

Electrochemical Sensor for Electrochemically Inactive β -D(+)-Glucose Using α -Cyclodextrin Template Molecules

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We report an electrochemical sensor for an electrochemically inactive organic compound using a self-assembled monolayer (SAM) formed on the gold surface from a solution of thiolated α -cyclodextrin (α -CD). The SAM makes up an array of ultramicroelectrodes, which capture electroactive molecules such as those of ferrocene. When this SAM-modified electrode is exposed to a solution containing an electrochemically inactive compound, e.g., glucose, the captured ferrocene molecules are replaced by the electroinactive molecules via an equilibrium established between the two compounds, lowering the current for ferrocene oxidation. The decrease in current is directly proportional to the amount of glucose added and the glucose can be analyzed up to ~ 0.80 mM, which is $\sim 1/10$ of a typical physiological concentration in blood serum. Formation constants of the surface-bound α -CD molecules with ferrocene and glucose are also reported.

Self-assembled monolayers (SAMs) are becoming increasingly important in engineering molecules for preparation of chemical sensors and nanostructured materials. Chailapakul and Crooks¹ demonstrated the concept of molecular templates by preparing mixed SAMs on gold electrodes using solutions containing appropriate ratios of alkanethiols and 4-aminothiophenol (4-ATP). Islands of electroactive 4-ATP aggregates formed among electrochemically inert alkanethiol SAMs acted as an microelectrode array and showed the selectivity for molecular sizes, as well as ionic charges of electroactive materials. This concept was later refined using thiolated cyclodextrin (CD) molecules,² which have better defined cavity sizes compared to the 4-ATP aggregates formed as a result of a thermodynamic compromise between the 4-ATP and alkanethiol molecules. When thiolated α - and β -CD molecules formed SAMs on gold electrodes, they showed molecular size selectivity for electroactive compounds such as *p*-benzoquinone (BQ), 2-methylnaphthaquinone, and anthraquinone, which have varied molecular sizes. The CD SAMs thus prepared were also used as templates for the preparation of nanodots of semiconductor materials and nanowires of conducting polymers.^{3–7}

In this study, we describe an electrochemical sensor for an electrochemically inactive compound using the α -CD SAM prepared on a gold electrode as a molecular template. We use β -D-glucose as an analyte as it is electrochemically inactive within the potential range studied and also forms a strong complex with α -CD, because α -CD is just a cyclic oligomer made of six glucose units. The glucose molecule with a width and length of 5.6 and 6.5 Å, respectively, fits snugly into the α -CD cavity, which has a cavity diameter and depth of 5.7 and 7.8 Å, respectively.⁸ This was also why α -CD was chosen in this study over β -CD, which is slightly larger than the glucose molecule. When the SAM-modified gold electrode is exposed to a solution containing both electroactive ferrocene and electrochemically inactive glucose, the current for ferrocene oxidation is determined by the equilibrium established between these two compounds due to their competition to form insertion complexes with α -CD. With glucose present in solution, the decrease in ferrocene oxidation current is directly proportional to the amount of glucose, which provides a basis for the electrochemical glucose sensor. Formation constants of the surface-bound α -CD molecules with ferrocene and glucose have also been determined.

EXPERIMENTAL SECTION

Reagent grade α -CD (Aldrich), ferrocene (Aldrich), β -D(+)-glucose (Sigma), dimethyl sulfoxide (DMSO, Aldrich), decanethiol (Aldrich), pentanethiol (Aldrich), ethanol (J. T. Baker), sulfuric acid (Junsei Chem. Co. Ltd.), and hydrogen peroxide (Samchun Chemical) were used as received. Doubly distilled, deionized water was used for all experiments. The primary alcohol groups on the lower rim of α -CD were thiolated according to the procedure reported previously;⁹ the structure of a thiolated CD is shown in Figure 1.

