Adsorption of Peptide Nucleic Acid and DNA Decamers at Electrically Charged Surfaces

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ABSTRACT Adsorption behavior of peptide nucleic acid (PNA) and DNA decamers (GTAGATCACT and the complementary sequence) on a mercury surface was studied by means of AC impedance measurements at a hanging mercury drop electrode. The nucleic acid was first attached to the electrode by adsorption from a 5-μl drop of PNA (or DNA) solution, and the electrode with the adsorbed nucleic acid layer was then washed and immersed in the blank background electrolyte where the differential capacity C of the electrode double layer was measured as a function of the applied potential E. It was found that the adsorption behavior of the PNA with an electrically neutral backbone differs greatly from that of the DNA (with a negatively charged backbone), whereas the DNA-PNA hybrid shows intermediate behavior. At higher surface coverage PNA molecules associate at the surface, and the minimum value of C is shifted to negative potentials because of intermolecular interactions of PNA at the surface. Prolonged exposure of PNA to highly negative potentials does not result in PNA desorption, whereas almost all of the DNA is removed from the surface at these potentials. Adsorption of PNA decreases with increasing NaCl concentration in the range from 0 to 50 mM NaCl, in contrast to DNA, the adsorption of which increases under the same conditions.

INTRODUCTION

The interactions of biological macromolecules with surfaces are of great significance in nature and in biotechnology, including medical biotechnology. Proteins have been studied quite extensively (Andrade and Hladky, 1986; Haynes and Norde, 1994; MacRitchie, 1978; Ramsden, 1995), whereas little information is available on the adsorption of nucleic acids to surfaces (Brabec and Paleček, 1972; Miller, 1961; Paleček, 1983), although such interactions are extensively utilized in various hybridization assays and could be the basis for the development of hybridization biosensors (Johnston et al., 1995; Mikkelsen, 1996; Wang et al., 1996; Xu and Bard, 1995).

Methods available for the studies of biomacromolecule adsorption have been recently reviewed (Ramsden, 1993). Recently, self-assembly of alkane thiol films on the liquid mercury has been studied by synchrotron x-ray scattering (Magnussen et al., 1996). It has been shown that strong interaction of the thiol group with the underlying disordered liquid dominates the order-promoting interactions of the alkyl chains. In this paper we use AC impedance measurements with a mercury electrode, which can provide information about macromolecule adsorption at the atomically smooth surface of the liquid mercury, which depends on the surface charge, in a wide range of potentials negative to the potential of the zero charge (pzc) as well as in the narrower range of potentials on the positive side from the pzc.

Electrochemical analysis of nucleic acids has usually been performed with the electrode dipped in the analyzed solution. We have shown (Paleček, 1986; Paleček and Fojta, 1994; Paleček et al., 1993; Teijeiro et al., 1993) that DNA and RNA can easily be immobilized (because of their strong adsorption) at the hanging mercury drop electrode (HMDE) and at carbon electrodes. The electrode can be washed and immersed in blank background electrolyte to measure the electrochemical responses of the nucleic acid immobilized at the electrode surface. This technique is called adsorptive transfer stripping voltammetry (AdTSV). A small drop of a DNA solution (4–10 μl) is sufficient for AdTSV analysis, compared to the 1–2 ml of DNA that is usually used in conventional voltammetric experiments. AdTSV is thus convenient for the studies of nucleic acid samples for which little material is available. In this paper we apply AdT in combination with AC impedance (Z) measurements to study the adsorption of DNA and peptide nucleic acid (PNA).

PNA is a DNA mimic in which the entire sugar-phosphate backbone is replaced by a pseudopeptide chain consisting of N-(2-aminoethyl)-glycine units (Nielsen et al., 1991). This compound is now intensively studied, as it is believed to be a very promising drug candidate with a number of potential applications. Studies of PNA can also provide a better understanding of DNA and RNA structural properties that are related to their biological roles. In contrast to DNA, PNA has an electrically neutral backbone and it is achiral, capable of recognizing its complementary sequence in DNA (Hyrup and Nielsen, 1996). PNA-DNA

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duplexes are more stable than the corresponding DNA-DNA duplexes (Egholm et al., 1993).

Adsorption as well as reduction and oxidation of DNA and RNA at mercury and carbon electrodes have been studied (reviewed in Paleček, 1983, 1996), but the literature concerning the electrochemical analysis of oligonucleotides is scarce (Brabec et al., 1989; Paleček, 1983, 1996; Paleček et al., 1990). Quite recently it has been shown that a DNA hybridization biosensor with PNA immobilized at the electrode as a probe is superior to biosensors with immobilized DNA probes (Wang et al., 1996). The literature on the electrochemical behavior of PNA is missing, however. In this paper we have studied adsorption of the decamers of DNA and PNA at the HMDE. We have found that the adsorption behavior of the DNA decamers qualitatively agrees with the earlier results obtained with chromosomal DNAs and various RNAs (Kaisheva et al., 1988; Paleček, 1983). On the other hand, the adsorption behavior of PNA greatly differs from that of RNA and DNA (in agreement with the electric neutrality of the PNA backbone) and shows a tendency for intermolecular interactions at the electrode surface.

