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SAND 2005-7590
Unlimited Release
Printed December 2005

Sensors for Cell Signaling Proteins

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Prepared by
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Sensors for Cell Signaling Proteins

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Abstract

Thiolated cyclodextrins have been shown to be useful as modifiers of electrode surfaces for application in electrochemical sensing. The adsorption of three different thiolated β-cyclodextrin (β-CD) derivatives onto gold (Au) electrodes was studied by monitoring ferricyanide reduction and ferrocene carboxylic acid (FCA) oxidation at the electrode surface using cyclic voltammetry. Electrodes modified with the β-CD MJF-69 derivative bound FCA within the CD cavity. The monolayer acted as a conducting layer with an increase in the oxidation current. On the other hand, the β-CD layer inhibited the reduction of ferricyanide at the electrode surface since ferricyanide is larger than the cavity of the β-CD derivative and thus unable to form an inclusion complex.

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Introduction

Much attention has been given to the design and synthesis of molecular based machines, shuttles, and molecular motors for use in drug delivery systems or as methods of building bio mimetic materials from a bottom-up approach. The synthesis of molecular assemblies enables new functions that cannot appear from a single molecule. Cyclodextrins are suitable candidates for such assemblies and have been used extensively for the construction of molecular based machines. Their rigid ring structure allows the ability to form complexes with small molecules (applicable to drug delivery) but in addition they have rotational symmetry and can undergo rotational and sliding motion (molecular shuttles). The host-guest complexation has also been explored between various cyclodextrins and ferrocene derivatives on electrode surfaces for sensor applications. Cyclodextrins are not electroactive however they do form inclusion complexes with suitable redox active guests based on the size of the ring structure. For example, cyclodextrin derivatives can bind ferrocene which, in the presence of a target molecule with a higher binding constant, can be displaced resulting in a decreased oxidation current for ferrocene.

In this late start LDRD, the adsorption of three different thiolated β-cyclodextrin (β-CD) derivatives onto gold (Au) electrodes was studied using cyclic voltammetry. The β-CD monolayer complexes small electroactive probes such as ferrocene and ferrocene carboxylic acid (FCA) but excludes the larger ferricyanide molecule. By monitoring the reduction of ferricyanide, the modified gold substrate can be characterized. Since the cavity of β-CD is smaller than ferricyanide, this layer acts as an insulating surface inhibiting electron transfer to the electrode causing a reduction in peak current. On the other hand, ferrocene and FCA complex with β-CD and now the monolayer acts as a conducting surface transferring electrons to the Au electrode.

This work focused on two objectives. First the characterization of the cyclodextrin film to determine if it effectively inhibited access of ferricyanide at the electrode surface. The second objective was to then characterize the host/guest interaction of the two smaller electroactive probes and determine the feasibility of using this complexation and release as an approach for electrochemical sensing of biological molecules. This work is still in its infancy but has received

additional funding in FY06 so that further development and characterization of electrochemically driven molecular complexes can be evaluated as electrochemical sensors.

Results

Three different thiolated β-CD derivatives (Figure 1) were used to modify Au electrodes. The thiolated β-CDs were bound to the surface via chemisorption of thiol groups to gold. Each β-CD differed in the length of the thiol linker and MJF103 was functionalized with ethylene glycol at the ring structure.

