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Short communication

Evidences of adenine–thymine Interactions at gold electrodes interfaces as provided by in-situ infrared spectroscopy

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ABSTRACT

The co-adsorption of complementary DNA bases adenine and thymine on gold thin-film electrodes from 0.1 M $HClO_4$ solutions in H_2O and D_2O is studied by surface-enhanced infrared absorption spectroscopy in the attenuated total reflection mode (ATR-SEIRAS). The comparison of the spectra in the range 1750–1550 cm^{-1} for co-adsorbed adenine and thymine at controlled potentials to those of the individual adsorbed bases shows the enhancement of the signals associated to the vibration modes of adenine and the inhibition of those of thymine. The results can be explained by invoking the rearrangement of both molecules on the electrode surface in order to facilitate the Watson–Crick (W–C) and/or Hoogsteen (HG) interactions between the bases. The co-adsorption seems to be a cooperative process in which a low surface concentration of each base can induce the rearrangement of the complementary base molecules on the surface.

1. Introduction

Adenine and thymine are complementary nucleic acid bases that provide replication and transcription of the genetic codes in living cells by double H-bonding either in the Watson–Crick (W–C) [1] or the Hoogsteen (HG) [2] configurations (Fig. 1a). The interactions between DNA bases play decisive roles also in enzymatic and protein organization processes.

The study of the interactions of these bases at the organized electrode interfaces can be interesting for understanding their activities in biological media and for biotechnology and nanotechnology applications.

In-situ infrared spectroscopy can provide evidences about the disposition of the molecules on the surface, the coordination sites with the metal and the intermolecular interactions. Particularly, surface-enhanced infrared absorption spectroscopy in the Kretschmann attenuated total reflection (ATR-SEIRAS) mode allows high sensitivity studies of adsorbed molecules with little interference from the solution [3].

We have studied adenine adsorption on gold electrodes in previous papers where an adsorption model was proposed consisting of slightly tilted adenine molecules coordinated to the metal by the N atoms of the amino group and by the N_7 atom [4–7]. On the other hand, for thymine adsorption on gold electrodes several adsorption states have been described depending on the applied potential [8–10]: a chemisorbed phase, at high potentials, in which the molecules coordinate to the metal with both oxygen atoms and a deprotonated N_3 atom in a vertically organisation, stabilized by π-stacking interactions and a condensed but weakly adsorbed adlayer stabilised by H-bonds, at lower potentials. Interestingly, the same atoms of adenine and thymine involved in the chemical interactions with the metal would also be participating in the W–C and HG interactions: $N_{10}H$ and N_1 (W–C interaction) or N_7 (HG interaction) of adenine and the oxygen on C_4 and N_3H of thymine.

In this paper the advantages of ATR-SEIRAS are explored in order to decide about the interactions of co-adsorbed adenine and thymine molecules on gold thin-film electrodes in acid media as compared to the separated adsorption of the two bases. Experiments were carried out both in water and deuterium oxide solutions and the spectra for deuterated and non-deuterated adsorbed species analysed.

2. Experimental

Working solutions were 0.1 M $HClO_4$ (Merck Suprapur) in Purelab ultra water or in D_2O (Sigma Aldrich 99.96%). Adenine and thymine (Sigma–Aldrich) were used as received. Adenine and thymine solutions with concentrations ranging from 1×10^{-5} to 1×10^{-3} M were prepared by spiking 1×10^{-2} M stock solutions made in the same supporting electrolyte. Solutions were deareated by bubbling argon.

The voltammetry experiments were performed with Au(111) single crystal electrodes prepared and cleaned as indicated in [6]. In the ATR-SEIRAS experiments, gold thin-film electrodes (ca 25 nm thick) were used, prepared by argon sputtering on one of the sides of a silicon prism bevelled at 60° (Pastec, Japan). Deposition rate was 0.01 nm s^{-1}. A gold foil and a reversible hydrogen electrode were used as counter and reference electrodes, respectively. All potentials are referred to the saturated calomel electrode.

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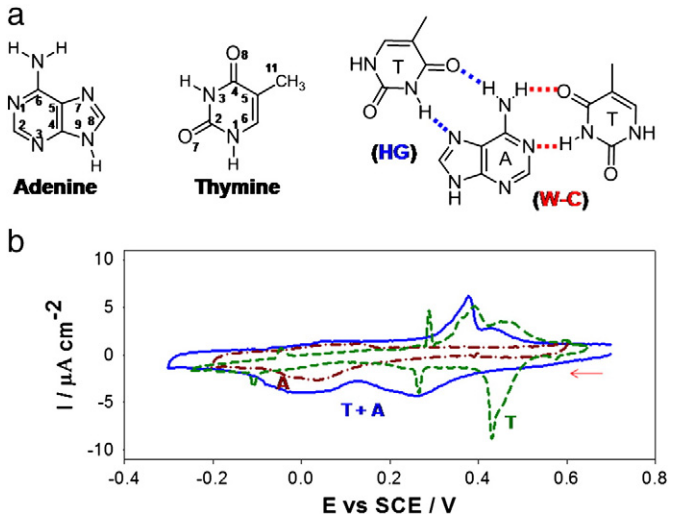

Fig. 1. a): Adenine and thymine molecules and W–C and HG interactions. b): Voltammograms obtained in (T) 1 mM thymine (- - -), (A) 0.01 mM adenine (-.-.-) and (T + A) 1 mM thymine + 0.01 mM adenine (——) 0.1 M $HClO_4$ solutions. v = 0.1 V/s.

