous ethanol. The electrode was then immersed in a 0.1% (w/v) thioctic acid solution in ethanol for at least 24 h and rinsed several times with water.

Electrochemical Measurements. Cyclic voltammetry experiments were performed with a BAS CV50W potentiostat/galvanostat (Bioanalytical Systems). All potentials were measured versus a Ag/AgCl reference electrode, and a platinum wire was used as an auxiliary electrode. All cyclic voltammograms (CVs)

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were run after the sample solutions were purged with nitrogen gas for 15 min. Solutions for cyclic voltammetry contained 0.1 M KCl and either 1.0 mM $K_3[Fe(CN)_6]$ or 1.0 mM $[Ru(NH_3)_6]Cl_3$. They contained Tris buffer of pH 7.4 unless stated otherwise. The protamine stock solutions also contained buffer and 0.1 M KCl. The concentration of protamine was varied by spiking small aliquots of 1.0 or 3.0 mg/mL protamine stock solutions into 10 mL of the KCl/marker solutions. Potential windows of +0.6 to −0.1 V and +0.1 to −0.5 V were used for the cyclic voltammetry with $[Fe(CN)_6]^{3-}$ and $[Ru(NH_3)_6]^{3+}$, respectively. Desorption studies to confirm the presence of a monolayer and to calculate the surface concentration of thioctic acid in the monolayers were performed by scanning the potential between the 0.0 and −1.4 V in 0.5 M KOH solution.

Titration of Heparin with Protamine. Titrations of various heparin solutions were performed by adding aliquots of a protamine solution (1.111 μg/mL in 0.1 M KCl/Tris buffer pH 7.4) to 10 mL of buffer solution (Tris, pH 7.4) containing 0.1 M KCl and 1.0 mM $[Ru(NH_3)_6]^{3+}$. The heparin solutions were prepared by pipetting a volume of a heparin stock solution and making it up to 10 mL with the buffer solution. The heparin stock solution contained 0.42 mg/mL heparin, 140 mM NaCl, 100 mM KCl, 2.8 mM $CaCl_2$, and 2.7 mM $MgCl_2$. The solution was stirred after the additon of the protamine and a CV recorded. This was done after each addition until the CV was quasi-reversible and there was no significant change in the reduction current of the marker any more. The current obtained at the peak potential of the most reversible CV was plotted against the protamine concentration to obtain a titration curve.

RESULTS AND DISCUSSION

Thioctic Acid SAMs on Gold Electrodes. Thioctic acid (also called lipoic acid or 1,2-dithiolane-3-pentanoic acid) has been reported to form SAMs on gold surfaces with its carboxylic acid groups facing the solution.[32,45] Exposed to solutions of high pH, these terminal functional groups become deprotonated. The resulting charge on the monolayer was used to control the response of these electrodes to electroactive species in solution.[32] Cheng and Brajter-Toth[32] reported that the reduction peak in the cyclic voltammograms of $[Ru(NH_3)_6]^{3+}$ at pH 1.5 and that for $[Fe(CN)_6]^{3-}$ at pH 7.4 and 9.1 are completely suppressed when electrodes modified with thioctic acid SAMs are used. They explained that at pH 1.5 the monolayer carries a positive charge, and therefore, the positively charged marker $[Ru(NH_3)_6]^{3+}$ is repulsed from the monolayer surface. At pH 7.4 and 9.1, access of $[Fe(CN)_6]^{3-}$ to the electrode surface was explained to be prevented by the negative charge of the carboxylate groups.

Given these results, we decided to test electrodes modified with thioctic acid SAMs as ion channel sensors for the detection of the polycation protamine. Multiple electrostatic interactions between the carboxylate groups of thioctic acid and the ammonium groups of protamine were expected. In choosing thioctic acid as a receptor, we also took into account the length of the alkyl chain anchoring the receptor to the gold electrode. Receptors with long chains tend to form tight monolayers that can completely block the electrode surface.[31,46] On the other hand, molecules with shorter chains have been reported to be suited for ion channel sensors,[38,47−49] provided that the resulting SAMs are sufficiently stable.

