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RAMAN SCATTERING FROM NUCLEIC ACIDS ADSORBED AT A SILVER ELECTRODE

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Adsorption of nucleic acids at a silver electrode polarized to −0.6 to −0.1 V (vs. Ag/AgCl) was investigated by means of surface enhanced Raman scattering (SERS) spectroscopy. Single-stranded polyriboadenylic acid and thermally denaturated DNA adsorbed at the silver electrode yield two intense bands at 734 and 1335 cm^{-1} on the SERS spectra. These bands, assigned to the vibrations of adenine residue rings, were much less intense if the SERS spectra were recorded for double-helical complex polyadenylic·polyuridylic acid and native DNA. Moreover, the courses of alkaline denaturation of DNA and its digestion by deoxyribonuclease I were observed by SERS spectroscopy. The results were interpreted as support for the view that intact double-helical segments of nucleic acids are not denatured or destabilized due to their adsorption at the positively charged and roughened surface.

1. Introduction

There is considerable published evidence that nucleic acids are extensively associated with charged biological surfaces such as various cell membranes, including 'microfaces' of protein molecules. It is therefore felt that an investigation of interactions of nucleic acids with surfaces might be instructive. The first attempts in this field were made more than 20 years ago when a polarized mercury surface was used as a model of biological surfaces. In 1961, Miller [1,2] investigated the behaviour of DNA and RNA on a polarized mercury surface. He measured the differential capacity of the electrical double layer between the dropping mercury electrode and NaCl solution containing the nucleic acid. He suggested that unfolding of DNA occurs on a mercury electrode charged slightly positively. However, Miller's results were reexamined, and it was concluded that the experimental data cannot be used as proof of an unfolding or denaturation of DNA on the surface [3]. Later, studies on adsorption of DNA at the mercury electrodes by means of linear sweep voltammetry and normal pulse polarography were published [4,5]. These investigations were performed under conditions when DNA can be electroreduced at the electrode (at acid and neutral pH). It was concluded from these studies [4,5] that the appearance of rather high reduction currents observed in voltammetric or pulse polarographic experiments with the solutions of double-helical DNA is connected with a surface denaturation or destabilization of DNA. These results and conclusion were also questioned [6,7] and on the contrary it was proposed that the latter voltammetric and pulse polarographic results were explicable without assuming a surface denaturation of DNA; interpretation in terms of changes in the surface concentration of DNA or of base fluctuations in adsorbed double-helical DNA was suggested.

Significant progress in discussion on denaturation of DNA on the mercury surface was achieved by a systematic study based on techniques with a large potential excursion during the drop lifetime (e.g., linear sweep voltammetry). The experimental

conditions were determined for obtaining the signals specific (appearing at various potentials) to single-stranded and double-stranded DNA on the mercury surface, even when the analysis of double-helical DNA samples was performed by means of the latter techniques [8]. Thus, it was possible to find, for instance, that at neutral pH double-helical DNA can be unwound on the mercury surface to a relatively small extent if the electrode is polarized to potentials around that of zero charge (PZC). The results obtained under the conditions when double-helical DNA was adsorbed on the electrode surface polarized to the potentials in a narrow region around -1.2 V vs. a saturated calomel electrode (SCE), i.e., at the negatively charged electrode surface, indicated a much greater extent of surface denaturation of DNA. Important results which unambiguously supported the preceding conclusions on the surface denaturation of DNA were obtained with the aid of a mercury pool electrode (MPE) [9]. A greater amount of the product of the DNA interaction with the MPE was isolated and subjected to analysis routinely used for determination of denatured material in double-helical DNA samples. This analysis convincingly demonstrated the presence of denatured DNA in the product, another important proof of the surface denaturation of DNA at the mercury surface.