MATERIALS AND METHODS

DNA decamers were synthesized by phosphoamidite chemistry, and PNAs were synthesized as described (Christensen et al., 1995). PNA monomers were purchased from PerSeptive Biosystems. Decamers of the following nucleotide sequences were used: 1) GTAGATCACT (amino- to carboxamide or 5′- to 3′-terminal, in the text denoted as PNA1 and DNA1, respectively); and 2) AGTGATCTAC (PNA2 and DNA2). The decamers are complementary and can form DNA-DNA, PNA-PNA, and hybrid PNA-DNA duplexes:

GTAGATCACT

CATCTAGTGA

Calf thymus DNA and supercoiled plasmid pUC19 DNA were isolated as described (Lilley, 1985; Paleček and Postbieglová, 1986); the pUC19 isolation procedure included two CsCl-ethidium bromide gradient centrifugations (Sambrook et al., 1989). Histone from chick erythrocytes was a gift of Dr. M. Štros (Brno, Czechoslovakia).

Preparation of DNA-DNA and PNA-DNA duplexes

To prepare a DNA2-DNA1 duplex, a mixture of both DNA decamers, each at a concentration of 50 μg/ml in 50 mM NaCl, 5 mM sodium phosphate (pH 7.0), was heated to 95°C in a water bath. After 1 min the sample was allowed to slowly cool down at room temperature. PNA-DNA hybrids and PNA2-PNA1 duplex were prepared by mixing of equivalent amounts of the given components (each at a concentration of 20 μg/ml) in 5 mM sodium phosphate (pH 7.0). The samples were kept at room temperature for at least 1 h before they were used.

Immobilization of DNA and PNA at the electrode

To prepare PNA- or DNA-modified HMDE, we proceeded in the same way as in AdTSV (Paleček, 1988; Paleček and Fojta, 1994). The electrode was immersed in a 5-μl drop of solution of the respective substance and deposited on a parafilm support for adsorption time t_A (usually 120 s). If not stated otherwise, DNA was dissolved in 0.1 M NaCl and 5 mM sodium phosphate buffer (pH 7.0), and PNA was dissolved in 5 mM sodium phosphate (pH 7.0) under the above conditions; the concentration of 10 μg/ml of both DNA2 or PNA2 was sufficient to reach full electrode coverage. After the adsorption step, the modified electrode was washed and transferred into an electrochemical cell containing deoxygenated blank supporting electrolyte. Before the current circuit was closed, argon was passed through the electrolyte for 60 s. The potential scan followed immediately after the initial potential was set (without "quiescent" time), if not stated otherwise. Details were as published (Paleček and Fojta, 1994; Paleček et al., 1993; Paleček and Postbieglová, 1986). Measurements with plasmid DNA- or histone-modified HMDE were performed in the same way. In experiments with cooled sample solution, a drop of the sample was deposited on the top of an parafilm-covered aluminum cylinder (33 × 55 mm). The sample and the cylinder were precooled in an ice bath for at least 10 min.

AC impedance measurements

AC impedance (Z) measurements can be used for the detection as well as for quantitation of adsorption of surface active substances at the electrode surface (Ramsden, 1993, and references therein). Adsorption of the nucleic acid at the electrode results in a decrease in the capacity C (ΔC) below the value C_0 (Fig. 1) yielded by the bare electrode (immersed in the background electrolyte). The decrease $\Delta C = C_0 - C$ is related to the adsorbed amount of the given nucleic acid. The measurements were performed using an AUTOLAB (EcoChemie, Utrecht, The Netherlands), equipped with Frequency Response Analyzer Software, at the following settings: amplitude 10 mV, equilibration time 1 s, integration time 0.2 s. The C–E curves

