Specific Binding of Concanavalin A to Glycolipid Monolayers on Gold Electrodes

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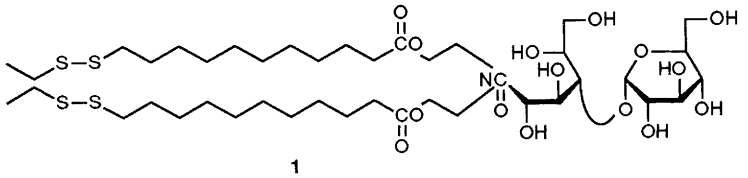
Formation of monolayers of disulfide-containing glycolipids by spontaneous adsorption onto gold electrodes and their specific binding properties to a lectin are described.

There has been considerable interest recently in self-assembled monolayers on solid substrates.[1–4] Our current interest has focused on the incorporation of functional groups such as polymerizable groups in the surface of monolayers on gold.[5] In this communication we describe the preparation of monolayer membranes of disulfide-containing glycolipids **1** and **2** on a gold surface *via* spontaneous adsorption and their specific binding properties to Concanavalin A (ConA), examined by an electrochemical method. ConA is well-known as a tetrameric protein with four carbohydrate binding sites, which specifically binds α-D-glucopyranosyl or α-D-mannopyranosyl moieties.[6–10] Recently Ringsdorf *et al.*, have re-



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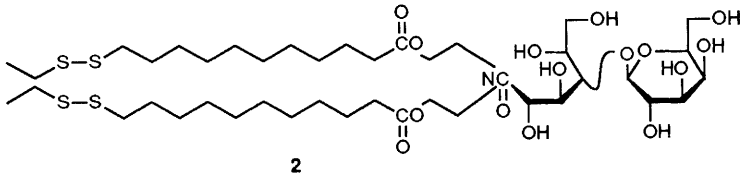

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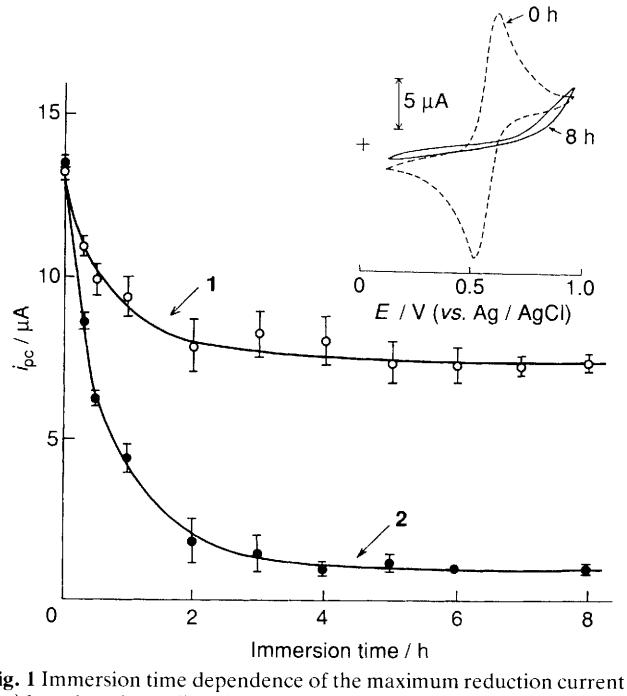

Fig. 1 Immersion time dependence of the maximum reduction current (i_{pc}) based on the cyclic voltammograms as is shown in the inset. After immersing the gold electrodes into an ethanolic solution of **1** (○) or **2** (●) (1 mmol dm^{-3}) for a prescribed period, the electrodes were mounted in a conventional three-electrode cell with 1 mol dm^{-3} $Fe(CN)_6^{4-}$ as the electroactive species and 1 mol dm^{-3} KCl as the electrolyte, and then the *i–E* curves were measured. Nitrogen was used for deaerating the solution. Measurement temperature 25 °C; scan rate, 100 mV s^{-1}; electrode area, 0.02 cm^2. The i_{pc} values are the average of four repeated runs. The error range is shown by bars.