The phenomenon of the surface denaturation of DNA at the mercury electrode polarized to around PZC and more negatively has been extensively studied under various experimental conditions (for a review see, e.g., ref. 10). However, a mercury surface is obviously very far from resembling a biological surface. So studies to find out whether the surface denaturation of DNA can also be observed on adsorbents of different chemical nature and/or adsorbents with a rather high positive surface charge represented another important contribution for clarifying the mechanism of interactions of nucleic acids with biological surfaces. The properties of adsorbed double-helical DNA were also studied on the surface of graphite electrodes, which were mechanically roughened [11–13]. The investigations were performed by means of voltammetry and the analysis of a greater amount of the product of the DNA interaction with a large graphite electrode. The results indicated no significant denaturation of DNA on the positively charged graphite surface. On the other hand, the results obtained with a graphite electrode polarized in the region of -0.8 to -0.3 V (vs. SCE) indicated interfacial denaturation of double-helical DNA at the negatively charged surface, similarly to the mercury electrode. The latter result thus supports the view that the conformational alteration in DNA adsorbed at a sufficiently negatively charged surface could take place independently of the chemical nature of the adsorbent.

In recent years a new spectroscopic technique, surface enhanced Raman scattering (SERS) spectroscopy, has been established as another powerful tool for studies of molecules adsorbed on metal substrates, under in situ conditions (for reviews, see refs. 14 and 15). However, SERS spectroscopy is not suitable for obtaining vibrational spectra of molecules adsorbed at mercury or graphite substrates. It is therefore impossible to compare directly the results describing the properties of DNA adsorbed at graphite and mercury electrodes by quite different and independent methods (SERS spectroscopy and voltammetry). The most intense Raman signals are usually found at an electrochemically pretreated silver electrode polarized to potentials around PZC and more positively [14,15]. The electrochemical pretreatment usually consists of one or more oxidation/reduction cycles that form silver chloride on anodization and re-form silver on cathodization. This electrochemical pretreatment leads to a surface roughness of the silver electrode. Thus, the silver electrode used in the SERS experiments may have some physical properties (roughness and surface charge density) very similar to those of the graphite electrode used in the experiments aimed at observing surface conformational changes in adsorbed DNA.

The data on the adsorption of some nucleic acids and their monomeric components on the silver surface obtained by means of SERS spectroscopy have already been reported [16–19]. The results on DNA were interpreted in terms of incomplete but significant destabilization of native DNA adsorbed at a roughened and positively charged silver electrode [17].

Here we report the SERS spectra of the syn-

thetic DNA-like double-stranded complex polyriboadenylic acid · polyribouridylic acid and natural mammalian DNA. The results indicate no significant destabilization of double-helical nucleic acid molecules as a consequence of their adsorption at a roughened and positively charged silver electrode.

2. Experimental

2.1. Materials

Adenine, uracil, their monomeric derivatives, calf thymus DNA, polyriboadenylic acid (poly(A)), polyribocytidylic acid (poly(C)), polyribouridylic acid (poly(U)) and deoxyribonuclease I (DNase I) were purchased from Sigma. The RNA content estimated in the DNA sample with the aid of orcinol [20] was less than 1%; the protein content, determined according to Lowry et al. [21], did not exceed 0.5%. The sedimentation coefficient, $s_{20,w}$ determined for native DNA in 0.1 M KCl was 22 S. The content of denatured DNA, estimated by differential pulse (DP) polarography [22], was lower than 1%. Denaturation was performed by heating DNA (in double the DNA concentration in which it was measured) in 0.01 M KCl for 10 min at 100°C and quick cooling in an ice bath. Both the homopolymer concentration (related to the mononucleotide content) and the DNA concentration were estimated spectrophotometrically. Double-helical complex poly(A) · poly(U) was prepared as previously described [23]. The content of single-stranded poly(A) in this complex determined pulse-polarographically [22,24] was lower than 1%.

