The Structure of Adenine Adsorbed at Sub-Saturation Coverage at Au(110)/Electrolyte Interfaces

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(Received 12 June 2008; Accepted 7 October 2008; Published 4 April 2009)

It is demonstrated using Reflection anisotropy spectroscopy (RAS) that at sub-saturation coverage adenine adsorbs on the Au(110)/electrolyte interface in a base-stacking configuration with the plane of the bases orientated vertically on the surface and with the long axis of the molecules parallel to the [110] direction. This orientation is the same as that determined for saturation coverage. We also show that RAS can be used to determine the adenine coverage of the Au(110) surface. [DOI: 10.1380/ejssnt.2009.225]

Keywords: Reflection spectroscopy; Adsorption kinetics; Gold; Biological molecules - nucleic acids; Low index single crystal surfaces; Metal-electrolyte interfaces

I. INTRODUCTION

In a previous study [1] we determined the three dimensional orientation of adenine adsorbed at saturation coverage at a Au(110)/electrolyte interface using reflection anisotropy spectroscopy (RAS). In the current work we show that at sub-saturation coverage adenine adopts the same orientation at the Au(110)/electrolyte as at saturation coverage and that RAS can be used to establish the fraction of the Au(110) surface covered by adenine.

II. EXPERIMENTAL

The experimental procedures have been described in detail earlier [1]. As in the previous work Au(110) single crystal surfaces were prepared by flame annealing and introduced into an electrochemical cell. Adenine was introduced into the cell in a solution of NaH₂PO₄, K₂HPO₄ phosphate buffer to yield concentrations of 0.1 µM, 0.5 µM, 20 µM and 100 µM at pH 7.1. Experimental results and a detailed analysis of the RAS spectra obtained with the 100 µM solution were presented earlier and the influence on the RAS profile of variations in the electrode potential were also described and analysed in detail [1]. In this work, we present results obtained with the 0.1 µM and 0.5 µM solutions.

RAS is a linear optical technique in which the difference of reflectivity at normal incidence of plane polarised light in two directions at right angles from the surface of a cubic material is measured. This geometry results in a cancellation of the bulk response by symmetry and RAS becomes a probe of the surface anisotropy. The technique has been reviewed [2] and has recently been applied to the study of amino acids [3], the DNA base cytosine and its monophosphate [4] and long sequences of single and double strand DNA adsorbed at Au(110)/electrolyte interfaces [5]. The RAS instrument used in this work was of the Aspnes design [6] and operated in the range 1.5 to 5.5 eV. The sensitivity of the instrument is known to fall off slightly beyond 5 eV. The measured RA signal is given by

\[
\text{Re}\left\{\frac{\Delta r}{r}\right\} = \text{Re}2 \left\{\frac{r_{[110]} - r_{[001]}}{r}\right\},
\]

where \(r_{[110]}\) and \(r_{[001]}\) are the reflectivities in the [110] and [001] directions in the (110) surface respectively and \(r/2\) is the average of these quantities.

In each experiment the RAS of the Au(110) crystal was recorded after the crystal had been inserted into the cell following flame annealing. The RAS technique is very sensitive to the morphology of the Au(110) surface [7-10] and there is some variation in the spectra obtained from the clean surfaces that probably arises from variations in the flame annealing procedure.

III. RESULTS

Figure 1(a) shows the RAS of the Au(110) surfaces prepared in two different experiments. The spectra are very similar giving confidence in the reproducibility of surfaces produced by flame annealing. Figure 1(b) shows the RAS obtained from the Au(110) surface following the addition of adenine in solutions of 0.1 µM and 0.5 µM. In each case, and also for the 20 µM and 100 µM solutions [1], the addition of adenine gives rise to an increase in the negative peak in the Au(110) RAS profile at 2.5 eV. Figure 2 shows the increase in the intensity of this negative peak with time following the addition of solutions of adenine of concentrations 0.1 µM, 0.5 µM, 20 µM and 100 µM to the electrochemical cell at pH7.1 with the Au(110) electrode held at 0.0 V. For all concentrations the RAS intensity increased to a maximum value with the rate of increase being determined by the concentration of the solution. The RAS results indicate that the coverage reached with the three highest concentrations are very similar, implying saturation, while that reached with the lowest concentration is significantly lower, a result which we interpret as arising from the exhaustion of the solution by adsorption on the crystal and on the walls of the electrochemical cell.