A gold disk electrode (area 0.065 cm²) polished successively with 0.3- and 0.05- μ m alumina slurry (Fischer) was used as a working electrode after cleaning by sonication in a piranha solution (70% concentrated H₂SO₄ and 30% H₂O₂) for 10 min, followed by

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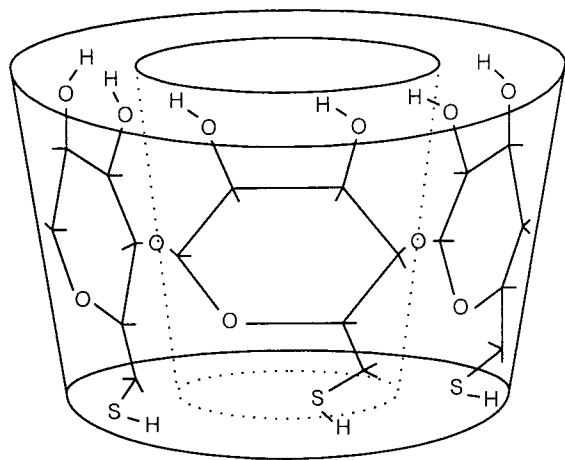


Figure 1. Structure of thiolated cyclodextrin (CD). α -CD has six glucose units.

washing with ethanol and water. The gold electrode thus prepared had a roughness factor of ~ 3.2 , which was determined by measuring the cathodic charge required for reducing the gold oxide formed during the cathodic scan after oxidation of the gold surface by anodic scan to 1.5 V versus the Ag|AgCl (in saturated KCl) electrode. The cathodic charge is estimated to be $430 \mu\text{C}/\text{cm}^2$ for reducing the gold oxide thus formed for an atomically flat surface, assuming a monolayer coverage. The roughness factor measured for the gold electrode used for quartz crystal analysis was measured to be 1.7. The electrode was electrochemically cleaned by potential cycling more than 10 times between -0.30 and 1.60 V versus the Ag|AgCl electrode in $0.50 \text{ M H}_2\text{SO}_4$ solution. The electrode was then dried by blowing with dry nitrogen after washing with water. A platinum spiral wire and Ag|AgCl electrodes were used as counter and reference electrodes.

The α -CD SAM was prepared on the gold surface by dipping a gold electrode for 12 h in a solution prepared by mixing corresponding DMSO (thiolated α -CD) and ethanol (decanethiol and ferrocene) solutions to make up final concentrations of 0.10 mM thiolated α -CD, 0.050 mM decanethiol, and 0.10 mM ferrocene. Decanethiol was used to seal the defect sites that can be formed between CD molecules footed on the gold surface, and ferrocene molecules were used to protect cavities of CD molecules by forming insertion complexes. Without ferrocene present, decanethiol molecules may anchor on the gold surface exposed to the solution through the α -CD cavities, plugging the cavities and preventing them from acting as ultramicroelectrodes. The SAM used for the ellipsometric measurement was prepared in the same manner but with pentanethiol employed as a sealant for the defect sites instead of decanethiol, which has about the same height of $\sim 7 \text{ \AA}$ as that of α -CD molecules.^{8,10} The α -CD SAM thus prepared was washed thoroughly to remove ferrocene molecules captured inside the cavities and excess alkanethiol molecules hanging on the SAM surface. For quartz crystal analyzer (QCA) measurement experiments, however, α -CD alone was used in DMSO to form a SAM without decanethiol and ferrocene.

Electrochemical measurements were made using an EG&G PAR model 273 or 283 potentiostat–galvanostat. The amount of

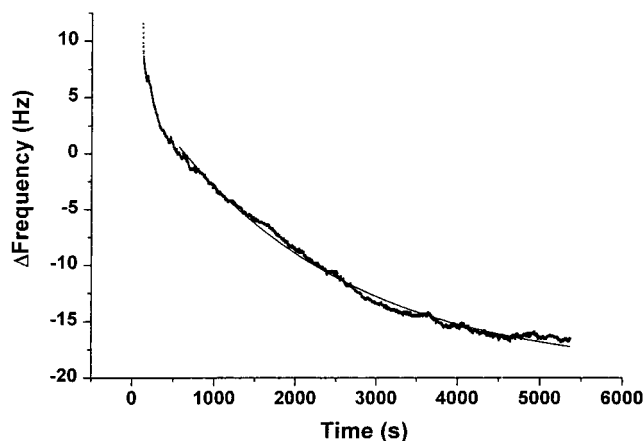


Figure 2. Decrease in frequency recorded during the adsorption of thiolated α -CD onto the gold surface in DMSO containing $50 \mu\text{M}$ thiolated α -CD. The area of the gold is 0.20 cm^2 .

CD molecules adsorbed on gold surfaces was measured with an EG&G/Seiko model 917 QCA using an AT-cut quartz crystal with a base frequency of 9 MHz. Ellipsometric measurements were made using a J. A. Woollam model M-44 ellipsometer on the α -CD SAM prepared on a 200-nm gold film on a glass by deposition with the electron beam, which already had a titanium layer of $\sim 10 \text{ nm}$.