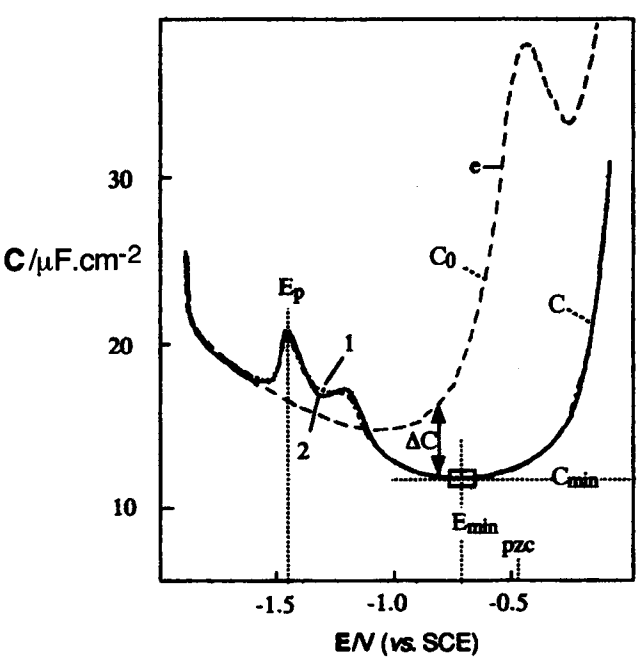

FIGURE 1 Comparison of conventional AdS and AdT C–E curves of denatured calf thymus DNA at full electrode coverage. *1*, AdS (electrode immersed in DNA solution); *2*, AdT (electrode with immobilized DNA layer immersed in blank supporting electrolyte); *e*, blank supporting electrolyte (0.3 M NaCl, 50 mM sodium phosphate, pH 8.5) measured with bare HMDE. DNA concentration 20 μg/ml; accumulation time t_A = 300 s. *pzc*, Potential of zero charge; E_p, peak potential; E_{min}, potential at which differential capacity C reaches its minimum value; C, differential capacity of the nucleic acid-modified HMDE at given potential; C_0, differential capacity of bare HMDE in the background electrolyte; $\Delta C = C_0 - C$. For details see Materials and Methods.

(dependence of the electrode double-layer capacity, C, on electrode potential, E) obtained at a frequency of 285 Hz are shown in this paper. Z was measured in 37-mV steps (corresponding to a scan rate of 8 mV/s) in the potential range from −0.05 V to −1.9 V, or in 30-mV steps (corresponding to 6.7 mV/s) in the range from −1.55 V to −0.05 V. A solution of 0.3 M NaCl, 50 mM sodium phosphate (pH 8.5) was usually used as the background electrolyte; measurements were performed at 4°C in a thermostatted cell against a saturated calomel electrode (SCE).

Potential of zero charge

The potential at which the charge is zero is called the potential of zero charge (pzc). pzc of a mercury electrode in 0.1 M KCl at 25°C is −0.506 V; at −1.6 V the charge of the electrode is $-20.03\ \mu C \cdot cm^{-2}$, and at −0.2 V the charge is $+11.50\ \mu C \cdot cm^{-2}$ (potentials against SCE) (Grahame et al., 1951). Experimentally the pzc can be determined from the dependence of the charging current on the electrode potential (Bard and Faulkner, 1980). By this method we have found that the pzc of the background electrolyte used in this paper (0.3 M NaCl, 0.05M sodium phosphate pH 8.5) is about −0.47 V at 4°C.

Calculation of surface coverage

The surface coverage (Θ) was calculated from the equation

$$\Theta = (C_o - C)/(C_o - C_s),$$

where the capacitance C_o corresponds to the bare electrode surface (i.e., the capacitance of the electrode double layer in the supporting electrolyte; Θ = 0), and C_s corresponds to the saturated coverage (i.e., Θ is not changed with the further increase in time of adsorption or with the higher concentration of surfactant, and is defined as Θ = 1; Frumkin, 1964). Near the potential E_{max} of maximum adsorption, the surface coverage does not change with the electrode potential E:

$$d\Theta/dE = 0,$$

so that at two different potentials (E_1 and E_2) close to the potential E_{max}, the value of Θ is not changed, and

$$\Theta_{E1} = \Theta_{E2}.$$

We have calculated the capacitance C_s corresponding to the saturated coverage using this relation; for DNA2 C_s = 10.75 $\mu F.cm^{-2}$, and for PNA2 C_s = 9.50 $\mu F.cm^{-2}$.

RESULTS AND DISCUSSION

We have shown (Paleček, 1986; Paleček and Fojta, 1994; Teijeiro et al., 1993) that the peaks of DNA obtained by cyclic voltammetry (CV) or differential pulse voltammetry (DPV) in the conventional way (with the electrode dipped in the DNA solution) do not substantially differ from those obtained by AdTS CV (Paleček, 1986; Teijeiro et al., 1993) and AdTS DPV, respectively (with DNA-modified electrode immersed in the blank background electrolyte; Paleček and Fojta, 1994). In agreement with the results of CV and DPV, the curves of denatured calf thymus DNA obtained by AdT and conventional Z measurements are almost the same (Fig. 1); no significant difference in the differential capacity, C, of the electrode double layer in a wide range of potentials was observed, and the heights of the adsorption/desorption peaks yielded by AdT were only slightly higher. The good agreement between the Z measurements with the electrode immersed in the analyzed solution and those obtained by AdT suggests that the observed peaks cannot merely correspond to adsorption/desorption peaks, usual in low-molecular mass compounds, reflecting a rapid exchange between the adsorbed and solution species (Jehring, 1974). In nucleic acids (and other polymers) these peaks can be due to adsorption/desorption of segments of the molecule (e.g., parts of DNA strands extending in solution may exchange rapidly with the trains of adsorbed DNA segments) (Paleček, 1983). In principle, the nucleic acid molecule may remain anchored at the surface, even if its major part extends to the solution; such a behavior of a DNA decamer is demonstrated at the end of this paper. To test the stability of the layers of PNA and DNA decamers, we adsorbed the given decamer (10 μg/ml, t_A = 120 s) at HMDE, and after the washing step we immersed the electrode in the background electrolyte for various periods of time t_B. Even after t_B = 30 min, no significant change in the AdTZ curves of DNA and PNA was observed. Repeated voltage scanning with the same PNA layer (three times) did not induce any change in the AdTZ curves (not shown). These results encouraged us to apply the AdTZ technique for the studies of the PNA and DNA decamers.