Desorption studies performed with the electrode modified with thioctic acid revealed the presence of the self-assembled monolayer, which desorbed at −0.964 V. The monolayer is close-packed with a surface concentration of 0.72×10^{-9} mol/cm^2 determined by the integration of the area under the desorption peak. This value is comparable to the reported value of $(1.5-2.0) \times 10^{-10}$ mol/cm^2 for ω-thioalkanoic acids and ferrocenes covalently attached to cystamine SAMs.[45,48,50]

Protamine Response with $[Fe(CN)_6]^{3-}$ as Marker. The responses of the thioctic acid-modified electrode to protamine at pH 7.4 (Tris buffer in 0.1 M KCl) with $[Fe(CN)_6]^{3-}$ as the marker ion are shown in Figure 1. It can be seen that, in the absence of protamine, there are no oxidation and reduction peaks in the cyclic voltammogram A. With the addition of small aliquots of protamine (3.33 μg/mL) to the sample solution, the current increased, resulting in the quasi-reversible cyclic voltammogram B. At the protamine concentrations of 5.00 and 6.67 μg/mL, the current further increased and reduction peaks were observed at +0.0 and +82 mV, respectively. A completely reversible peak at +105 mV (E) for $[Fe(CN)_6]^{3-}$ reduction was obtained at even higher concentrations of protamine (16.7 μg/mL). The presence of protamine in the sample solution clearly facilitates the reduction of the negatively charged $[Fe(CN)_6]^{3-}$ at the monolayer-modified electrode surface. This result can be explained by binding of protamine to the thioctic acid monolayer. Protamine with its positive charge binds to the monolayer, reducing the negative excess charge on the monolayer and, at high protamine concentrations, may even give the electrode surface an excess positive charge. Without the electrostatic repulsion from the negatively charged carboxylate groups, $[Fe(CN)_6]^{3-}$ can easily access the electrode surface and is reversibly reduced. As shown in Figure 2, the reduction current depends on the concentration of the protamine at pH 7.4 and 9.1 with linear concentration ranges of 3−8.0 (0.7−1.8 μM) and 6.7−11.5 μg/mL (1.5−2.6 μM), respectively. The concentration range of the response at pH 7.4 is at lower concentrations than the corresponding range for the response at pH 9.1. This is expected because at pH 9.1 more COOH groups are deprotonated, resulting in more negative sites on the monolayer surface and, therefore, stronger protamine binding. However, it was observed that repeated scanning of the potential from +0.6 to −0.1 V for a monolayer-modified electrode that had been used to detect protamine caused an increase in the $[Fe(CN)_6]^{3-}$ reduction current even when this cycling was performed with the electrode dipped in a solution containing no protamine. This may be due to an interaction between the $[Fe(CN)_6]^{3-}$ marker and the adsorbed protamine on the electrode surface (vide infra) since the phenomenon of increasing current was not observed for a fresh monolayer-modified electrode in the absence of protamine. Also, this behavior was not observed when $[Ru(NH_3)_6]^{3+}$ was used as marker.

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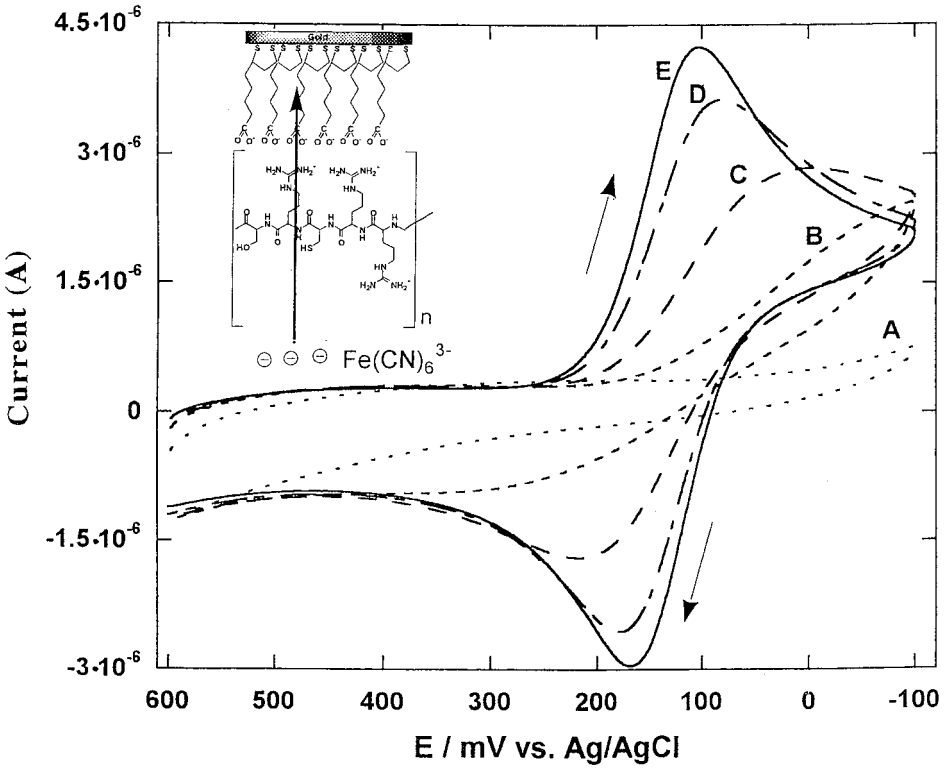