RESULTS AND DISCUSSION

The amount of thiolated α -CD adsorbed on the gold electrode was measured by monitoring the decrease in frequency with the QCA after a solution of thiolated α -CD was injected to make up the final concentration of $50 \mu\text{M}$ for α -CD. Figure 2 shows the frequency decrease recorded as a function of time after injection of the α -CD solution in DMSO. The amount of α -CD was calculated to be $\sim 9.5 \times 10^{13} \text{ molecules}/\text{cm}^2$ from the total decrease in frequency, which is slightly larger than the amount corresponding to a monolayer coverage considering that the β -CD monolayer would have $6.8 \times 10^{13} \text{ molecules}/\text{cm}^2$ on an Au(111) surface. Fitting the data to the Langmuir adsorption isotherm gives an adsorption rate of $6.2 \text{ M}^{-1} \text{ s}^{-1}$, which is much slower than that reported for β -CD.² We believe this is because the α -CD molecules have a higher solubility than β -CD.^{8,10}

Corroborating evidence for the formation of slightly thicker films than a monolayer was also obtained from the ellipsometric measurement of the film thus formed. The average film thickness measured by ellipsometric measurement was 11.2 \AA , which is $\sim 40\%$ higher than the expected molecular height of α -CD, $\sim 7.8 \text{ \AA}$. This result, along with the weight measurement described above, indicates that a self-assembled film slightly thicker than a monolayer was obtained under the experimental conditions used here. We believe that additional α -CD might have been deposited on the monolayer, forming some aggregates. Under certain conditions, the aggregate formation was reported to be more severe than we report here, resulting in a multilayer film of as thick as three layers.²

Figure 3 shows a series of cyclic voltammograms (CVs) recorded for oxidation of ferrocene at the α -CD modified electrode. Well-defined SAMs were reported to have been formed at gold electrodes pretreated similarly to the one used in this study,

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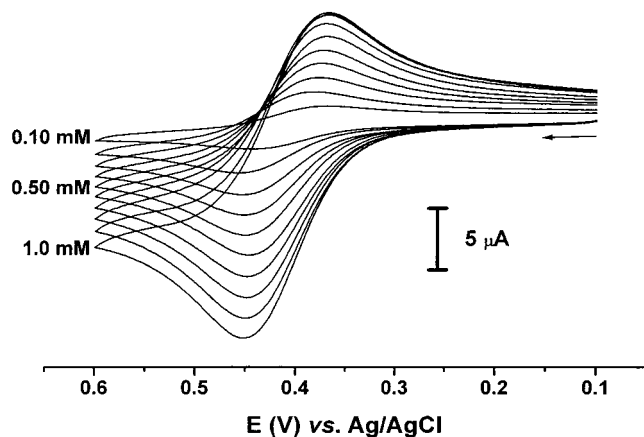


Figure 3. A series of CVs recorded at 100 mV/s at various ferrocene concentrations (0.10–1.0 mM) in in water–ethanol (= 9/1 v/v) solution containing 0.20 M NaClO₄ as supporting electrolyte.

although the roughness factors for similarly prepared gold electrodes would be expected to be ~ 3.2 as was the case for the electrode used for this study (see Experimental Section).^{1–7} Recently, we also observed formation of well-defined SAMs of various thiols on the same electrode as was used in this study.¹¹ Thus, the SAM on the gold electrode acts as ultramicroelectrodes whose exposed areas would be $\sim 24 \text{ \AA}^2$ with its diameter of $\sim 5.5 \text{ \AA}$. The ultramicroelectrodes also act as hosts for the guests, ferrocene molecules (see below).

We used ethyl alcohol as a solvent for these measurements to be able to dissolve ferrocene to a desired concentration. The CVs recorded for ferrocene at the α -CD-modified electrodes show that the electron-transfer reaction is reversible with CV peak separations of $\sim 60 \text{ mV}$ under the experimental conditions employed here. The anodic peak current was directly proportional to the ferrocene concentration up to 1.0 mM, while the cathodic peak current tended to level off from $\sim 0.80 \text{ mM}$. We believe this is because the ferrocenium ions produced upon anodic scan are rejected from cavities at higher concentrations because of their positive charges. While the oxidized products are rejected from the cavities, neutral reductants (ferrocene) diffuse in to the cavities from the solution and the increase in anodic current is maintained at even an increased concentration. The relatively hydrophobic CD cavities may be capable of trapping neutral ferrocene molecules up to 1.0 mM, whereas only a limited amount of ferrocenium ions can stay inside the cavities due to the hostile environment inside the cavity.

