RAMAN SCATTERING FROM ADENINE, URACIL AND THEIR DERIVATIVES ADSORBED AT A SILVER ELECTRODE

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Adsorption at a silver electrode of adenine, uracil and their monomeric nucleosides and nucleotides from neutral solutions of KCl was studied by means of Raman spectroscopy and the measurement of the double-layer capacitance. It was found that all compounds investigated except uridine 5′-monophosphate were strongly adsorbed at a positively charged electrode. Analysis of the surface Raman spectra was performed with the aim of obtaining qualitative information on the orientation and configuration of nucleic acid components in the adsorbed state. The results of this analysis were compatible with the conclusions on adsorption of these compounds drawn on the basis of previous measurements with the mercury electrode.

A considerable body of evidence suggests that the biological role of nucleic acids may be related to the adsorption and other interfacial behaviour of polynucleotides at charged interfaces. With a view to understanding the interfacial behaviour of these complex biomacromolecules, attempts are being made to explain the interfacial behaviour of their monomeric constituents[1-5].

Adenine, uracil and their monomeric derivatives are found naturally in living organisms, for instance as components of nucleic acids. In ribonucleic acids adenine can be hydrogen bonded to uracil. We have already reported on the adsorption of these compounds at a mercury electrode from aqueous solutions[3,4,6,7]. Differential capacitance and electrocapillary measurements were used to obtain a fairly detailed picture of the behaviour of these compounds at mercury electrodes, *i.e.* primarily on the metal surface polarized to around the potential of zero charge (p.z.c.) and more negatively.

In recent years a new spectroscopic technique, surface enhanced Raman scattering (SERS) spectroscopy, has been established as another powerful tool for studies of adsorbed molecules on metal surface (for review see refs[8,9]). This technique allows one to obtain vibrational spectra of molecules adsorbed on metal substrates *in situ*. The most intense Raman signals are usually found at a silver electrode when it is subjected to an oxidation/reduction cycle that forms silver chloride on anodization and reforms silver on cathodization[8,9].

Some data on the adsorption of major components of nucleic acids (except uracil and its derivatives) on the silver electrode obtained by means of SERS have already been reported by Sequaris, Koglin and their coworkers[10-13]. In this work we investigated adsorption of adenine, uracil, their nucleosides and nucleotides on silver electrode with the aid of SERS and the capacitance measurement. The aim was to obtain further data on the interfacial behaviour of these compounds on a positively charged metal surface.

EXPERIMENTAL

Raman spectra were recorded on a Jasco model R-800 laser Raman spectrophotometer equipped with Spectra Physics model 164 Argon laser in the spectral range 200 to 1 600 cm^{-1}. Laser excitation wavelength was 514·5 nm. In all experiments the laser power was approximately 60 mW at the cell and the laser beam was incident on the silver surface at c. 70°. The Raman scattered light was collected in the plane of incidence and perpendicular to the direction of the laser beam[9,11] (the angle of incidence is defined in the same way as *e.g.* in the papers of Birke and coworkers[9] and Pettinger and coworkers[14]). Scan parameters were as follows: slit width 3 cm^{-1}, scan rate 0·5 Å/s and 0·05 s counting interval. The 5 ml capacity glass cell contained a platinum wire counter electrode and a silver/silver chloride reference electrode. The working electrode was a plate (1·0 × 0·5 cm) made of polycrystalline silver sheathed in a teflon holder. Before each experiment this electrode was first mechanically polished to a 1 μm finish with alumina, ultrasonically cleaned in distilled water and then electrochemically roughened. The roughening procedure consisted of two oxidation/reduction cycles (ORC) performed in the background electrolyte in the absence of adsorbate in room lighting. During the ORC the potential variation was −0·1 V → 0·2 V → −0·1 V at the voltage scan rate 50 mV/s. The voltage between the silver electrode and the reference electrode was controlled by means of the potentiostat of a Fuso Model 312 Polarograph (Fuso Co., Kawasaki, Japan). All SERS measurements were carried out with the pretreated silver electrode polarized within −0·1 V to −0·6 V in a medium of 0·1 mol l^{-1} potassium chloride with 1 mmol l^{-1} phosphate buffer, pH 7·0.