2.2. Methods

Raman spectra were recorded on a Jasco model R-800 laser Raman spectrophotometer equipped with a Spectra Physics model 164 argon laser in the spectral range 200–1600 cm^{-1}. The laser excitation wavelength was 514.5 nm. The laser power at the cell was approx. 60 mW in the experiments with monomers and about 100 mW in those with macromolecules. The laser beam was incident on the silver surface at approx. 70°. The Raman scattered light was collected in the plane of incidence and perpendicular to the direction of the laser beam [15,25] (the angle of incidence is defined in the same way as, e.g., in the papers of Birke et al. [15] and Pettinger et al. [25]). 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 spectroelectrochemical 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 to 0.2 to −0.1 V at a voltage scan rate of 50 mV/s. The voltage between the silver electrode and the reference electrode was controlled by means of a potentiostat model 312, mfr. Fuso Polarograph (Fuso Co., Kawasaki, Japan). All SERS measurements were carried out with the pretreated silver electrode polarized within −0.1 to −0.6 V in a medium of 0.1 M KCl with 1 mM phosphate buffer, pH 7.0.

The measurements of the differential capacitance of the silver electrode were carried out with the aid of phase-sensitive 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 5 mV/s d.c. ramp 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 an approx. 5 mm disk of polycrystalline silver. It was first polished mechanically with emery paper (no. 1500), ultrasonically cleaned in distilled water and then polished electrochemically. The latter polishing was done by cyclic voltammetry between −1.0 and 0.15 V at a volt-

age scan rate of 20 mV/s performed in the background electrolyte until a steady-state voltammogram was obtained.

DP polarographic measurements were carried out with an EG and G PAR model 384 B Polarographic analyzer in connection with a model 303A static mercury drop electrode. The polarograms were recorded with a voltage scan rate of 2 mV/s, drop time of 2.0 s and a pulse amplitude of −50 mV. Details of the application of this method in studying the structure of nucleic acids have already been published [10,26]. All potentials reported in this paper are quoted against silver/silver chloride (saturated KCl) reference electrode if not otherwise stated.

The viscosity was measured with a four-gradient Ubellohde type viscometer [27].

3. Results and discussion

3.1. SERS spectroscopy of double-helical complex poly(A) · poly(U)

Our first experiments with the SERS spectroscopy of nucleic acid samples were carried out with a simple synthetic model of natural DNA. The complex of poly(A) · poly(U) composed of two homopolynucleotides poly(A) and poly(U) has a double-helical DNA-like structure in solution (it has a conformation belonging to the family of A-forms). The geometry of this double helix is somewhat different from that of canonic B-form, which prevails in natural DNAs under physiological conditions. Nevertheless, a common feature of the conformations A and B is the fact that the complementary bases in opposite chains are hydrogen bonded and hidden inside the intact macromolecule, while the sugar-phosphate backbone is on the periphery.

In order to interpret more easily the results obtained with the complex poly(A) · poly(U), the SERS spectra for its monomeric components (adenine, uracil and their nucleosides and nucleotides) were recorded first. Details on the SERS spectra of these compounds will be published elsewhere [28]. Here we report only that adenine, adenosine and adenosine 5′-monophosphate adsorbed at the pretreated silver electrode to −0.6 to −0.1 V gave rise to two prominent bands in SERS spectra at 735 and 1334 cm^{-1} (fig. 1a). These bands are attributable to adenine ring vibrations [16,28,29]. Uracil also yielded prominent bands around 799 and 1276 cm^{-1}, while uridine gave rise to two more intense bands around 797 and 1248 cm^{-1} (fig. 1b). These bands are attributable to uracil ring vibrations [28,29]. Under the same experimental conditions no surface Raman signals were observed for uridine 5′-monophosphate (UMP). The latter behaviour of UMP was suggested [28] to be connected with much lower adsorbability of UMP at the silver electrode compared with other monomeric compounds subjected to SERS spectroscopy in this work.