The $\log(I_p)$ versus $\log(\nu)$ plot (not shown) obtained from the data scan rate dependency in Figure 3, where ν is the scan rate, had a slope of 0.70, indicating that the electrochemical oxidation of ferrocene at the α -CD-modified electrode displays a mixed behavior for both diffusion- and the surface-controlled processes. In an ideal situation, a diffusion-controlled process would show a slope of $1/2$ while the surface-controlled reactions would have a slope of 1.0 for the $\log(I_p)$ versus $\log(\nu)$ plot. This suggests that the ferrocene molecules are fixed inside the cavities in contrast to *p*-BQ, which shows a diffusion-controlled behavior on a β -CD covered gold electrode.² However, the diffusion- and surface-controlled processes reach equilibrium as the formation constant

between ferrocene and α -CD is not large enough (see below). Also, the fact that the ferrocenium ion produced upon oxidation would be released from the cavities due to its hydrophilic nature inside the hydrophobic cavity might have affected the scan rate dependency as well, as already pointed out above. These observations indicate that *p*-BQ molecules access and leave the β -CD cavities relatively freely by diffusing in and out of the CD cavities, whereas the cyclopentadienyl ring of the ferrocene molecule is captured more strongly inside the α -CD cavities forming insertion complexes. The *p*-BQ is reported to form an insertion complex with α -CD in solution with a formation constant of ranging from 8 to 24.^{12,13} At a low *p*-BQ concentration, the complexation is not strong enough to show the surface-bound behavior during its reduction.

In efforts to study how strongly the ferrocene and glucose molecules would interact with α -CD molecules adsorbed on the gold surface, an electrochemical technique was used.^{14,15} When an electroactive guest molecule, i.e., ferrocene (Fc), forms a host–guest complex with a host molecule adsorbed on the electrode surface, the following relation has been derived from the Langmuir isotherm,^{14,15}

$$\frac{[\text{Fc}]}{I_p} = \frac{1}{K_f C} + \frac{[\text{Fc}]}{C} \quad (1)$$

Here I_p is the CV peak current at a guest concentration (ferrocene), C is a constant, and K_f is the formation constant. Of course, the host molecule here is α -CD adsorbed on the electrode surface while the electroactive guest molecule is ferrocene in solution.

When an electrochemically inert molecule such as glucose forms a stronger complex with the host, it will replace the electroactive guest molecule, lowering the current due to the oxidation or reduction of the electroactive guest. The difference in current, ΔI , observed in the presence and absence of the electrochemically inert molecule is related to its formation constant with the host molecule on the surface via the following equation,^{14,15}

$$\frac{[\text{Glu}]}{\Delta I} = A \left\{ \frac{K_f [\text{Fc}] + 1}{K_{\text{Glu},f}} + [\text{Glu}] \right\} \quad (2)$$

where

$$A = \frac{1 + K_f [\text{Fc}]}{c[\text{CD}]_0} \quad (3)$$

Here $K_{\text{Glu},f}$ is the formation constant of the host molecule with glucose, $[\text{CD}]_0$ is the concentration of the host molecules on the gold surface, which is constant under the experimental conditions used here, and c is the current per 1 mol of ferrocene molecule. Equation 1 allows the formation constant K_f to be determined from

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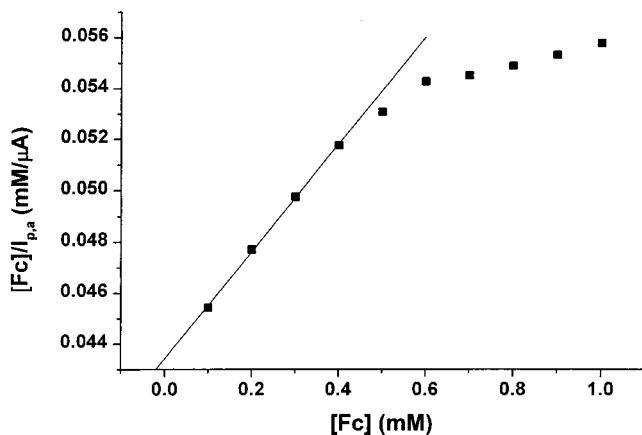


Figure 4. $[Fc]/I_{pa}$ vs $[Fc]$ plot. The currents used here were obtained in water–ethanol solutions containing various ferrocene concentrations at a scan rate of 100 mV/s.

the $[Fc]/I_p$ versus $[Fc]$ plot, and similarly, the formation constant of α -CD with glucose $K_{Glu,f}$ can be obtained from eq 2 by appropriately plotting the experimental data.