Infrared experiments were performed in the Kretschmann configuration using a Nicolet 6700 spectrometer, equipped with a narrow-band MCT-A detector. Spectral resolution was 4 cm^{-1}. 100 interferograms were accumulated in the single-beam experiments at each potential. Background spectra were registered under the same conditions either in the base-free supporting electrolyte or before de addition of the second base. The spectra are presented as the ratio $-\log(R_2/R_1)$, where R_2 and R_1 are the reflectance values corresponding to the sample and reference single-beam spectra, respectively. A CHI Instrument potentiostat was used for potential control.

The gold electrode surfaces were treated in order to get the lifting of the reconstruction of the (111) domains in the supporting electrolyte by slowly scanning the potential from −0.1 V to 0.7 V, at which they are kept for a few minutes. The two bases were added at electrode potentials at which both can chemically adsorb.

3. Results

3.1. Voltammetry results

Typical voltammograms obtained in thymine solutions (Fig. 1b) show a peak at 0.440 V corresponding to chemical desorption, followed by characteristic transition of phase couple of peaks, which delimit the potential region corresponding to a condensed layer of physically adsorbed thymine [8]. The voltammograms in adenine solutions show that this base remains chemically adsorbed in a wider potential range, the desorption peak appearing around 0.060 V. Addition of adenine to chemically adsorbed thymine induces the shift of thymine desorption peak to lower potentials and the disappearance of the transition of phase signals.

3.2. ATR-SEIRAS spectra in deuterium oxide

Some results are given in Fig. 2a and b. The more significant signals in the infrared spectra of adenine and thymine appear in the region 1750–1450 cm^{-1}. Chemically adsorbed deuterated adenine shows a surface active signal around 1650 cm^{-1}, assigned to a skeletal stretching mode within the pyrimidine ring [11] while the spectrum of chemically adsorbed deuterated thymine provides three signals at 1649, 1581 and 1567 cm^{-1} related to the stretching modes of the $C_2 = O$, $C_4 = O$ and $C_5 = C_6$ double bonds [12,13]. At lower potentials, at which thymine is physically adsorbed the double-bond stretching modes of this molecule are not surface active because of the flat orientation of the molecular plane on the electrode [8–10].

Two sets of experiments have been performed in order to compare the spectra of co-adsorbed deuterated bases to those of the individual adsorbed bases, each experiment starting with a different base. The effects of adding adenine to adsorbed thymine are illustrated in parts

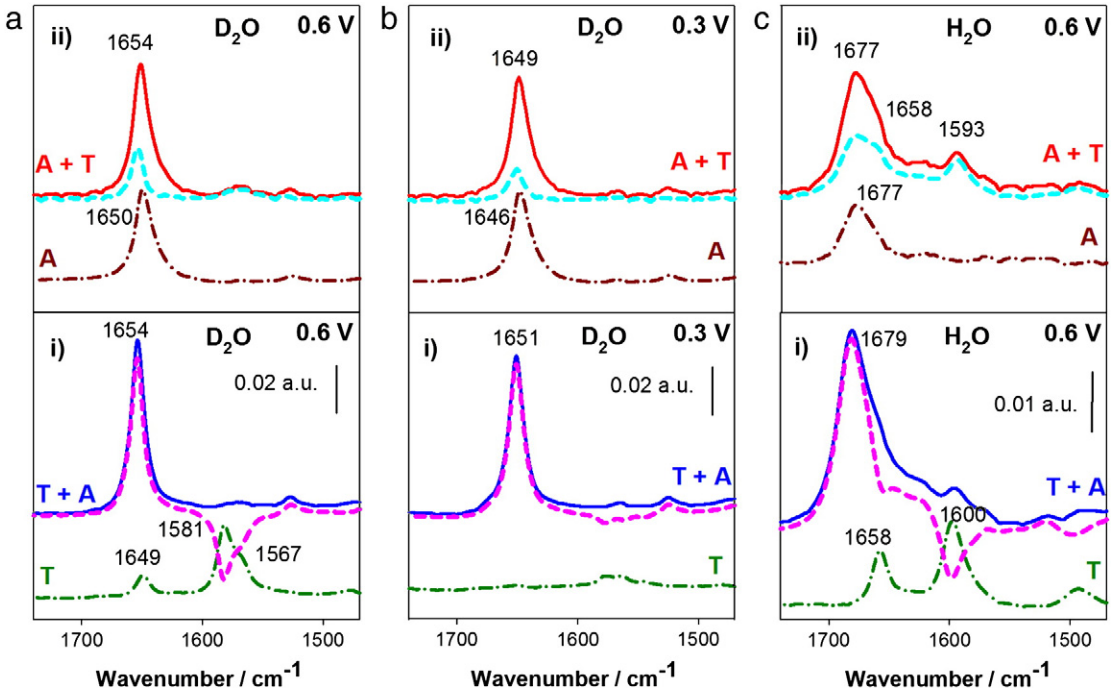

Fig. 2. ATR-SEIRAS spectra collected at indicated potentials after addition of adenine (till 0.01 mM) to 1 mM thymine solution (——, i) or after addition of thymine (till 1 mM) to 0.01 mM adenine solution (——, ii). Test electrolyte: 0.1 M $HClO_4$ in D_2O (Fig. 2a and b) and in H_2O (Fig. 2c). Each spectrum is referred to that collected in the supporting electrolyte at the same potential in the absence of the bases except the bold-dashed lines, which are referred to the corresponding spectrum collected in solutions containing only thymine (– – –, i)) or only adenine (– – –, ii)). The spectra collected at the same potentials in the individual base solutions, 1 mM thymine (-.-.-) or 0.01 mM adenine (-.-.-) are also given.