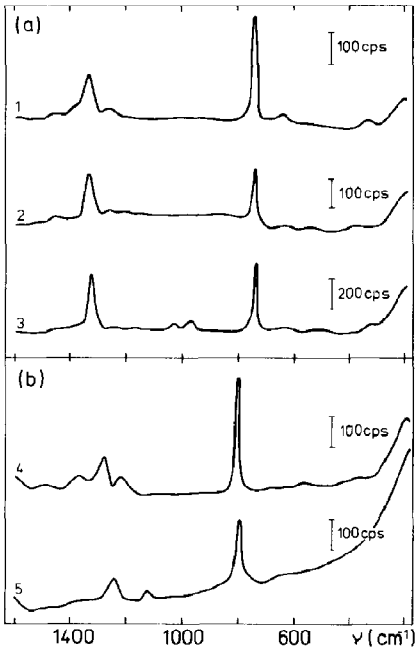

Fig. 1. Surface Raman spectra of monomeric components of nucleic acids adsorbed at a silver electrode polarized to −0.6 V in 0.1 M KCl with 1 mM phosphate buffer, pH 7.0. (a) Curve 1, 5×10^{-3} M adenine; curve 2, 1.5×10^{-2} M adenosine; curve 3, 2.5×10^{-2} M adenosine 5′-monophosphate. (b) Curve 4, 3×10^{-2} M uracil; curve 5, 5×10^{-2} M uridine. Waiting time at −0.6 V was approx. 10 min.

The SERS spectra at the silver electrode pretreated by the oxidation/reduction cycles and

polarized within −0.6 to −0.1 V were recorded for neutral solutions of poly(A), poly(U) and their double-helical complex at bulk concentrations of 1×10^{-4} M and higher. Single-stranded poly(A) gave rise to two intense bands in the SERS spectrum (fig. 2a) like its monomeric constituents [16] (cf. fig. 1). The maximum intensities of these prominent bands were observed at −0.6 to −0.3 V. If single-stranded poly(U) was subjected to SERS spectroscopy under the same experimental conditions no Raman scattering could be observed. Adsorption of single-stranded polynucleotides at a silver electrode was also studied by double-layer capacitance measurements. It was found that poly(A), similarly to its monomeric constituents, appreciably lowered the double-layer capacity in the region of potentials used for the SERS experiments. Poly(U), however, even at a concentration of 2 mM caused only a negligible reduction of the double-layer capacity, which suggests much weaker adsorption of poly(U) at the silver electrode compared with poly(A). It was interesting that UMP also showed much weaker adsorbability at the silver electrode compared with adenine, its monomeric derivatives, uracil and uridine.

The formation of the double-helical complex poly(A)·poly(U) resulted in a marked decrease in intensities of both most intense SERS bands of poly(A) (fig. 2b). This result indicates that mixing poly(A) with poly(U) in equimolar concentrations leading to the formation of the double-helical complex resulted in a marked lowering of the number of adenine residues in poly(A) capable of interacting with the silver electrode sufficiently to cause the SERS effect. This marked lowering could also take place, for instance, due to a competition in adsorption at the silver electrode between poly(A) and poly(U). Even though this possibility does not seem very likely because of the much weaker adsorbability of poly(U) (compared to poly(A)), this eventuality was also examined by means of the measurements of SERS spectra of the model mixture containing single-stranded poly(A) and poly(C) at the same concentration (1 mM) (poly(A) does not form a complex with poly(C)). If this mixture was subjected to the SERS spectroscopy under the same experimental conditions, the presence of poly(C) in the mixture influenced the intensity of the SERS bands yielded by single-stranded poly(A) only slightly. Moreover, the most intense bands of the complex poly(A)·poly(U) were obtained if the electrode was polarized to −0.6 to −0.3 V, but the extent of the lowering of the intensity of the bands of poly(A) due to the formation of double-helical complex (fig. 2b) was independent of the electrode potential throughout the potential range used in the SERS experiments. It can therefore be concluded that the results of SERS spectroscopy of the complex poly(A)·poly(U) exclude the possibility of extensive surface denaturation of this double-helical macromolecule adsorbed on the surface of the silver electrode polarized within −0.6 to −0.1 V. The SERS band at 734 cm^{-1} corresponding to adenine ring vibrations yielded by poly(A)·poly(U) was about 10-times lower than that of single-stranded poly(A) (fig. 2). If we admit that this small intensity band appeared due to an incomplete surface denaturation or destabilization of the double helix of poly(A)·poly(U), then this surface phenomenon could not involve more than roughly 10% of the adsorbed double-helical

