

# Mercaptopyridine Modified Single Crystal Gold Electrodes: Structures and Electron-Transfer Reactions of Metalloproteins

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In recent years, various functional electrodes for metalloprotein electrochemistry have been developing by many groups including the authors'.<sup>1-4</sup> In the present paper, surface structures of useful functional electrodes for metalloprotein electrochemistry are discussed.

Au(111) and Au(100) single crystals were prepared by the flame-annealing-quenching method developed by Clavilier et al.<sup>5</sup> In situ electrochemical STM measurements of modified electrode surfaces were carried out in a 0.05 M HClO<sub>4</sub> solution using a Nanoscope E and an electrochemically etched tungsten tip.<sup>6</sup> The tunneling current used was around 2 nA. Surface-enhanced IR absorption spectroscopy (SEIRAS)<sup>7</sup>, HR-HEELS, and impedance measurements were also used to evaluate the surface structures of the modified electrodes. Cyclic voltammetry of cytochrome c at a single crystal electrode was carried out at 25 °C using the meniscus (or hanging electrolyte) method.<sup>8,9</sup> Platinum plate (1.5 x 0.7 cm) was used as a counter electrode. An Ag/AgCl (saturated KCl) was used as a reference electrode. A BAS CV-50W electrochemical analyzer was used for electrochemical measurements. Horse heart cytochrome c (from Sigma) was purified further by ion exchange chromatography. 3-Mercaptopyridine and its disulfide were synthesized from 3-aminopyridine according to the literature<sup>10</sup> using improved procedures.

By using the atomically flat gold single crystal surfaces, since surface functions become much clear and simple, surface structures of various modified electrodes have been studied at the molecular level.<sup>6, 8, 9, 11-14</sup>

On a 4-PySH modified Au(111) electrode a well-defined voltammetric response of cytochrome c was observed.<sup>2, 8</sup> For preparation of a suitable 4-PySH modified surface, a very small amount of sulfide impurity in a 4-PySH-modifier solution should be taken into account.<sup>9</sup> The STM image for the 4-PySH modified Au(111) electrode showed the rectangular unit cell of a  $p(5 \times \sqrt{3}R-30^\circ)$  structure with an interaction of two neighboring 4-PySH.<sup>6</sup> On the other hand, the 2-PySH modified Au(111) electrode showed a  $p(4 \times \sqrt{7}R-40.9^\circ)$  structure with no dimer formation, suggesting that 2-PySH adsorbed at both thiolate S and pyridine N atoms.<sup>14,15</sup> The suggested structure was also confirmed by SEIRAS, HR-HEELS, impedance measurements and the electrochemical reductive desorption behavior.<sup>9, 11, 14</sup> On a 2-PySH modified electrode, poor electrochemical response of cytochrome c was seen as was on a thiophenol modified electrode. The 2-PySH modified surface has no pyridine nitrogen at the solution side, while the 4-PySH modified surface has pyridine nitrogen faced to the solution, through which cytochrome c in solution interacts with the electrode. Similar disulfide formation for 4-PySH ( $p(\sqrt{2}R-45^\circ \times 5R-53.1^\circ)$ )<sup>12</sup> and adsorption at both thiolate S and pyridine N atoms for 2-PySH ( $(\sqrt{2} \times 3\sqrt{2})R-45^\circ$ ) were also observed on 4-PySH and 2-PySH modified Au(100) electrodes, respectively. Again, no electrochemical response of cytochrome c was

observed on a 2-PySH modified Au(100) electrode. These results suggest that for the rapid electrochemical response of cytochrome c, the pyridine nitrogen faced to solution is preferable.

Electrochemical responses of cytochrome c on 3-mercaptopyridine modified Au(111) and Au(100) electrodes were examined, because the pyridine nitrogen of 3-PySH is expected to be oriented toward the solution when 3-PySH adsorbed on gold through the thiolate-Au interaction as is observed for other mercaptopyridines. Actually, 3-PySH modified Au(111) and Au(100) electrodes showed excellent voltammetric responses for cytochrome c. Clear electrochemical responses of cytochrome c were obtained in a wide pH region (3.5-9.0) on 3-PySH modified electrodes. High-resolution STM image of the surface structure of 3-PySH modified Au(111) electrode suggested a  $(6 \times \sqrt{3}R-30^\circ)$  structure.<sup>16</sup>

The observed surface structures obtained by modifying with mercaptopyridines were the same as those obtained by modifying with corresponding disulfides. Similar results were obtained on vacuum evaporated Au(111) single crystal film electrodes.

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