Figure 4 shows a $[Fc]/I_p$ versus $[Fc]$ plot for the ferrocene– α -CD complex according to eq 1. It is seen in Figure 4 that the $[Fc]/I_{pa}$ values for ferrocene concentrations level off from ~ 0.60 mM ferrocene, suggesting that more ferrocene molecules are present than is needed by the stoichiometry of the host–guest complex reaction. This agrees with the discussion made above with respect to the concentration dependency for the reversal peak of ferrocene oxidation. From the linear part shown in Figure 4, a formation constant between the α -CD and ferrocene complex is calculated to be 484 M^{-1} . This is very similar to the formation constant of 493 M^{-1} , which was obtained between α -CD and ferrocene, both in a $\text{D}_2\text{O}-\text{C}_2\text{D}_5\text{OD}$ ($= 9/1 \text{ v/v}$) mixed solvent, by measuring the chemical shift of the pentadienyl proton in the nuclear magnetic resonance (NMR) spectra taken in the presence of different amounts of α -CD.¹⁶

Similarly, from the plot according to eq 2 (not shown), the formation constants of glucose with immobilized α -CD are calculated to be 1640 M^{-1} in water–ethanol ($= 9/1 \text{ v/v}$) solution containing 0.10 mM ferrocene and 0.20 M NaClO_4 . Here, we reduced the amount of ethanol to increase the concentration of glucose at a relatively low ferrocene concentration. Unfortunately, we were not able to measure the formation constant for the complex formed between α -CD and glucose by the NMR method, because the chemical shifts for the protons of both compounds were so similar. This is readily expected from the fact that α -CD is just the cyclic oligomer of glucose.

As seen from this result, glucose forms a significantly stronger complex with α -CD than ferrocene does, although the ferrocene– α -CD complex would be stronger, while the glucose– α -CD complex would be weaker, in the mixed solvent than in ethanol. Thus, glucose in solution is expected to replace ferrocene captured in the α -CD cavities. While it is generally true that the formation constant of a complex between an organic compound and CD molecules would be larger in aqueous media than in organic

solvents, there are cases reported in the literature in which the formation constant is larger in DMF than in water.^{17,18} The formation constant measured for the complex between β -D-glucose and α -CD, both dissolved in water, is reported to be 36 and 447 M^{-1} ,^{19,20} both of which are significantly smaller than the ones we obtained from our experiments. We believe this is because (1) the solubility of glucose is significantly smaller in water–ethanol mixed solvent than in water, which would make association process more favorable, and (2) we already have decreased the entropy of the system by immobilizing the α -CD on the gold surface, making a further decrease in entropy of the reaction easier. Similar results had been reported for complexes formed between *o*- and *p*-methyl red and α -CD in the solution phase and on the surface,¹⁴ as well as complexes for phthalic acid esters and α -CD.¹⁵ Only a few formation constants have been reported thus far, however, for insertion complexes formed between immobilized α -CD and guest molecules, while many formation constants have been reported for the complexation reaction in solution phase.^{12,13,19–21}

Figure 5 shows a series of CVs recorded in the presence of various amounts of glucose with the concentration of ferrocene kept constant at 0.10 mM in water–ethanol ($= 9/1 \text{ v/v}$) solution containing 0.20 M NaClO_4 . It is seen clearly that the CV currents decrease when the concentration of glucose is increased in solution. The CV currents recorded as a function of the glucose concentration are described by an equation,

$$I_p = 2.91 (\pm 0.05) - 3.55 (\pm 0.13)[\text{Glu}] \quad (4)$$

where I_p is in microamperes and $[\text{Glu}]$ is in millimolar. The linearity is good, but the glucose population becomes almost saturated in the α -CD cavities at concentrations higher than ~ 0.80 mM, resulting in a low current. Thus, the highest concentration that can be determined by this method is a little higher than $1/10$ of the physiological concentration in blood serum.