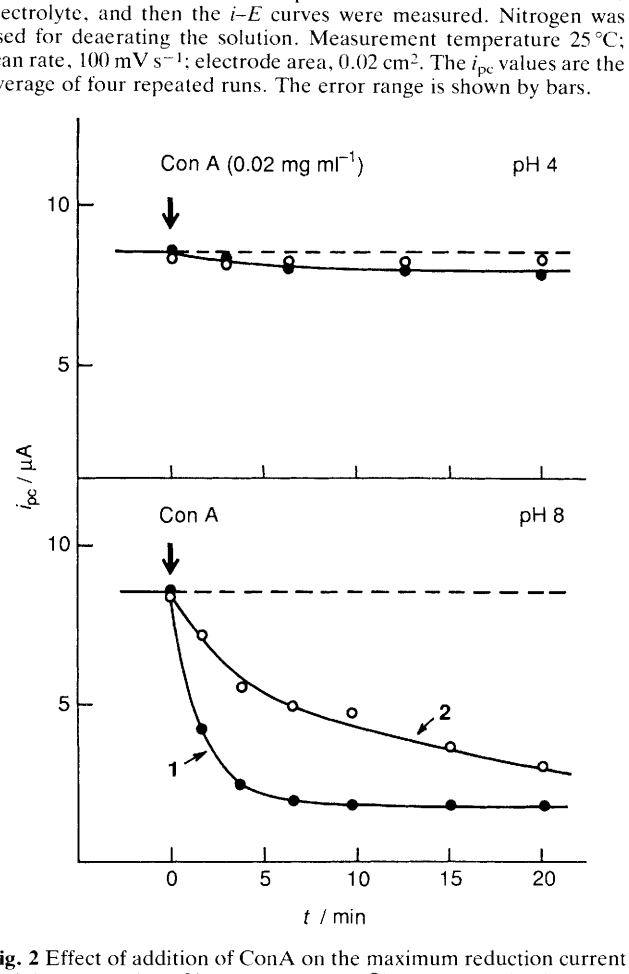

Fig. 2 Effect of addition of ConA on the maximum reduction current (i_{pc}) for **1**-modified (●) and **2**-modified (○) electrodes: (top) at pH 4 and (bottom) at pH 8. The cyclic voltammograms were measured for the electrolyte solutions in which a small amount of ConA (0.02 mg cm^{-3}) was contained. Other experimental conditions are the same as those in Fig. 1.

ported binding properties of a protein (Streptavidin) to the biotin-functionalized monolayers on gold through scanning tunnelling microscopy (STM) observation.[11]

The novel amphiphiles **1** and **2**, which contain α-D-glucopyranosyl-D-gluconamide and β-D-galactopyranosyl-D-gluconamide as the head group, respectively, were prepared as follows. 3-Azapentamethylene bis[(11-ethyldithio)undecanoate] was synthesized by condensation of 11-ethyldithioundecanoic acid, which was obtained by the reaction of ethyl ethanethiosulfinate[12] and 11-mercaptoundecanoic acid, with bis(2-hydroxyethyl)amine. Maltonolactone[13] or lactonolactone[13] was allowed to react with 3-azapentamethylene bis[(11-ethyldithio)undecanoate] to give the final compound **1**† or **2**,† respectively. Glycolipid monolayers were formed by immersing polished gold electrodes in ethanolic solutions of **1** or **2** (1 mmol dm^{-3}) at ambient temperature. Electrochemical measurements were carried out with a CV-1B cyclic voltammograph (BAS). All potentials were measured with respect to an Ag/AgCl (saturated NaCl) reference electrode.

Fig. 1 inset shows typical cyclic voltammograms for the **2**–gold electrode with 1 mmol dm^{-3} $Fe(CN)_6^{4-}$ as the electroactive species and 1 mol dm^{-3} KCl as the electrolyte. The *i–E* curve of the electrode exposed to a solution of **2** gives an apparent decrease in the redox current peaks compared with that for the bare electrode, indicating that the monolayer blocks the electrochemical communication of $Fe(CN)_6^{4-}$ with the electrode. In order to determine the condition under which steady-state monolayers are formed, the maximum reduction currents (i_{pc}) on the basis of the *i–E* curves were plotted against immersion time in Fig. 1, in which the data for **1** were included. The steady-state adsorption was reached after 2–3 h for both lipids. There was a significant difference in the i_{pc} value at the steady-state monolayer between the lipids, *i.e.* the i_{pc} value for **2** was much lower than that for **1**. The same trend was observed for the electrodes prepared by a horizontal deposition of Langmuir-monolayers of the lipids. Since the structural difference between the lipid molecules exists only in their hydrophilic portion, the difference in electrochemical barriers observed above should be ascribed to that in a steric bulkiness between the sugar residues of lipids, which might affect molecular packing of the monolayers in adsorption processes. In fact, the molecular area, estimated from the surface pressure–area isotherms on water, was larger for **1** (0.53 nm^2 $molecule^{-1}$) than for **2** (0.43 nm^2 $molecule^{-1}$) at a surface pressure of 30 mN m^{-1}, at which the monolayers were in condensed phase.

To examine the binding ability of ConA to these monolayers, two electrodes, modified with **1** or **2**, were prepared, which had the same i_{pc} value (*ca.* 8 μA) (Fig. 2). When ConA (0.02 mg cm^{-3}) was added to the electrolyte solutions at pH 4, neither a significant change in i_{pc} nor a difference between the lipids was observed, probably because ConA could not retain the tetrameric form at such a pH region.[14,15] In contrast, at pH 8, at which ConA is in a tetrameric form, the i_{pc} values markedly decreased by adding ConA, suggesting binding of it to the glycolipid monolayers. The initial binding rate of ConA, estimated by the extent of decrease in the i_{pc} value with time, is apparently different between the monolayers: the initial binding to the monolayer of **1**, carrying α-D-glucopyranosyl moiety is about four times faster than to the β-D-galactopyranosyl-functionalized monolayer **2**. This means that binding sites of ConA recognize and predominantly bind with the α-D-glucopyranosyl head group of monolayer **1**, and also shows good correlation with the specific binding ability of ConA.[6–10]

In conclusion, the lipid monolayer specifically responsive to a lectin (ConA) was formed on the gold electrodes.

† Satisfactory elemental analyses were obtained.

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