Figure 1. Cyclic voltammograms of $[Fe(CN)_6]^{3-}$ measured with a gold electrode chemically modified by a self-assembled monolayer of thioctic acid (Tris buffer, pH 7.4; 0.1 M KCl; scan rate, 0.1 V/s). Protamine concentration: (A) 0.00, (B) 3.33, (C) 5.00, (D) 6.67, and (E) 10.00 μg/mL.

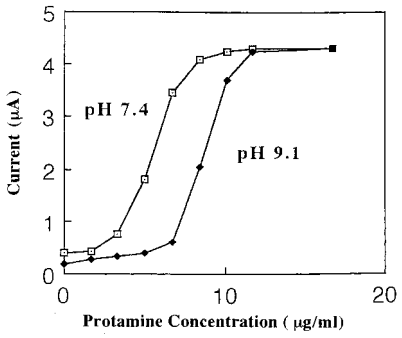

Figure 2. Dependence of sensor response on protamine concentration when using 10 mM $[Fe(CN)_6]^{3-}$ as marker: (a) pH 7.4; (b) pH 9.1. Scan rate, 0.1 V/s. Current observed at + 105 mV vs Ag/AgCl for pH 7.4 and + 111 mV vs Ag/AgCl for pH 9.1.

Interestingly, CVs were reversible even for high protamine concentrations. Steric blocking of the electrode surface by the large analyte protamine does not occur.

Protamine Responses with $[Ru(NH_3)_6]^{3+}$ as Marker. The response of the ion channel sensor to protamine with $[Ru(NH_3)_6]^{3+}$ as marker is shown in Figure 3. CVs were obtained at a scan rate of 0.1 V/s. A pH of 7.4 (Tris buffer in 0.1 M KCl) was used because it is close to the pH of blood. It can be seen that as aliquots of protamine are added to the buffer, the current of $[Ru(NH_3)_6]^{3+}$ reduction decreases, the reduction peak potential shifts to more negative values, and the CVs become quasi-reversible. These changes are opposite to those that were observed for $[Fe(CN)_6]^{3-}$ and can also be ascribed to binding of the positively charged protamine to the negatively charged thioctic acid monolayer. The presence of protamine on the electrode surface results in electrostatic repulsion of $[Ru(NH_3)_6]^{3+}$. The dependence of the sensor response on the protamine concentration at pH 7.4 is shown in Figure 4. The reduction current at the potential of −0.16 V in these CVs was used to assess the protamine responses. This potential is the peak potential of the reversible $[Ru(NH_3)_6]^{3+}$ reduction in the absence of protamine. Figure 4 shows an abrupt drop in current in the protamine concentration range between 4.4 and 5.0 μg/mL for the initial exposure of the electrode to protamine (Figure 4a) and between 2.7 and 3.3 μg/mL protamine for subsequent exposures of the electrode to protamine solutions (Figure 4b). Similar changes after the first protamine contact were also obtained even when 30 potential scans were applied on a monolayer-modified electrode before protamine was added. We speculate that a small amount of protamine irreversibly binds to the electrode surface. Covalent binding of protamine, which has thiol groups, may be needed to eliminate pinholes in the SAM of thioctic acid.