The measurements of differential capacitance of silver electrode were carried out with the aid of phase sensitive alternating current (a.c.) voltammetry using the same background electrolyte as for the SERS measurements. A.c. voltammetry was performed on a PAR Model 174 A polarographic analyzer coupled with a PAR Model 174/50 AC polarographic analyzer interface and a PAR Model 5204 lock-in analyzer. For a.c. voltammetry a d.c. ramp of 5 mV/s and a modulation voltage of 80 Hz and 10 mV peak-to-peak was employed for all experiments. The silver electrode for capacitance measurements was a c. 5 mm disk of polycrystalline silver. It was first polished mechanically with emery paper (No 1 500), ultrasonically cleaned in distilled water, and then polished electrochemically. The latter polishing was by cyclic voltammetry between −1·0 and 0·15 V at a voltage scan rate of 20 mV/s, performed in the background electrolyte until a steady-state voltammogram was obtained.

All potentials quoted are *vs* Ag/AgCl (sat. KCl) reference electrode. All measurements were carried out at 25°C.

RESULTS

The surface Raman scattering spectra at the silver electrode pretreated by the oxidation/reduction cycles were recorded for neutral solutions of adenine, uracil and their

monomeric derivatives at their bulk concentrations of $1 . 10^{-4}$ mol l^{-1} and higher (Figs 1 and 2). If the Raman spectra were recorded without the electrode pretreatment they showed only a background curve without any detectable bands.

Adenine, adenosine and adenosine 5'-monophosphate (AMP) adsorbed at the pretreated silver electrode give rise to two prominent bands in SERS spectra at 735 and 1 334 cm^{-1} (Fig. 1). These bands, along with low intensity bands appearing in the range of 300–700 cm^{-1} and 1 200–1 600 cm^{-1}, are attributable to adenine ring vibrations[11,17,18]. AMP also yields less intense Raman signals at around 1 000 cm^{-1} due to vibrations of the sugar and phosphate residues[11,17,18]. The maximum intensities of all surface Raman bands of adenine and adenosine are observed at −0·6 V, while in the case of AMP the maximum intensities are obtained at c. −0·35 V. The qualitative character of the surface Raman spectra of adenine and AMP does not change with the bulk concentration of the adsorbate (Figs 1*a*,*c*

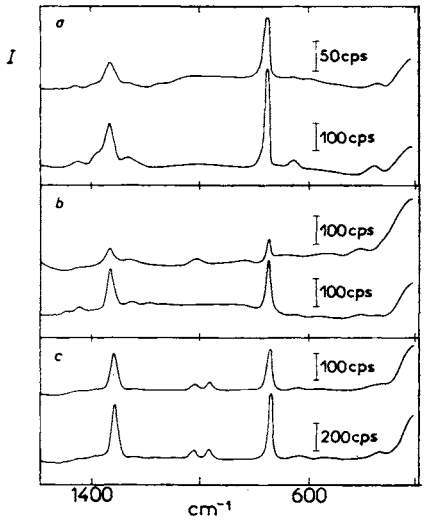

FIG. 1

Surface Raman spectra of adenine and its derivatives adsorbed on a silver electrode polarized to −0·6 V in 0·1 mol l^{-1} potassium chloride with 1 mmol l^{-1} phosphate buffer, pH 7·0. *a* Adenine at a concentration of $5 . 10^{-4}$ mol l^{-1} (upper curve) and $5 . 10^{-3}$ mol l^{-1} (lower curve). *b* Adenosine at a concentration of $5 . 10^{-4}$ mol l^{-1} (upper curve) and 0·015 mol l^{-1} (lower curve). *c* Adenosine 5'-monophosphate at a concentration of $5 . 10^{-4}$ mol l^{-1} (upper curve) and 0·025 mol l^{-1} (lower curve)