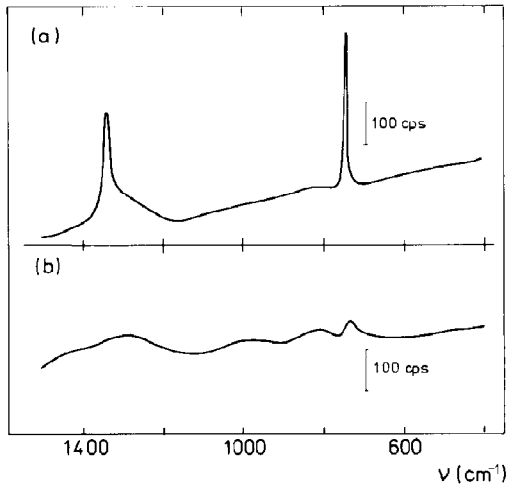

Fig. 2. Surface Raman spectra of 1 mM single-stranded poly(A) (a) and 2 mM double-stranded poly(A)·poly(U) (b) adsorbed at a silver electrode polarized to −0.6 V in 0.1 M KCl with 1 mM phosphate buffer, pH 7.0. Waiting time at −0.6 V was about 20 min.

material. However, the latter result can also be easily explained without assuming any surface denaturation or destabilization of the double-helical complex. The source of the bases responsible for the appearance of the 734 cm^{-1} SERS band could be the regions of the double-helical molecules, which in some way already had a distorted structure in the bulk of solution. In order to clarify this problem we also investigated adsorption of natural DNA by means of SERS spectroscopy at a silver electrode.

3.2. SERS spectroscopy of calf thymus DNA

The SERS spectra of double-helical and thermally denatured DNAs were obtained with the silver electrode polarized to −0.6 to −0.1 V (fig. 3). Thermally denatured DNA, in which most of the bases are exposed to the solvent, gave rise to intense SERS signals at 734 and 1334 cm^{-1} (fig. 3a), apparently corresponding to vibrations of adenine residue rings. The fact that other base residues bound in denatured DNA yielded no or weak SERS signals has not yet been clarified. There are some indications that adenine residues are more strongly adsorbed at the silver electrode than the residues of other major bases occurring in natural mammalian DNAs [17]. More studies will be necessary to answer this question unequivocally.

The sample of native DNA also yielded both bands at 734 and 1334 cm^{-1} (fig. 3b), but they were much less intense than those of denatured DNA. This difference between the SERS spectroscopic behaviour of native and denatured DNA suggests that SERS spectroscopy can also monitor the helix-coil transition of DNA in the bulk solution. Alkaline denaturation of DNA was therefore observed by means of SERS spectroscopy (734 cm^{-1} band) and absorbance measurements at 260

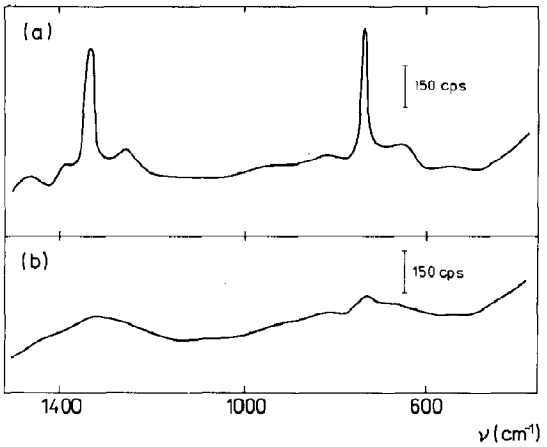

Fig. 3. Surface Raman spectra of thermally denatured DNA (a) and native DNA (b) adsorbed at a silver electrode polarized to −0.1 V in 0.1 M KCl with 1 mM phosphate buffer, pH 7.0. DNA concentration was 0.2 mg/ml. Waiting time at −0.1 V was approx. 40 min.