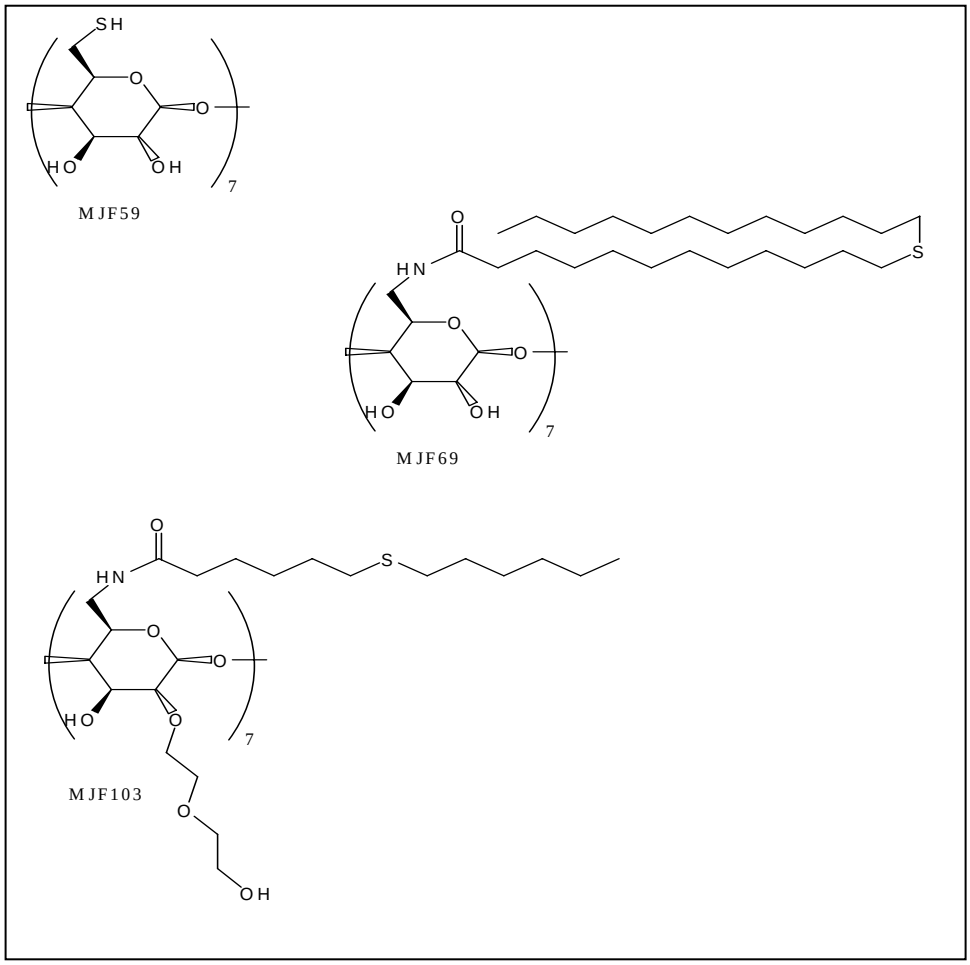

Figure 1: Three Thiolated β-Cyclodextrin derivatives using various surface and rim fuctionalization groups.

The adsorbed β-CDs were then tested for their ability to inhibit the access of ferricyanide to the electrode surface since ferricyanide is larger than the cavity of the β-CDs and is therefore

incapable of complexing with the β-CD host. For these studies 1 mM and 0.01 mM ferricyanide, in a 0.2 M KCl solution was used and the redox behavior of ferricyanide was monitored using potentiometric cyclic voltammetry. To monitor host-guest interactions, either ferrocene or FCA was used at concentrations of less than 1% since these two electroactive probes complex with β-CD and interact with the gold surface via the alkyl linker.

Characterization of β-CD derivative MJF-59

The first surface studied was that of a Au electrode modified with β-CD MJF-59.

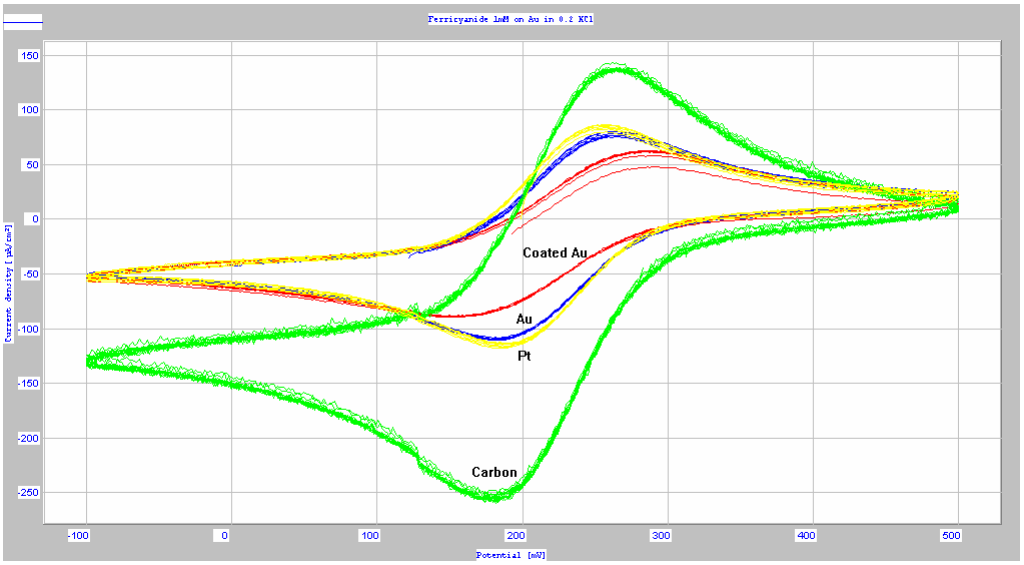

Figure 2: Multiple CV runs in 1.0 mM Ferricyanide/0.2 M KCl using various surfaces compared to a coated (MJF-59) Au surface.