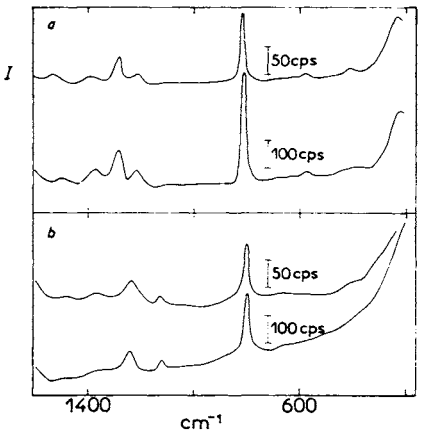

FIG. 2

Surface Raman spectra of uracil and uridine adsorbed at a silver electrode polarized to −0·6 V in 0·1 mol l^{-1} potassium chloride with 1 mmol l^{-1} phosphate buffer, pH 7·0. *a* Uracil at a concentration of $5 . 10^{-4}$ mol l^{-1} (upper curve) and 0·03 mol l^{-1} lower curve. *b* Uridine at a concentration of $5 . 10^{-4}$ mol l^{-1} (upper curve) and 0·06 mol l^{-1} (lower curve)

and 2). This indicates no change in the orientation of these molecules adsorbed at the silver electrode in the whole range of the bulk concentrations used in our work (Figs 1 and 2). In the case of adenosine, however, the less intense band in the region of sugar residue vibrations[17] appears only at the lower bulk concentrations of this nucleoside (Fig. 1*b*). At a bulk concentration higher than c. 2 mmol l^{-1}, no band corresponding to ribose vibrations is observable. This result indicates that the configuration of adenosine molecules adsorbed at the silver electrode is dependent on the fractional surface coverage. At lower coverages the sites in the ribose moiety responsible for the appearance of the band at 1 044 cm^{-1} are attached to the electrode surface, while at the large surface coverage, these sites are apparently moved completely out of the plane of the electrode surface.

Uracil also yields two prominent bands around 799 and 1 276 cm^{-1}, while uridine gives rise to two more intense bands around 797 and 1248 cm^{-1} (Fig. 2). These bands, along with low intensity bands appearing in the ranges 300–600 cm^{-1} and 1 200 to 1 500 cm^{-1} are attributable to uracil ring vibrations[17]. In the case of uridine, the low intensity band at 1 130 cm^{-1} attributable to vibrations of the sugar moiety has been observed independently of uridine bulk concentration in the whole range of uridine concentrations used in this work ($5 . 10^{-4}$–0·09 mol l^{-1}) (Fig. 2*b*). Under the same experimental conditions no surface Raman signals can be observed for uridine 5'-monophosphate (UMP). The qualitative character of the surface Raman spectra of uracil and uridine does not change with the bulk concentration of the adsorbate. The maximum intensities of all surface Raman bands of the latter compounds are observed at −0·6 V, if the electrode is polarized to more positive potentials, the scattering from the adsorbed molecules becomes less intensive.

The addition of adenine, adenosine, AMP, uracil and uridine to the background electrolyte to concentrations of $5 . 10^{-4}$ mol l^{-1} and higher caused appreciable lowering of the double layer capacitance of the silver electrode in the region of potentials used for the SERS experiments (−0·1 V – −0·6 V). In this potential region no pit or peak appeared on the capacitance curves of these compounds at the silver electrode; this indicates that probably no reorientation of these adsorbates induced by a change of the electrode potential took place. UMP, however, even at the concentration of 60 mmol l^{-1}, caused only negligible lowering of the double layer capacity, which suggests much lower adsorbatility of UMP at the silver electrode in comparison with other compounds investigated in this work.

In order to gain some insight into the electrosorption of mixtures of complementary bases the adsorption from two component mixtures of adenine and uracil was studied at the silver electrode using the SERS spectroscopy. These investigations were initiated because of our earlier findigs[15] that these complementary bases are hydrogen bonded when they are adsorbed flat at the mercury electrode under conditions of fully or almost fully covered electrode surface. As shown by Lord and Thomas[16] this phenomenon could be reflected in Raman scattering spectra. Thus

solutions were prepared in which the ratios of uracil to adenine concentrations were 4·0, 10·0 and 30·0 (the concentration of uracil was always 30 mmol l^{-1}, which is a concentration close to that corresponding to saturation of the solution by uracil). It was remarkable that only Raman bands of adenine ring vibrations (Fig. 1*a*) were observed, the bands of uracil (Fig. 2*a*) being totally absent.