Adsorption of PNA strongly differs from DNA

The dependence of C on the polarization potential E measured (by the AdT technique) with single-stranded DNA2 and PNA2 decamers with identical sequence of nucleotides at full coverage of the electrode is shown in Fig. 2 (the experimental conditions under which the full coverage was reached are shown in Fig. 3). The C–E curve of the DNA decamer (Fig. 2, *curve 1*) shows in the potential range between 0.0 V and −1.0 V (against SCE) a typical decrease in C (ΔC) below the values of the bare electrode in the background electrolyte (C_0), followed by a peak with a peak potential E_p = −1.25 V (similar to calf thymus DNA, Fig. 1). ΔC observed with PNA in the potential range from 0.0 to −0.74 V (Fig. 2, *curve 3*) is smaller than ΔC produced by DNA in the same potential range (Fig. 2, *curve 1*). At potentials more negative than −0.7 V, ΔC of PNA strongly increases (whereas ΔC of DNA decreases).

PNA produced no peak, suggesting that the PNA molecules remained strongly adsorbed at the electrode surface through the potential range from −0.05 to about −1.8 V. The adsorption is so strong that the adsorption/desorption process is unable to respond to the excursions of the alternating voltage, and the desorption peak cannot be observed, as observed with polylysine (Miller and Grahame, 1956). The results obtained in the potential range from −0.05 to about −1.0 V are in good qualitative agreement with the ionic nature of the investigated decamers. It has been shown (Brabec and Paleček, 1972; Jelen and Paleček, 1985; Paleček, 1983) that single-stranded DNA is adsorbed at the mercury electrode in a wide potential range, mainly via the hydrophobic bases. At potentials more positive than pzc

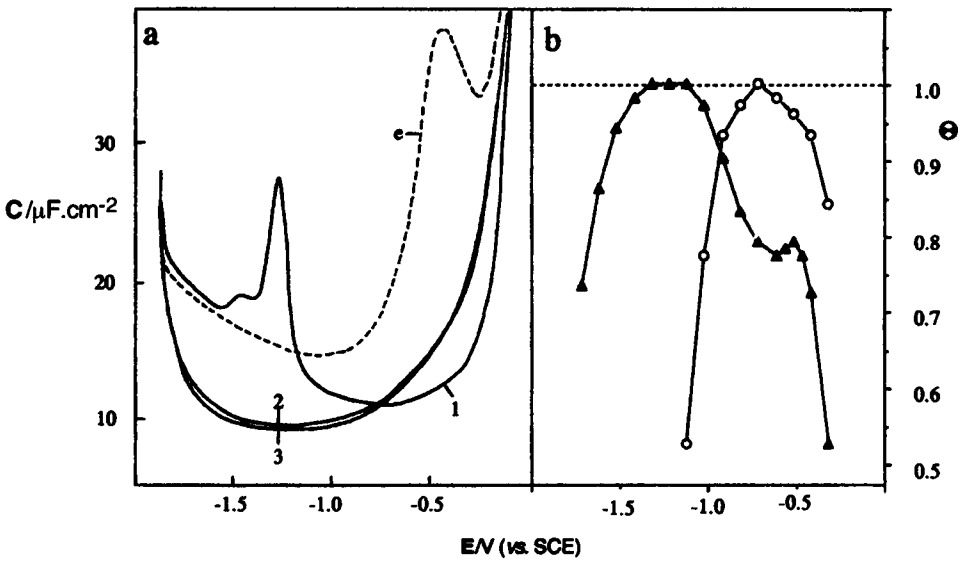

FIGURE 2 (*a*) AdT *C–E* curves of DNA and PNA decamers. The electrode was fully covered with *1*, DNA2; *2*, PNA1; *3*, PNA2. DNA at a concentration of 10 μg/ml (3.66 μM) was adsorbed from a 4-μl drop of 100 mM NaCl, 5 mM sodium phosphate (pH 7.0), and PNAs (10 μg/ml) were adsorbed from 5 mM sodium phosphate (pH 7); t_A = 120 s; the DNA or PNA solutions were cooled during adsorption and capacity measurements (see Materials and Methods). *e*, Blank background electrolyte. (*b*) The dependence of the degree of surface coverage, Θ (calculated from the curves shown in *a*), on electrode potential. ▲, PNA2; ○, DNA2.

(pzc of the background corresponded to −0.47 V; see Materials and Methods), the negative charges of DNA contribute to its adsorption (Brabec and Paleček, 1972; Brabec et al., 1996; Paleček, 1983, 1996). No such contribution can be expected for PNA with an electrically neutral backbone; moreover, the free amino group at the terminus of the PNA molecule is partially protonated and may be repelled from the positively charged surface. It is not surprising, therefore, that the adsorption of PNA at the positively charged surface is weaker than that of DNA. As the electrode potential is shifted to more negative values, the negatively charged DNA is repelled from the electrode, showing its adsorption/desorption peak at about −1.25 V. The PNA does not display any peak, and the minimum C (C_{min}, see Fig. 1) is observed at the potential corresponding roughly to the E_p of DNA1 (Fig. 2). The curve of PNA1 differs only slightly from that of the complementary PNA2. In agreement with the results obtained with calf thymus DNA (Fig. 1), conventional Z measurements of PNA1 and DNA1 (with the electrode dipped in the nucleic acid solution) produced *C–E* curves that were practically identical to those yielded by AdTZ (not shown). The measurements of PNA2 in 0.3 M $NaNO_3$ (i.e., in the medium not containing phosphate and Cl^- ions, which may specifically adsorb and interact at positive potentials with the electrode mercury) produced practically the same results (not shown).