We attempted to analyze the glucose concentration in an actual sample; the sample should be diluted to $1/10-1/20$ to secure that the current falls in the measurable range. The blood sample was taken from one (S.J.C.) of the authors a few hours after the lunch, and the blood plasma was separated from the blood serum by centrifuging the sample in a test tube for 30 min. A 1.00-mL aliquot was placed in a 10.00-mL volumetric flask and diluted to the mark after appropriate amounts of ferrocene and NaClO_4 were added to make up final concentrations of 0.10 mM ferrocene and 0.20 M NaClO_4 , respectively. CVs were then recorded using the same electrode as was used for the calibration curve. The glucose concentration in the blood was $4.8 (\pm 0.3) \text{ mM}$, which corresponds to $81 \pm 3 \text{ mg/dL}$. The blood sample gave a reading of the glucose concentration of 79 mg/dL when One Touch Ultra Blood Glucose Monitoring System (Lifescan Canada, Ltd., a subsidiary of Johnson & Johnson) was used.

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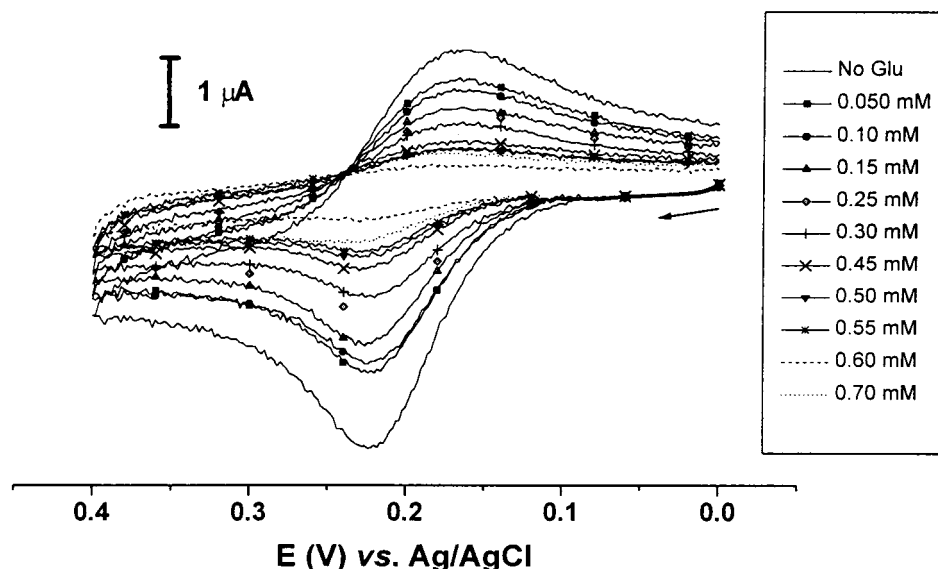


Figure 5. CVs recorded for ferrocene oxidation as a function of the added glucose concentration in water–ethanol solution containing 0.10 mM ferrocene and 0.20 M NaClO₄. The scan rate was 100 mV/s.

CONCLUSION

We have demonstrated in this work that an electrochemical sensor can be assembled for an electrochemically inactive organic compound by using an appropriate CD molecular template on a gold electrode. The sensor we demonstrated in this work employing glucose as an example for the electrochemically inactive compound is based on the molecular template prepared from thiolated α -CD because the glucose molecules fit right into the template molecules and compete well with the electrochemically active compound, ferrocene, for the formation of an insertion complex. The glucose sensor we constructed in this work offers a novel concept of how an electrochemically inert organic compound can be analyzed and also a molecular size selective sensor can be designed. For other electrochemically inert organic compounds whose molecular sizes are larger than that of glucose, other template molecules such as various sizes of CDs, calixarenes, and cucurbiturils may be used. The biggest challenges for these cage- or basket-shaped compounds would be the thiolation

at appropriate positions of the molecules. However, the most important criteria for choosing an electroactive compound for a given analyte would be the formation constant as well as molecular sizes. Work along this line is in progress in our laboratory.

ACKNOWLEDGMENT

A grateful acknowledgment is made to the Korea Science and Engineering Foundation (KOSEF) for supporting this research through the Center for Integrated Molecular System located at POSTECH and to the Ministry of Education of Korea for graduate stipends for B.-G.C. and S.-J.C. through the BK21 program.

Received for review July 16, 2001. Accepted February 21, 2002.

AC0107913