DISCUSSION

Although the mechanism of the SERS enhancement is not yet completely understood, a number of papers concievably demonstrate that SERS can offer at least a qualitative indication of adsorption and determination of surface orientation of adsorbates at metal surfaces[8,9,11]. The discussion that follows is an attempt to interpret our SERS results from the point of view of the surface orientation of adenine, uracil and their derivatives adsorbed at a positively charged silver electrode.

Nucleic acid bases (*adenine and uracil*): It has been shown regarding the adsorption behaviour of these compounds at the mercury electrode that they adopt a flat surface orientation at lower bulk concentrations in a broad region of potentials around the p.z.c.[2-4,6,7]. They can adopt the perpendicular orientation only at their bulk concentrations close to the saturation limit (adenine above c. 3 mmol l^{-1} and uracil above c. 24 mmol l^{-1}) and only in a relatively narrow range of potentials around the p.z.c. at neutral pH[3,4,6]. No qualitative change in surface Raman spectrum is observed if the bulk concentration of the base is changed in the range from $1 . 10^{-4}$ mol l^{-1} to its saturation limit (Figs 1*a*, 2*a*). Moreover, our spectra were recorded at potentials rather far from the p.z.c. It thus seems reasonable to conclude that adenine and uracil when adsorbed at a silver electrode adopt a flat surface orientation.

Nucleosides (*adenosine and uridine*): The following properties of nucleosides have to be considered when interpreting the interfacial behaviour of these compounds:

a) Adenosine exists preferentially in the *anti* conformation[9] (Fig. 3) but the steric barrier to interconversion between the *anti* and *syn* conformation is small[19,20]. Also the energy difference between the two conformations is very small[21]. Accordingly, it is energetically and sterically easy for adenosine to adopt either the *syn* or *anti* conformation. Uridine also exists in the *anti* conformation but, contrary to adenosine, the steric barrier to interconversion between the *anti* and *syn* conformations is large, and also somewhat larger energy difference between the *syn* and *anti* forms may exist for uridine in comparison with adenosine[20,22,23].

b) D-Ribose is much less adsorbable at the aqueous solution/metal interface than more hydrophobic adenine and uracil[7]. The results of our surface Raman spectroscopy experiments with nucleosides can be rationalized as support for our previous

conclusions on the surface orientation of these compounds adsorbed at a mercury electrode[4,7]. It appears that uridine, independently of its bulk concentration or surface coverage, and adenosine at a bulk concentration corresponding to smaller surface coverage are adsorbed with the planar base residue in a flat orientation on the electrode adopting *anti* conformation. Then the sugar residue can be adsorbed only approximately perpendicular to the electrode surface. Molecular models of adenosine and uridine (Fig. 3) reveal that if these nucleosides adopt the latter surface configuration then the adsorbed nucleoside molecules attach not only with the base plane but also with C′(1), C′(4), ring oxygen O′(1) and C′(5) CH_2OH groups of the sugar residue in close proximity to the plane of the electrode surface. On the other hand, in the case of adenosine at large surface coverage, rotation about the N(9)—C′(1) bond (Fig. 3) could occur due to displacement of sugar residues from the surface by more adsorbable adenine residues. Adenosine would adopt *syn* conformation in the surface with the sugar ring oxygen, C′(3), C′(5) elevated above the plane