From the capacitance-potential curve (Fig. 2 *a*) we have calculated the surface coverage Θ (Fig. 2 *b*). With DNA, Θ reaches its maximum at the potential around −0.6 V, i.e., near pzc. At this potential the DNA is adsorbed at the mercury surface through the hydrophobic bases. With PNA, the dependence of Θ on the electrode potential E shows two maxima. The first one around −0.5 V might correspond to the adsorption of PNA via bases similarly as with DNA. The second maximum appears near −1.2 V. Θ of PNA at this potential is higher than Θ at −0.5 V (Fig. 2 *b*), and C_s is lower for PNA (9.50 $\mu F.cm^{-2}$) than for DNA (10.75 $\mu F.cm^{-2}$), suggesting tighter packing of the PNA molecules at the surface.

PNA binds tightly to the surface of the negatively charged mercury electrode

Fig. 4 shows AdTZ curves of a positively charged histone and a supercoiled plasmid DNA (scDNA). The curve of scDNA resembles that of calf thymus (Fig. 1) and decamer DNAs (Fig. 2), showing, however, only a very small peak at −1.22 V, probably due to a limited accessibility of bases in a covalently closed circle of scDNA. Histone shows the smallest ΔC at the positively charged surface among the molecules tested. The C_{min} of histone (Table 1) is observed at about −0.79 V, i.e., ~140 mV more negative than the maximum ΔC of DNA. At −1.25 V PNA shows its C_{min}, and the adsorption of histone is strongly diminished (as compared to the potentials around −0.79 V). The presence of C_{min} of histone at potentials more negative than those of the C_{min} of DNA (Table 1) can be due to the net positive charge of the histone molecule; similar behavior was observed with polylysine (Miller and Grahame, 1956). The substantially more negative C_{min} of PNA (at about −1.25 V) is unusual and might be connected with an attraction between the PNA molecules at the electrode surface at higher surface coverages (Delahay, 1965).

Compared to DNA and histone, PNA shows the smallest C_{min}; this could mean that the adsorbed PNA has a lower dielectric constant and/or lower polarizibility or that PNA molecules are at the electrode in a more compact arrangement than DNA or histone. Such an arrangement would make the penetration of water molecules and the solvent ions to the electrode surface more difficult, thus giving rise to a lower C_{min} (Miller and Grahame, 1956). A more compact packing of PNA at the electrode can be expected because of the absence of the electric charge in the PNA