Since protamine is routinely used to neutralize heparin in blood at the end of surgery,[3] it entails that its determination should not be affected by blood electrolytes. In this respect, promising preliminary results were obtained. The sensor did not respond significantly to Na^+, Ca^{2+}, and Mg^{2+} when protamine was detected in the presence of 140 mM Na^+, 5.0 mM Ca^{2+}, or 4.8 mM Mg^{2+}. The protamine responses shifted slightly to a lower concentration range when the concentration of the supporting electrolyte KCl was lowered from 0.1 to 0.01 M. However, this appears to be an effect of the ionic strength and not a specific effect from K^+, another important blood electrolyte.

Using diluted horse serum (1/21 dilution), the sensor clearly responded to protamine when a high scan rate of 51.2 V/s was

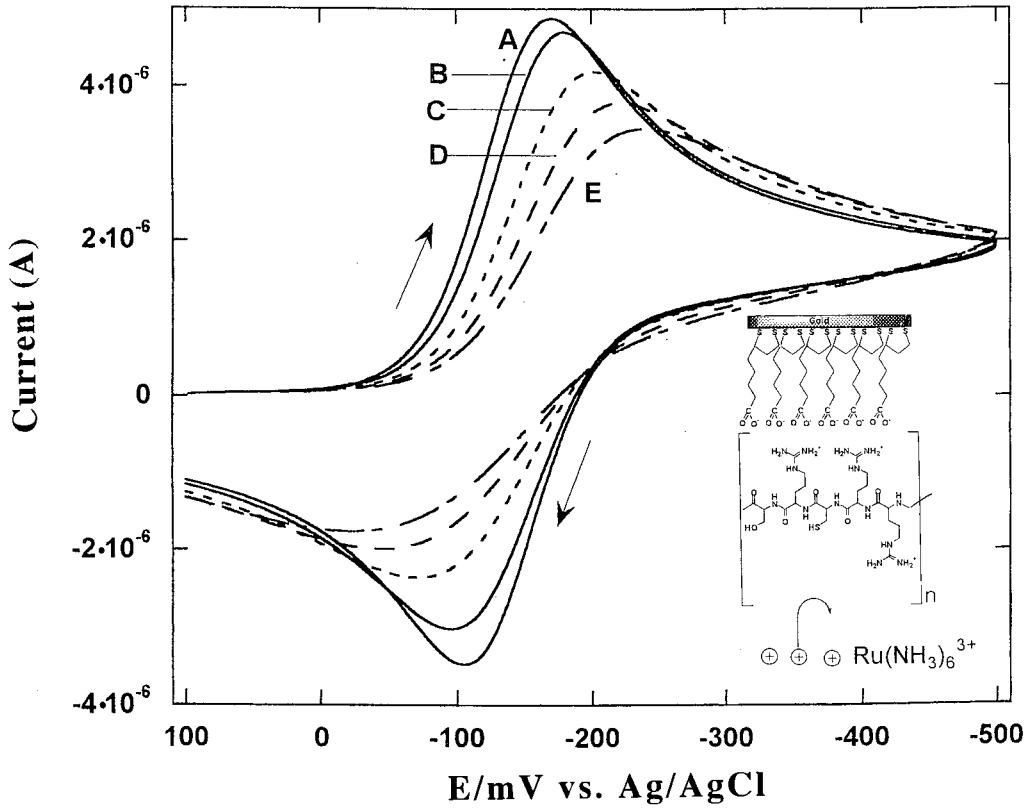

Figure 3. Cyclic voltammograms of $[Ru(NH_3)_6]^{3+}$ measured with an electrode modified by a thioctic acid SAM (Tris buffer, pH 7.4; 0.1 M KCl; scan rate, 0.1 V/s). Protamine concentration: (A) 0.00, (B) 2.78, (C) 2.83, (D) 2.88, and (E) 4.44 μg/mL.