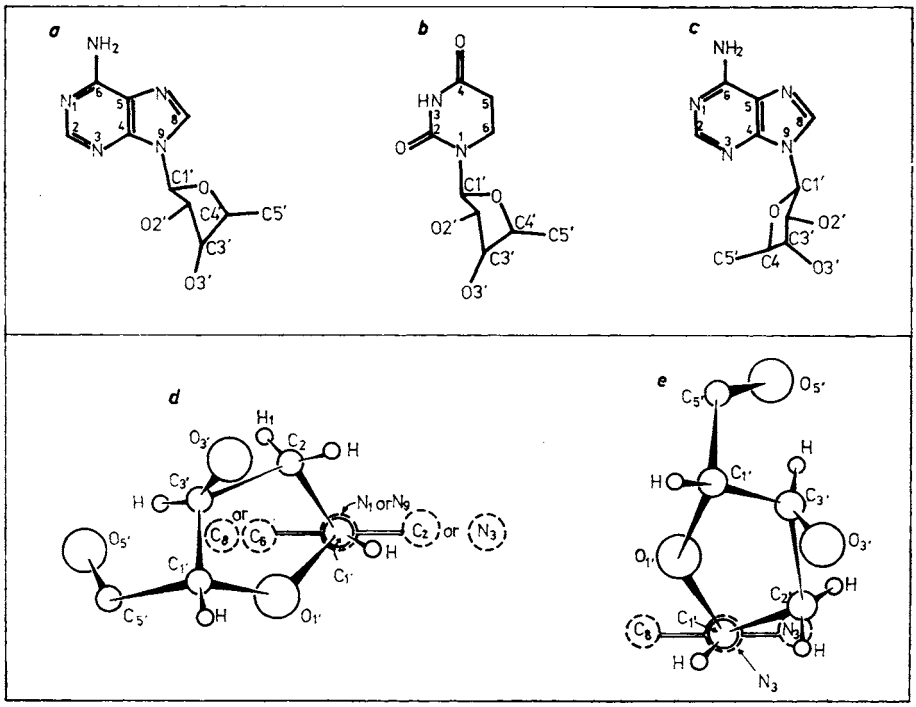

FIG. 3

Schematic illustration of conformations of nucleosides. *a*, *b* and *d*: The *anti* conformation. *c* and *e*: The *syn* conformation. *a*—*c*: The plane of the base is parallel to the plane of the page. *d*, *e*: The plane of the base is viewed end-on with the glycosyl bond between C′(1) and N(1) (for uridine) or N(9) (for adenosine) of the base perpendicular to the page

of the electrode, having only C'(1) and C'(2) in close proximity to the plane of the surface. Thus the surface Raman spectra of adenosine (Fig. 1*b*) and uridine (Fig. 2*b*) could be rationalized by the fact that the appearance of the weak bands in the spectrum of adenosine and uridine around 1 000 cm^{-1} corresponds to vibration of the part of ribose residue attached with O'(1), C'(4) or C'(5) in close proximity to the plane of the surface.

Nucleotides (AMP): The surface Raman spectra of AMP (Fig. 1*c*) demonstrate the attachment of these molecules with sugar group and negatively charged phosphate group in close proximity to the silver electrode surface independently of the bulk concentration of this compound. Thus this behaviour can easily be rationalized by the fact that AMP is adsorbed at the silver electrode with adenine residue attached to the surface in a flat orientation adopting anti conformation even at high bulk concentrations. It seems reasonable to expect that the *anti* conformation of AMP adsorbed at higher bulk concentrations is stabilized by an attractive electrostatic force between the negatively charged phosphate group and the positively charged surface of the silver electrode. This electrostatic interaction could prevent the displacement of sugar residues from the surface by adenine residues, as suggested for the adsorption of adenosine at higher surface coverages.

The fact that we failed to record SERS signals for UMP could reflect very low adsorbability of this compound at the silver electrode confirmed by the differential capacitance measurements. A much lower adsorbability of UMP compared with uridine has already been observed at a mercury electrode[6].

Mixture of complementary bases: The results of this work that were obtained when the adsorption from the mixtures of complementary bases was investigated indicate that only adenine molecules were present at the silver electrode surface. This could be due to significantly stronger adsorption at the metal/solution interface of adenine in comparison with uracil. Adenine molecules could thus displace the uracil ones from the surface. Much stronger adsorption of adenine in comparison with uracil was also observed at the mercury solution interface[4,6,15].

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