The adsorption of the adenine has very little effect on the RAS of the Au(110) surface below ~2.3 eV (Fig. 1(b)). However from 2.5 eV onwards the negative amplitude of the RAS is increased notably and beyond 3.5 eV the signal continues in a sequence of negative undulations rather

*This paper was presented at the 14th International Conference on Solid Films and Surfaces (ICSFS-14), Trinity College Dublin, Ireland, 29 June - 4 July, 2008.
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ISSN 1348-0391 © 2009 The Surface Science Society of Japan (http://www.sssj.org/ejssnt) 225
FIG. 1: (a) RA Spectra of Au(110), (b) RA Spectra of Au(110) + 0.1 µM (solid line) and + 0.5 µM (○) of 1 mM adenine at 0.0 V vs. SCE and (c) adenine spectra obtained by subtraction of the corresponding Au(110) spectra plus the RA difference spectrum from the 100 µM concentration experiment (×) [1].

than following the broad positive feature centred on 4.5 eV that appears in the spectrum of the clean surface. In addition to enhancing the amplitudes of the negative peaks that occur in the RAS of the clean surface at 2.5 eV and 3.5 eV the addition of the adenine gives rise to new broad negative peaks at 4.2 eV and 5.3 eV. The changes that the addition of adenine induces in the RAS of the Au(110) surface are more clearly brought out when the spectra of the clean surface is subtracted from the spectra of adenine on the Au(110) surface as shown in Fig. 1(c). The difference RA spectrum obtained in the earlier study of adenine adsorbed from the 100 µM concentrated solution is also shown in Fig. 1(c).

Important clues to the orientation of molecules adsorbed on surfaces can be obtained from the dependence of the RAS on the angle, θ, between the polarisation direction of the incident light and a crystal axis in the surface plane [2, 4]. We performed an angular rotation of the RAS obtained from adenine adsorbed from the 0.1 µM solution. The spectra are shown in Fig. 3 and it is important to note that the spectra collapse to zero across the whole spectral range for θ = 0° or 90° when the polarisation of the incident light is parallel to one of the crystal axes in the surface plane.

IV. DISCUSSION

The RAS profiles of the Au(110) surfaces shown in Fig. 1(a) are very similar to the RAS profile obtained in the earlier work [1] and to RAS profiles of Au(110) 2×1 surfaces obtained in other studies in both electrochemical [2, 11] and ultra high vacuum environments [12–15]. The interpretations of the origin of the spectral features in the RAS profile of this surface have been reviewed in detail [2].

The changes induced in the RAS of the Au(110) surface by an adenine solution (Fig. 1(b)) indicates that the increase in the strength of the RAS signal at 2.6 eV with time and with the concentration of the solution (Fig. 2)
can be explained by assuming that the adenine adsorbs on the Au(110) surface until a saturation coverage is reached or the solution is exhausted. The results of Fig. 2 indicate that saturation is achieved for the three highest concentrations studied and that the solution is exhausted for the lowest concentration studied. The volume of the cell was 50 ml which for the lowest concentration of 0.1 µM gives a total of $3 \times 10^{15}$ adenine molecules. The area of the Au(110) crystal was 0.5 cm² and if all the molecules in the weakest solution adsorbed on this surface this would give a very high density of 3 molecules per 5 Å². However it is likely that the adenine molecules would also reach and adsorb on the rear surface of the Au crystal reducing this estimate of the surface concentration by a factor of two. More importantly, given the large area of the cell, any adsorption on the cell walls would dramatically reduce this estimate of the surface density indicating a less than fully saturated surface and certainly bringing the estimated coverage to values comparable with the estimates of $\sim 42$ Å² for the area occupied by one adenine molecule on the gold surface [16–18]. We conclude that this exhaustion of the solution prohibits formation of a monolayer coverage.