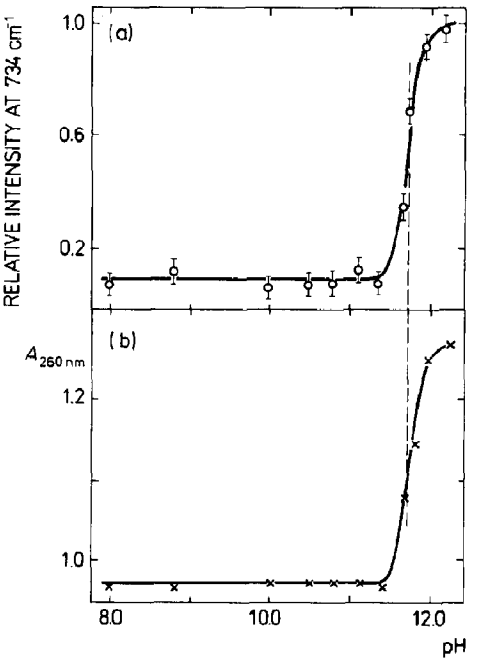

Fig. 4. (a) The dependence of the relative intensity of the surface Raman band at 734 cm^{-1} yielded by double-helical DNA solution on pH. Relative intensity is defined as the ratio of the intensities of native and thermally denatured DNAs obtained at pH indicated in the graph. DNA concentration was 0.1 mg/ml. Other conditions were the same as those listed in fig. 3. (b) The dependence of the absorbance of native DNA at 260 nm ($A_{260\,nm}$) on pH. DNA concentration was 49 μg/ml. SERS and absorbance measurements were carried out in the medium of 0.1 M KCl with 1 mM phosphate buffer. pH of the solutions was adjusted by addition of NaOH.

nm ($A_{260\,nm}$) (fig. 4). While the latter dependence exclusively reflects the bulk solution properties of DNA, SERS spectroscopy should, on the other hand, yield information on the properties of DNA only in the interface situation. This assumption was verified by experiments with the same nucleic acid samples but without the silver electrode present in the SERS cell or with the silver electrode, which was not pretreated with oxidation/reduction cycles. In these cases the Raman scattering spectrum showed only the background curves without any detectable bands. The dependence of $A_{260\,nm}$ on pH showed a form typical of the helix-coil transition with a melting point at pH 11.75 (fig. 4b). The relative SERS intensity at 734 cm^{-1} was independent of pH in the range where no change in $A_{260\,nm}$ was observed. The helix-coil transition in the bulk solution induced by a pH increase resulted in an increase of the relative intensity of the SERS band at 734 cm^{-1}, showing exactly the same transition pH (fig. 4a) as that obtained from the $A_{260\,nm}$ measurements. This convincingly demonstrates that the stability of double-helical DNA molecules adsorbed on the silver electrode against denaturation was the same as that in the bulk solution. Thus, it strongly supports the view that the DNA double helix was not destabilized due to its adsorption at the silver electrode polarized to the potentials corresponding to a rather highly positively charged surface. If there were any significant destabilization in the adsorbed DNA double helix, the melting pH obtained from the SERS measurements would have to be lower than that obtained from $A_{260\,nm}$ measurements.