Figure 2 shows the cyclic voltammograms of ferricyanide at the MJF-59 Au modified electrode and bare Au, Pt, and Carbon electrodes in 0.2 M KCl. Comparing the coated (red) and bare Au (blue) electrodes, there is a reduction in peak current although it is not completely inhibited indicating incomplete coverage of the surface by the β-CD monolayer. For the addition of FCA (Figure 3), an oxidation peak near +400 mV is present however only a small reduction current occurs at +200 mV (blue curve). This is compared to a CV scan from the same MJF-59 modified electrode in the absence of FCA (red curve) and a typical FCA voltammogram from a bare platinum electrode (pink curve). Although FCA is capable of complexing with the β-CD, and thus an increased oxidation current would be expected, the small current response could be

explained by a number of factors. First, FCA is only slightly soluble in water therefore the concentration is relatively low. Following the anodic scan, the ferrocenium ions produced are repelled from the β-CD cavity due to their charged state thus the cathodic peak could level off.

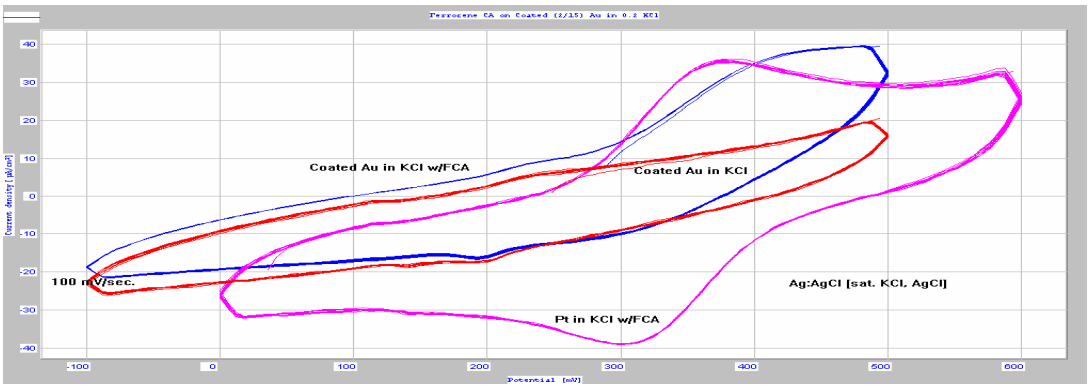

Figure 3: Two CV scans of <1 mM Ferrocene carboxylic acid in 0.2 M KCl from a coated (MJF-59) Au surface as compared to a polished Pt surface, and one CV scan of the coated Au surface in 0.2 M KCl supporting electrolyte.

In addition, since the MJF-59 did not assemble as a uniform monolayer, ferrocenium ion could react with the exposed Au, poisoning the electrode surface.

Characterization of β-CD derivative MJF-103

The β-CD derivative MJF-103 is functionalized with ethylene glycol at the cavity (see Figure 1). On the cyclic voltammograms carried out at the MJF-103 modified electrode, inhibition of electron transfer occurred in all cases using FCA, ferrocene, and ferricyanide. This can be explained by two possibilities either the ethylene glycol moieties sterically inhibited any interaction with the cyclodextrin cavity or the ethylene glycol complexed within the β-CD cavity (data not shown).

Characterization of β-CD derivative MJF-69

Assembly of MJF-69 gave complete coverage of the Au electrode. This was verified by complete inhibition of electron transfer in the reduction peak current of ferricyanide. Figure 4 shows two cyclic voltammetric scans; one showing the redox behavior of FCA (blue curve) and the other taken in the presence of ferricyanide after the FCA scan (red curve).