The observation of a RAS profile from molecules adsorbed at a surface proves that the molecules adopt an anisotropic alignment at the surface. In the earlier work we showed that the optical response of the adenine molecule in this spectral range arises from three well-defined dipole transitions in the plane of the molecule that have significantly different orientations with respect to the axes of the molecule [1]. As explained earlier [1, 4] if a molecule has at least two strong optical transitions that are orientated in significantly different directions in the plane of the molecule with respect to the molecular axes then the observation that the RAS of the molecule adsorbed on the Au(110) surface cell goes to zero across the whole spectral range at $\theta \sim 0°$ or 90° establishes that the molecule is orientated essentially vertically on the Au(110) surface with the optical axes of the molecule coincident with those of the Au(110) surface which are along the [001] and [110] directions. Consequently the results of Fig. 3 establish that the adenine molecules adsorbed on the Au(110) surface from the 0.1 µM solution adopt a vertical orientation on the surface with their optical axes coincident with the optical axes of the Au(110) surface. A similar conclusion was found in the earlier study of the RAS observed from adenine adsorbed from the 100 µM solution [1]. Furthermore the analysis of the RAS profile observed from adenine adsorbed from the 100 µM solution in terms of a three phase Fresnel model [2, 4, 7, 19–21] established that the adenine molecules were oriented on the Au(110) surfaces with the long axis of the molecule along the [110] direction. A similar analysis of the RAS profiles obtained following adsorption from the 0.1 µM and 0.5 µM concentrated solutions (Fig. 2) reaches the same conclusion. A comparison of the RAS observed from the 100 µM concentrated solution and a similar concentration of adenosine 5’-monophosphate established that the adenine adsorbs through the N(7) atom (Fig. 4) [1] and the similarity of these spectral profiles with those obtained from the 0.1 µM and 0.5 µM concentrated solutions (Fig. 1) establishes that this is generally the case.

In the earlier work we concluded that at saturation coverage the adenine molecules formed a based stacked monolayer on the Au(110) surface with an orientation determined by the geometry of the substrate [1]. We also showed that the RAS profile of the monolayer and in particular the relative intensity of the two broad negative peaks at 4.2 eV and 5.3 eV is very sensitive to the orientation of the molecules in the plane normal to the surface, i.e. the angle $\alpha$ in Fig. 4. The relative intensity of the two high energy features in the RAS obtained from adsorption from the 100 µM solution is consistent with the molecule adsorbing through the N(7) atom and also through the NH$_2$ group, a result that agrees with the conclusion of Xiao et al. [22] from Surface Enhanced Raman Spectroscopy of adenine adsorbed on polycrystalline Au.

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We now draw attention to the fact that the RAS profiles observed from adenine adsorbed from both concentrations studied in this work differ from each other and from the profile reported in the earlier work (Fig. 1(c)). These differences arise in the region of the spectrum dominated by the broad peaks at 4.2 eV and 5.3 eV that are sensitive indicators of the orientation of the molecules on the surface. The results shown in Fig. 3 establish that the molecules adsorbed on the Au(110) from the 0.1 µM solution are oriented vertically on the surface with optical axes coincident with those of the Au(110) substrate. Thus at both saturation and subsaturation coverages the adenine molecules adopt a similar vertical orientation on the Au(110).

We now consider the difference between the RAS profiles obtained from the 0.1 µM and 0.5 µM concentrated solutions (Fig. 1). Since the latter corresponds to a saturation coverage of adenine while the former results from a sub-saturation coverage, the likely explanation for this difference in profiles is that at sub-saturation coverage there is a significant contribution to the RAS profile from areas of the Au(110) surface that are not covered by adenine. If this is so, and if the orientation of the adenine is the same at saturation and sub-saturation coverage, which would be expected if the molecules adsorb by forming rows
of close stacked bases, then it should be possible to represent the observed RAS profile at sub-saturation coverage as a linear sum of the profiles of the clean Au(110) surface and the RAS profile obtained at saturation coverage, i.e. RAS of \((\text{Au}(110) + 0.1 \mu \text{M adenine}) = A^*\{\text{Au}(110)\} + B^*\{\text{Au}(110) + 0.5 \mu \text{M adenine}\}\). \(A\) and \(B\) are independent multiplicity factors where \(A = 0.07\) and \(B = 0.9\).