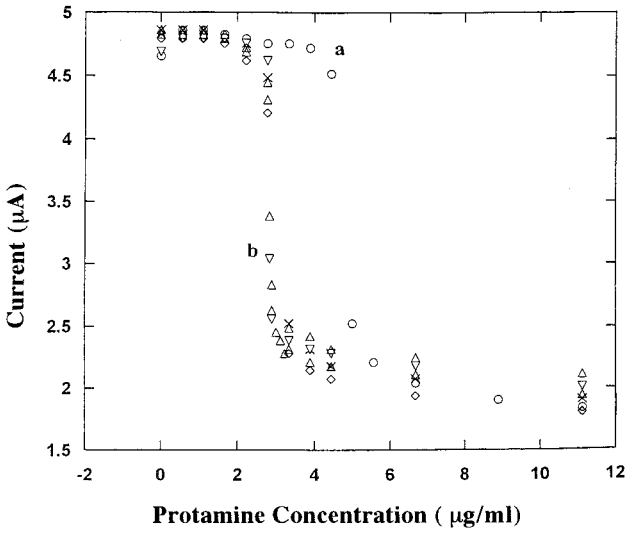

Figure 4. Sensor response at pH 7.4 using 1.0 mM $[Ru(NH_3)_6]^{3+}$ as marker: Current dependence on protamine concentration. The empty circles (○) represent the first exposure to protamine; other symbols represent measurements after subsequent exposure to protamine. Current observed in CV at −162 mV (scan rate, 0.1 V/s).

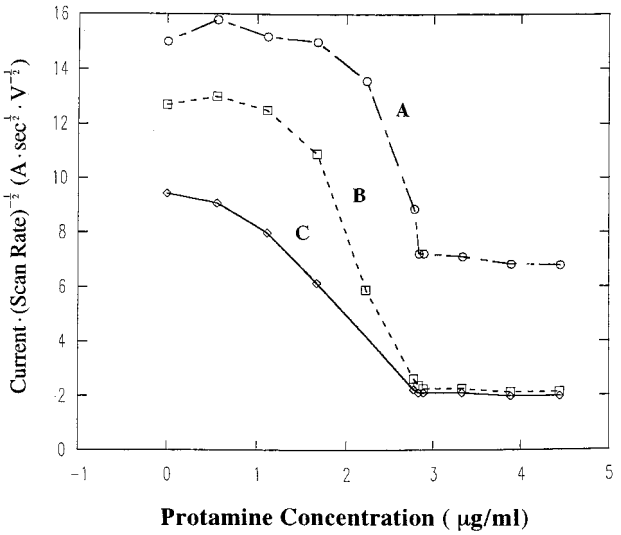

Figure 5. Effect of scan rate on the dynamic range of the protamine response (1.0 mM $[Ru(NH_3)_6]^{3+}$ as marker; Tris buffer in 0.1 M KCl, pH 7.4). (A) 0.1, (B) 5.12, and (C) 51.2 V/s. For enhanced comparability, the *y*-axis represents the ratio of measured currents and the square root of the scan rate.

used (Figure 6). However, the protamine calibration plot did not show an abrupt drop in current with increasing protamine concentration as was the case when the solution contained no horse serum. This may be due to binding of macromolecules from the serum to the electrode surface, preventing protamine from readily binding to the negative sites of the thioctic acid monolayer. At the low scan rate of 0.1 V/s, however, the sensor became less sensitive to protamine.

Increasing the ionic strength of the measurement solution from 0.1 to 0.3 and 1.0 M by adding KCl gave a result similar to that when horse serum was added to the measurement solution (Figure 7). Association of K^+ with the thioctic acid monolayer and Cl^- with heparin may prevent the protamine from readily binding to the monolayer. This would explain the absence of an abrupt drop in current with increasing protamine concentration. In fact, the calibration of this protamine sensor in the presence

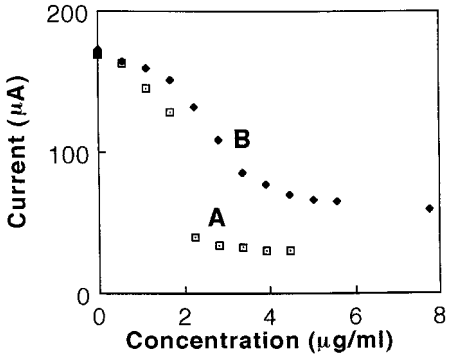

Figure 6. Concentration dependence of the protamine response using $[Ru(NH_3)_6]^{3+}$ as marker (1 mM) at a scan rate of 51.2 V/s. (A) Response in Tris buffer in 0.1 M KCl, pH 7.4. (B) Response in 1/21 diluted horse serum.