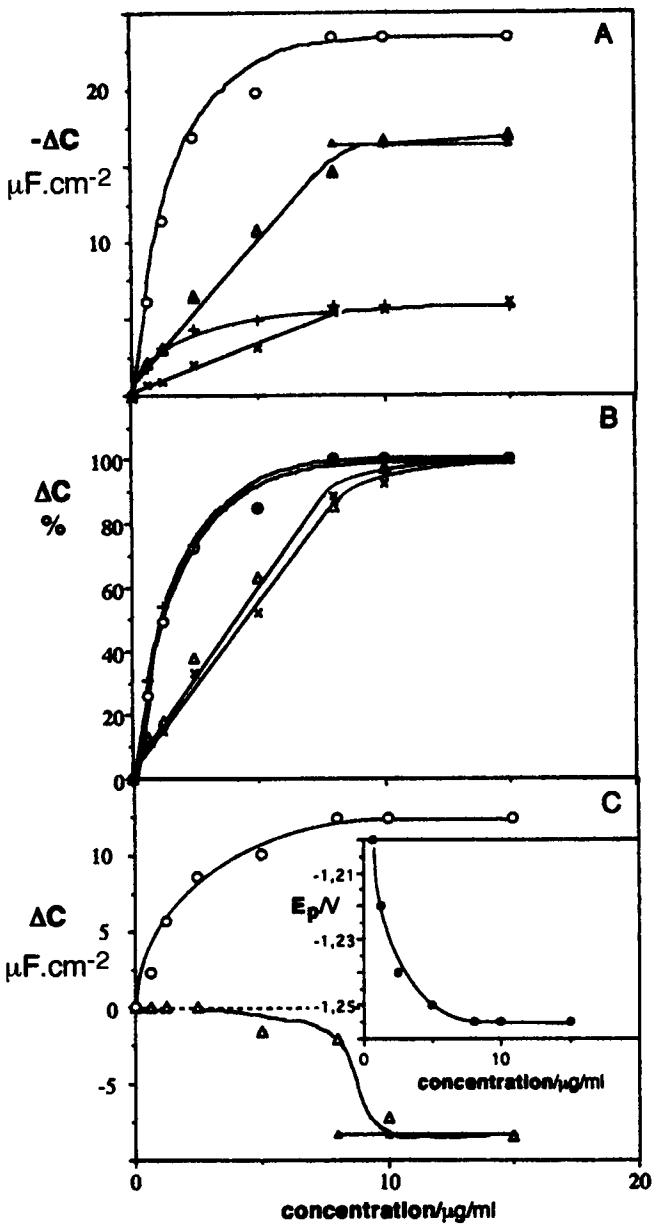

FIGURE 3 The dependence of ΔC on concentrations of PNA2 and DNA2. (*A*) Decrease in the capacity measured at −0.37 V (△, ▲, ○) and −0.80 V (×, +). (*B*) Plot of the same data shown as a percentage (values obtained at the NA concentration of 15 μg/ml were taken as 100%). (*C*) Height of the peak yielded by DNA2 (○) and ΔC produced by PNA2 at the potential of the DNA peak (−1.25 V) (△, ▲). (*Inset*) Concentration dependence of the DNA peak potential (E_p). DNA (○, +) was adsorbed from 100 mM NaCl, 5 mM sodium phosphate (pH 7); PNA was adsorbed from the above solution (△, ×) or from 5 mM sodium phosphate alone (▲). Adsorption was performed at room temperature. In *A*, $\Delta C = C - C_0$; other conditions were as in Fig. 2.

backbone, which prevents closer contacts between the adsorbed DNA molecules.

Intermolecular interactions of PNA at the surface are supported by the observed high surface coverage around −1.2 V (Fig. 2 *b*) and by the S-shaped concentration dependence of ΔC at −1.25 V (Fig. 3 *C*). The S-shaped form

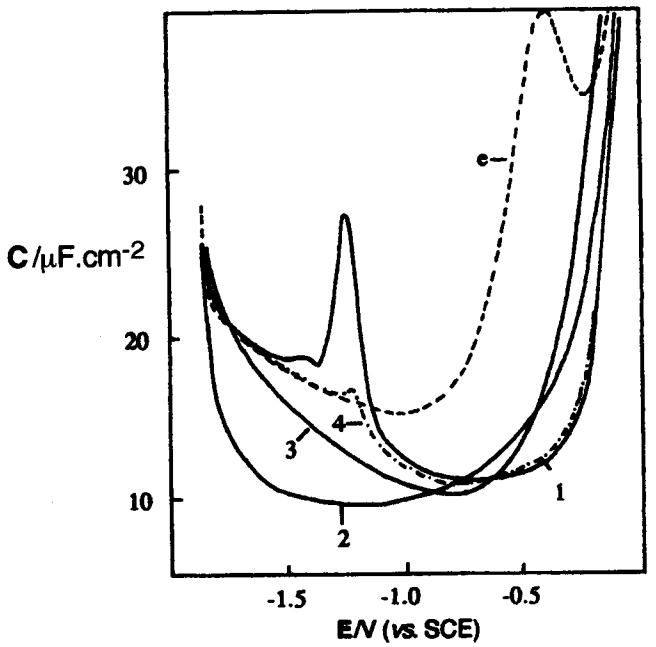

FIGURE 4 AdT *C–E* curves of DNA2 (*1*), PNA2 (*2*), histone (*3*), and supercoiled pUC19 plasmid DNA (*4*). Concentrations: DNA2 and PNA2, 10 μg/ml; pUC19 DNA, 110 μg/ml; histone, 30 μg/ml. DNAs were adsorbed (under conditions securing full coverage of the electrode) from 0.1 M NaCl, 5 mM sodium phosphate (pH 7), and PNA and histone were adsorbed from 5 mM sodium phosphate at room temperature. *e*, Background electrolyte. Other conditions were as in Fig. 2.

of the adsorption isotherm is considered evidence of large lateral attractions between the adsorbed molecules (Frumkin, 1964; Brabec et al., 1996). Such an isotherm was observed with nucleic acid bases, where the lateral interaction was so strong that the two-dimensional condensation of the adsorbed bases took place and a compact layer of the bases was formed at the surface (Retter et al., 1989; Vetterl, 1965, 1966, 1968; Vetterl and de Levie, 1991). Similar forces may act between the bases of the neighbor PNA molecules as a result of the close contact of the PNA molecules at the electrode surface. Under partial coverage of the electrode (Fig. 3), the C values at potentials more negative than −1.0 V are approaching those of the background ($\Delta C = 0$) (Fig. 5), whereas in the same curve an appreciable ΔC is observed at potentials around −0.8 V, suggesting that at high surface coverage the intermolecular interactions help to keep PNA adsorbed at the surface at highly negative potentials.