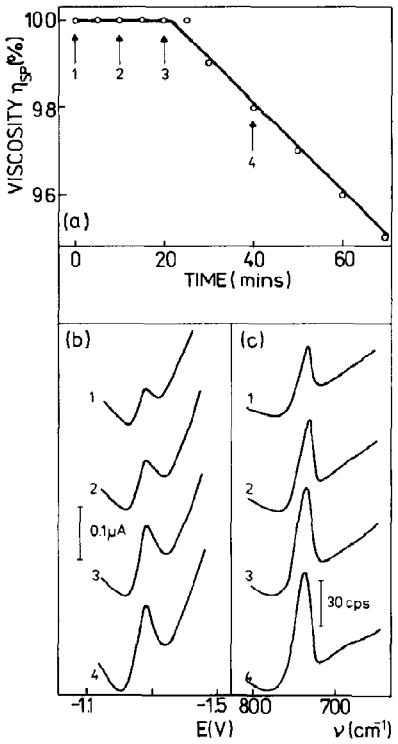

Fig. 5. Behaviour in time of DNA digested by deoxyribonuclease I followed with aid of viscometry, DP polarography and SERS spectroscopy. (a) Dependence of viscosity of DNA treated with DNase I on incubation time. A sample of 246 μg DNA/ml in 0.01 M $MgSO_4$ with 0.01 M NaCl plus 0.02 M sodium phosphate, pH 7.0, was incubated at 25°C with 1×10^{-3} μg DNase I/ml. One portion of this mixture was incubated in the viscometer. Samples for DP polarographic and SERS measurements were withdrawn from the other portion in the intervals indicated by arrows in the graph (a), the enzymatic reaction being stopped by adding 1/40 vol. of 1.0 M sodium citrate. The viscosity of the sample was checked. (b) DP polarograms. Measurements were carried out at a DNA concentration of 0.2 mg/ml in 0.3 M CsCl with 0.1 M sodium phosphate, pH 6.8. (c) SERS spectra. The conditions of the measurements were identical to those listed in fig. 3.

SERS spectra were also recorded for double-helical DNA into which single-strand breaks had been introduced by DNase I (fig. 5c). The introduction of single-strand breaks into double-helical DNA increases the number of distorted (but not single-stranded) regions in this biomacromolecule [26]. It has been shown [10,26] that the bases in these distorted double-helical regions are more exposed to the solvent than those in the remainder of the double helix. A sensitive probe for monitoring single-strand breaks in double-helical DNA is the DP polarographic peak II at −1.29 V (fig. 5b) [10,26]. Moreover, the viscosity of the DNA solution digested by DNase I was observed simultaneously. As evident from these viscometric measurements (fig. 5a), after 10 and 20 min of incubation (samples 2 and 3, respectively in fig. 5a), the molecular mass of DNA had not yet decreased. The digestion of DNA by DNase I resulted in a parallel increase of both DP polarographic peak II

and the SERS band at 734 cm^{-1}. This result indicates that the appearance of the band in the SERS spectra of the samples of double-helical DNA at 734 cm^{-1} is connected with the ring vibrations of adenine residues in distorted double-helical regions present in DNA already in the bulk solution.

On the SERS spectrum yielded by the sample of native DNA a very intense band also appeared at around 240 cm^{-1} (not shown). This band has already been assigned to a silver-phosphate group vibration [18], thus indicating that the sugar-phosphate backbone of DNA was anchored at the electrode surface. It was interesting that such a band, although less intense, was also yielded by the sample of thermally denatured DNA. This behaviour of thermally denatured DNA could be connected with the presence of short double-stranded segments in its sample, which could appear due to partial renaturation or aggregation during the preparation of the denatured sample.

4. Conclusions

This work confirms the conclusions already drawn by other authors [16,19] that double-helical nucleic acids are adsorbed at the positively charged silver electrode and that this adsorption can at least be qualitatively observed by SERS spectroscopy. The results of this work, which describe alkaline denaturation of DNA observed by SERS spectroscopy and absorbance measurements (reflecting only the bulk solution properties of DNA), strongly support the view that intact double-helical segments of DNA at a positively charged and roughened surface are not destabilized or denatured due to their adsorption. The experiments, in which DNA digested by DNase I was subjected to SERS spectroscopy, revealed that a surface conformational alteration leading to higher accessibility of the base residues in DNA adsorbed at the silver electrode may appear only in its double-helical regions, with a structure already in some way distorted in the bulk solution.

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