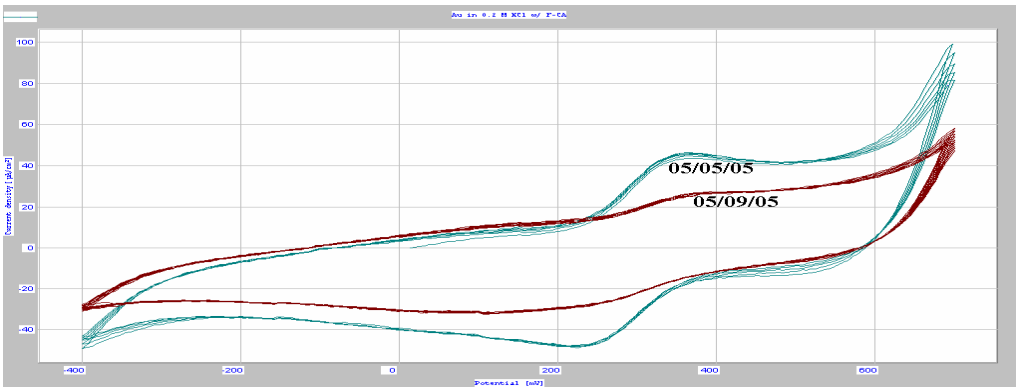

Figure 4: CV scan from a freshly deposited di-alkyl sulfide thiolated β-cyclodextrin (MJF-69) on an Au surface in FCA/0.2 M KCl (blue) compared to CV scan from the same coating in a 1 mM ferricyanide/0.2 M KCL (red), scan rates 100 mV/sec., vs. SCE [sat. KCl].

Finally the stability of these films after long term storage was evaluated by storing the modified electrodes in either DI water, 0.2 M KCl, or under dry nitrogen for 12 days. After this time cyclic voltammograms of FCA at the modified electrodes were obtained and are shown in Figure 5. Similar redox behavior occurs in all three conditions.

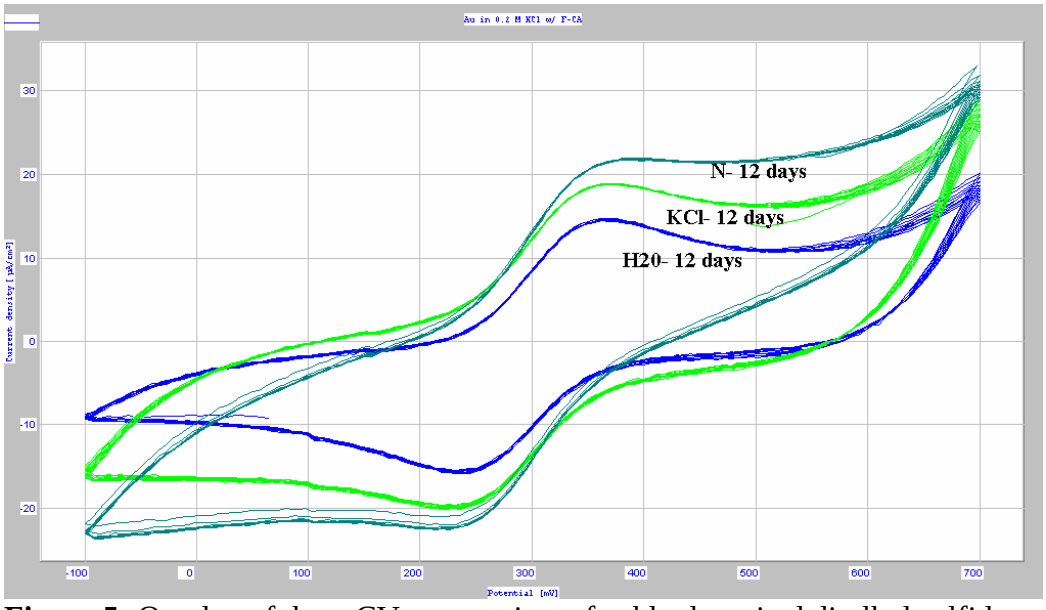

Figure 5: Overlay of three CV scans using a freshly deposited di-alkyl sulfide thiolated β-cyclodextrin (MJF-69) on a Au surface from FCA/0.2 M KCl after 12 days storage in various media. The blue scan resulted from exposing the coated Au surface in DI water, the green scan from storage in 0.2 M KCl, and the dark green scan from storage in dry nitrogen.

Conclusions

Three different β-CD derivatives were assembled on gold electrodes and where characterized based on their ability to inhibit access of ferricyanide to the electrode surface and to complex the small electroactive probe FCA. The MJF-69 modified gold surface proved to be a good insulating substrate for ferricyanide and a conductive substrate for FCA. This work will continue in FY06 under an existing LDRD focused on the development of sensor technologies for detection of cell signaling proteins. These films will be immobilized on different transducers and will be evaluated as possible recognition films for the target proteins.

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