Dependence on concentration of PNA and DNA

The height of the DNA peak (at −1.25 V) and ΔC increase with DNA2 concentration, reaching the saturation level at the same concentration (Fig. 3 *C*). The peak potential (E_p) shifts with the DNA concentration to more negative values at partial surface coverage. This shift of E_p has been observed with many surface-active substances (Breyer and Bauer, 1963; Jehring, 1974). At negative potentials the

TABLE 1 Some parameters of PNA, DNA, and histone *C-E* curves*

	E_p (V)	E_{min} (V)	C_{min} (μF.cm^{-2})	$\Delta C_{-1.55}$ (μF.cm^{-2})	$\Delta C_{-0.24}$ (μF.cm^{-2})
PNA2	*x*	−1.14	9.20	−10.4	−11.75
DNA2-PNA1	−1.25	−0.82	9.75	0.00	−14.75
Histone	*x*	−0.79	9.75	−1.75	−4.00
DNA2	−1.25	−0.65	11.00	+0.25	−19.00

*For an explanation see Fig. 1, *x*, no desorption peak.

adsorbed substance is removed from the surface because of the competition with the cations and the solvent dipolar molecules strongly attracted to the electrode. For negatively charged molecules such as DNA, electrostatic repulsion from the (negatively charged) surface contributes to the desorption forces (Miller and Grahame, 1956, 1957). At higher concentrations of DNA a more solvent molecules and ions electrostatically attracted to the electrode are necessary to remove the adsorbed DNA from the surface, resulting in the shift of E_p of the DNA desorption peak to more negative values. The slope of the dependence of ΔC on the concentration of DNA is substantially steeper than that of PNA (Fig. 3, *A* and *B*), and higher concentrations of PNA are necessary to reach full coverage of the surface.

Dependence on ionic strength

Earlier we have shown that with chromosomal DNA moderate ionic strengths are necessary to obtain the most efficient adsorption at the negatively charged mercury electrode (Brabec and Paleček, 1972; Paleček, 1983; Paleček and Postbieglová, 1986). In agreement with this finding, the DNA2 decamer shows the lowest ΔC at −0.37 V if it is adsorbed from 5 mM sodium phosphate (pH 7.0) in the absence of NaCl (Fig. 6). More efficient adsorption, manifested by larger ΔC, is observed with increasing concentration of NaCl up to 100 mM; in the range from 100 mM to 500 mM, ΔC is almost independent of NaCl concentration. In contrast, the PNA decamer displays the highest ΔC in the absence of NaCl and a decrease in adsorption with increasing NaCl concentration up to 50 mM; at higher NaCl concentrations the PNA adsorption is almost independent of the ionic strength.

PNA and DNA are anchored at the electrode, even at highly negative potentials

At potentials more negative than the DNA peak potential, ΔC of DNA is zero, which might suggest that DNA is fully desorbed from the surface. Such a conclusion is usually correct for low-molecular-mass compounds, but need not be true with polymers and oligomers, which may remain an-

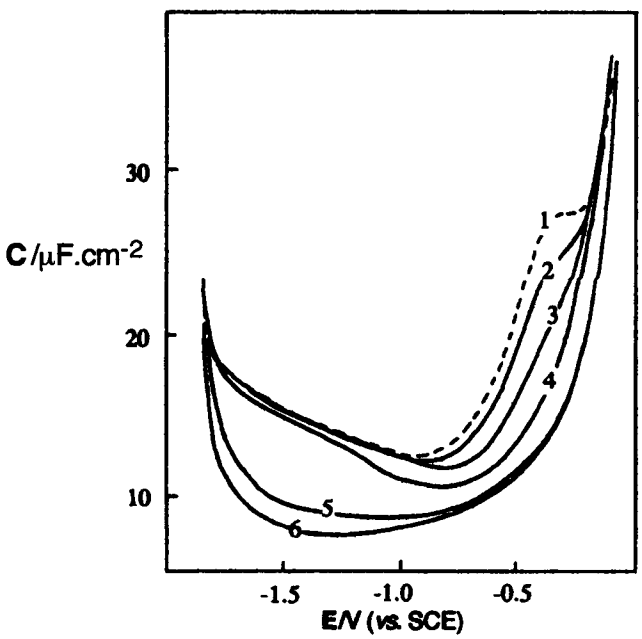

FIGURE 5 Dependence of AdT *C–E* curves on PNA2 concentration. Before the transfer into the supporting electrode, the HMDE was immersed in 0.1 M NaCl, 5 mM sodium phosphate (pH 7) containing PNA2 at concentrations of 0 μg/ml (*1*), 1.25 μg/ml (*2*), 2.5 μg/ml (*3*), 5 μg/ml (*4*), 10 μg/ml (*5*), and 15 μg/ml (*6*) at room temperature for 120 s. Other conditions were as in Fig. 2