The bottom dashed line is the experimental intensity ratio of the 4.2 eV and 5.3 eV transitions is expected to vary with the angle normal to the surface, the angle \(\alpha\) (Fig. 4) and the geometry of the dipole transitions with respect to the molecular axes (the angle \(\phi\) in Fig. 4) and the geometry of the RAS experiment made it possible to predict how the relative intensity of the 4.2 eV and 5.3 eV peaks in the RAS profile varied with the rotation of the molecule in a plane normal to the surface, the angle \(\alpha\) in Fig. 4. This information is captured in Fig. 6 in which the middle curve shows how the ratio of the intensity of the 4.2 eV and 5.3 eV transitions is expected to vary with the angle \(\alpha\). The bottom dashed line is the experimental intensity ratio of these two features in the RAS profile obtained from the 100 \(\mu\)M solution. This line intersects the curve at \(\alpha = -35^\circ\) and \(+45^\circ\) the former value being consistent with the expectation from the work of Xiao et al. [22] that the NH\(_2\) group will touch the Au surface (Fig. 4).

The relative intensity of the 4.2 eV and 5.3 eV transitions given by fitting the RAS profile obtained from the 0.5 \(\mu\)M solution using the procedure described earlier [1] is 2.81 and this ratio is shown by the upper dotted line in Fig. 6. This line intersects the middle curve at \(+20^\circ\) and \(+30^\circ\). We conclude that a rotation of the molecule in a plane normal to the surface from \(\alpha = -35^\circ\) to \(+20^\circ\) or \(+30^\circ\) could explain the difference in the RAS profiles observed from the 0.5 \(\mu\)M and 100 \(\mu\)M concentrated solutions. Unfortunately one cannot be certain of this conclusion since the uncertainties in the relative intensity of the various contributions to the optical response of the adenine molecule place very large error bars on the accuracy of this calculation. These error bars are represented by the higher and lower curves in Fig. 6. However it is reasonable to expect that slight changes can occur in the orientation of molecules at saturated coverage’s caused by the drive to increase the saturated coverage since RAS has been shown to be sensitive to similar changes in the orientation of stacked planes of 9-anthracene carboxylic acid (9ATC) on the Cu(110) surface [24].

V. SUMMARY

It has been shown that adsorption from a 0.1 \(\mu\)M concentrated solution gives rise to a sub-saturation adenine coverage of the Au(110) surface. The variation of the RAS intensity with the polarisation direction, \(\theta\), of the incident light establishes that at sub-saturation coverage the adenine molecule is oriented vertically on the Au(110) surface with the optical axes of the molecules coincident with those of the Au(110) substrate.

It is possible to simulate the RAS profile obtained from the 0.1 \(\mu\)M concentrated solution by combining the profile obtained from the 0.5 \(\mu\)M solution with that of the bare Au(110) surface. The success of the simulation establishes that at sub-saturation coverage the surface is composed of 7% bare Au(110) and 93% adenine on Au(110) and that the molecules adsorbed from the 0.5 \(\mu\)M concentrated so-
olution are also orientated vertically on the surface. The difference in the spectral profiles of the RAS obtained from the 0.5 µM and 100 µM concentrated solutions could arise from a rotation of the molecules in a plane normal to the surface from $\alpha = -35^\circ$ to $+20^\circ$ or $+30^\circ$ though the uncertainties in the relative intensity of the optical transitions of the adenine molecule place very large error bars on this estimate.

Acknowledgments

This work was supported by the UK EPSRC. M. C. Cuquerella acknowledges the support of the EU through a Marie Curie Fellowship. C. I. Smith and M. C. Cuquerella also acknowledge support from the UK North West Development Agency through the North West Science Fund.

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