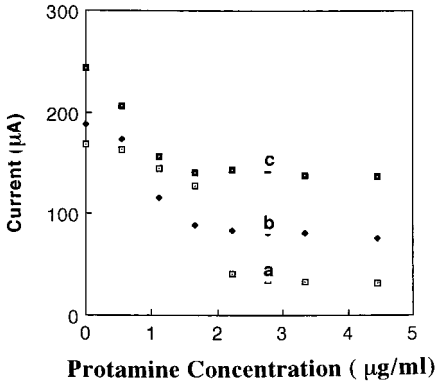

Figure 7. Concentration dependence of protamine response using $[Ru(NH_3)_6]^{3+}$ marker at a scan rate of 51.2 V/s. (A) Response in Tris buffer in 0.1 M KCl, pH 7.4. (B) Response in Tris buffer in 0.3 M KCl, pH 7.4. (C) Response in Tris buffer in 1.0 M KCl, pH 7.4.

of some amount of serum or in a background of high ionic strength may be an advantage. Furthermore, the protamine sensor was found to be about 1000 times more selective to protamine over Polybrene, another polycation that neutralizes the anticoagulant activity of heparin. On the basis of the fact that the total charge of Polybrene (+40 to +80, as calculated by assuming the average molecular weight of Polybrene to be between 7 500 and 15 000) is much higher than that of protamine (about +20), this high protamine selectivity can be explained by considering the structural differences between the two polycations. While the positive charge is distributed over the outer surface of protamine, the charge is to some degree buried in Polybrene. This appears to weaken the interaction between the thioctic acid monolayer and the Polybrene polycation.

After the electrode had been exposed to protamine, it was observed that washing the electrode with pure water did not cause the reduction current of $[Ru(NH_3)_6]^{3+}$ in buffer solutions without protamine to return to its original value. It seems that protamine was not removed from the monolayer surface by washing with water alone. The electrode was therefore washed with 0.1 M KCl solutions of pH 5.1 (The pH was adjusted by the addition of a little amount of HCl to the solution.). The washing was performed by shaking a test tube containing the KCl solution and the electrode. This solution was used with the view that the K^+ or H^+ would neutralize the charge of the monolayer carboxylates and cause the release of the attached protamine. Washing the

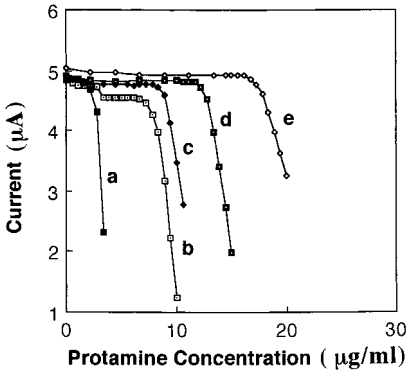

Figure 8. Titration curves of heparin-protamine titrations. Heparin concentration: (a) 0.00, (b) 1.68, (c) 2.52, (d) 2.94, and (e) 4.20 μg/mL. The titrations were repeated three times.

electrode for 2 min in a 0.1 M KCl solution of pH 5.1 caused the reduction current of $[Ru(NH_3)_6]^{3+}$ to return to its original value, which suggests the removal of protamine from the electrode surface. The electrode thus can be used repeatedly, which enhances the possibility of its use for real analytical applications.

The responses of ion channel sensors are based on the interaction between the electroactive marker, on one hand, and the receptor monolayer and surface-bound analyte, on the other hand. This interaction influences the kinetics of the redox reaction of the marker by changing its concentration in the electrical double layer at the electrode surface and by shifting the potential at which the electroactive marker is reduced or oxidized.[51,52] In the case of quasi-reversible CVs, higher scan rates are more appropriate for observing this influence because at high scan rates the rate-limiting effect of diffusion on the redox current becomes smaller. As a result, the effect of the surface-bound analytes on the CVs becomes larger. Therefore, the scan rate was varied from 0.1 to 50 V/s, expecting to result in a broadening of the concentration range of detectable protamine. As the scan rate was increased from 0.1 to 50 V/s, lower concentrations of protamine, which could not otherwise be easily detected at lower scan rates, became accessible. For example, a protamine concentration of 1.67 μg/mL that could hardly be detected at a scan rate of 0.1 V/s can be more easily detected by increasing the scan rate. The plot of the current over the square root of the scan rate against the protamine concentration, as shown in Figure 5, indicates an increase in the dynamic range as the scan rate is increased. The double layer charging current at the high scan rate of 51.2 V/s was found to be around 7 μA, which is about one-twentieth of the peak current due to the redox reaction. Even at this high scan rate, the charging current does not contribute more significantly to the total current. Determination of protamine at a scan rate of 51.2 V/s shows a response from 0.5 to 2.8 μg/mL, which is equivalent to 0.1−0.6 μM protamine.

Titration of Heparin with Protamine. Since protamine has a high affinity for heparin and since it is used clinically to neutralize heparin activity, it was of interest to perform preliminary studies on the use of the protamine sensor to detect the end point in titrations of heparin with protamine. Figure 8 shows a titration

(51) Takehara, K.; Takemura, H.; Ide, Y. *Electrochim. Acta* **1994**, *39*, 817.
(52) Takehara, K.; Takemura, H. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1289.

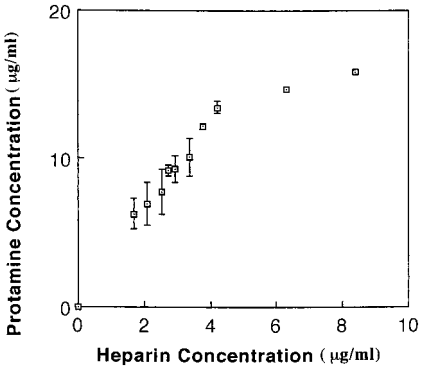

Figure 9. Protamine concentration vs heparin concentration in heparin−protamine titrations.

curve of a heparin−protamine titration. As the heparin concentration is increased, the concentration of protamine required to neutralize heparin becomes larger. The end point of the titration was determined by extrapolating the two linear portions of the titration curve. The point of intersection was taken as the end point. The end points so determined were plotted against the heparin concentration in order to obtain a calibration curve and the amount of protamine needed to bind heparin. The calibration curve, as can be seen in Figure 9, has two linear regions, one at low heparin concentrations (1.68−4.20 μg/mL) and the other at heparin concentrations above 4.20 μg/mL. The linear portion at low heparin concentrations indicates that 3.3 μg/mL protamine will neutralize 1.0 μg/mL heparin. This is slightly different from the value of 1.05 μg of protamine to 1.0 μg of heparin reported by Ma and co-workers.[53] This difference in value may be due to differences in the type of heparin and protamine used in the two different works. These preliminary results suggest possible heparin determination by titration with protamine using the protamine sensor as an end point detector.

Very recently, we also attempted to determine heparin by attaching protamine electrostatically to thioctic acid as a receptor. A report about that work is in preparation.[54]

(53) Ma, S.-C.; Yang, V. C.; Fu, B.; Meyerhoff, M. E. *Anal. Chem.* **1993**, *65*, 2078.

(54) Gadzekpo, V. P. Y.; Bühlmann, P.; Xiao, K. P.; Aoki, H.; Umezawa, Y., in preparation.

Lifetime. Preliminary observations showed an electrode to be very stable without any observable change in performance as long as it was stored in pure water in a stoppered test tube. This observation was made over a period of 4 months during which the electrode was used for the measurement of over 450 CVs.

CONCLUSIONS

An ion channel sensor has been used for the first time to detect polyions. Detection of protamine, a polycation, using $[Ru(NH_3)_6]^{3+}$ as electroactive marker and electrodes modified with SAMs of thioctic acid has been used to demonstrate the potential of ion-channel sensors in the detection and analysis of polyions. Lower concentrations of protamine can be detected by increasing the scan rate. The sensor is about 3 orders of magnitude more selective to protamine over Polybrene and its response is not affected by the blood electrolytes Na^+, K^+, Ca^{2+}, or Mg^{2+}. Protamine can be detected with this sensor in a diluted horse serum when a high scan rate is used. The electrodes were also used to detect the end point in heparin−protamine titrations. The electrode is stable over a period of 4 months when stored in pure water in a stoppered container. With these results, efforts can now be directed at the development of analytical applications using ion channel sensors for other polyions, such as heparin, which is an anticoagulant that is used during surgery.

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