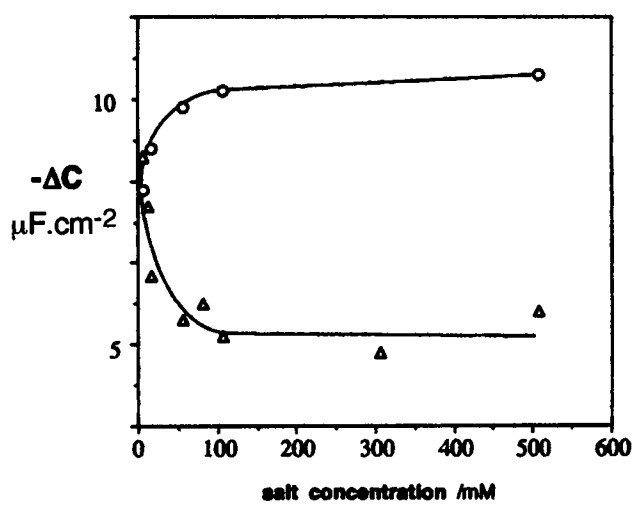

FIGURE 6 Dependence of ΔC at −0.39 V of PNA (△) and DNA (○) on the ionic strength of solution from which PNA and DNA were adsorbed at HMDE. The solutions contained 5 mM sodium phosphate buffer (pH 7.0) and NaCl at concentrations given in the graph; t_A = 120 s, concentration of PNA or DNA 5 μg/ml, adsorption at room temperature. Other conditions were as in Fig. 2.

chored at the surface by a small portion of the molecule (which negligibly influences C), with the rest of the molecule extending to the solution. To test whether the DNA is present at the electrode surface at highly negative potentials, we adsorbed DNA2 at open current circuit and scanned the potential either from −0.05 V in the negative direction or from −1.55 V to positive potentials. In both cases we observed characteristic C–E curves of DNA, but scanning to positive potentials resulted in a smaller peak (at E_p by 40 mV less negative) and smaller ΔC (Fig. 7 *A*) than with the potential scanning in the opposite direction. In the same experiment with PNA2, the C–E curves were almost independent of the scanning directions (Fig. 7 *B*). These results suggest that at −1.55 V both DNA and PNA are still tethered to the electrode. We exposed the electrode with the adsorbed NA layer to −1.55 V for different periods of time from 0 to 300 s; after 60 s and 300 s the ΔC of DNA at −0.37 V corresponded to 85% and 16%, respectively, of its original value, in contrast to the ΔC of PNA, which remained almost unchanged, even after 300 s (Fig. 8). At both the full and partial coverages of the electrode, ΔC of PNA at −0.37 V was almost independent of time of exposure to −1.55 V up to 300 s. We may thus conclude that prolonged exposure of DNA to highly negative potentials results in a full removal of DNA decamers from the surface, whereas PNA molecules remain adsorbed under the same conditions.

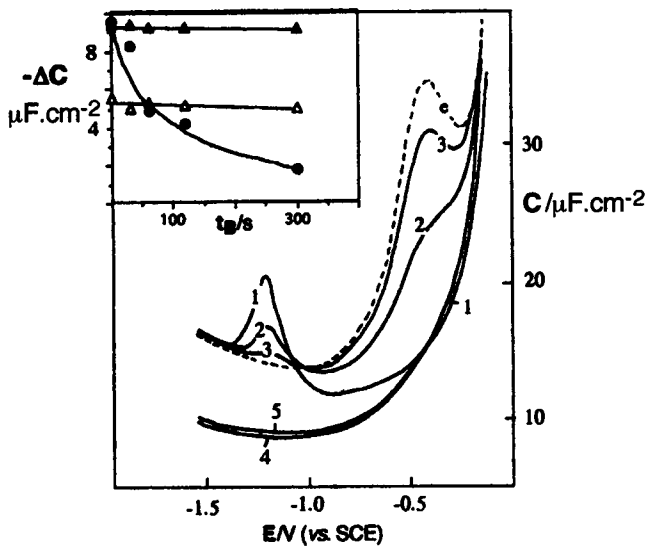

FIGURE 8 Influence of the time t_B on AdT C–E curves of DNA2 (*1–3*) or PNA2 (*4, 5*). The HMDE was fully covered with DNA or PNA before the potential E_B (−1.55 V) was applied for the time t_B. After the elapse of time t_B the potential was scanned from −1.55 V to −0.05 V; t_B = 0 (*curves 1, 4*), t_B = 60 s (*curve 2*), t_B = 300 s (*curves 3, 5*). (*Inset*) Dependence of ΔC at −0.39 V on t_B. ●, DNA2, full coverage; ▲, PNA2, full coverage; △, PNA2, partial coverage (4 μg/ml PNA in 5 mM sodium phosphate, t_A = 90 s, room temperature). Other conditions were as in Fig. 2.

Adsorption of DNA-PNA duplexes

The C–E curves of the PNA single-stranded decamers and the PNA duplex do not differ substantially (Figs. 2, 3, 9). Furthermore, the differences between the C–E curves of the DNA single strands (Figs. 2 and 3) and the DNA1-DNA2 duplex (Fig. 9) are rather small. On the other hand, the C–E curves of the hybrid PNA1-DNA2 and DNA1-PNA2 duplexes (Fig. 9) differ markedly from both PNA and DNA. Composed of one polyanionic DNA strand and one PNA strand with an electrically neutral backbone, the PNA1-DNA2 and DNA1-PNA2 duplexes display, in the ranges −0.05 to −0.42 V and −0.95 to about −1.6 V, ΔC values intermediate between those of DNA and PNA. In the range from −0.42 to −0.95 V, the ΔC values of the DNA-PNA duplexes are higher than those of DNA-DNA or PNA-PNA duplexes. The C–E curve of a mixture of PNA2 and DNA2 almost coincides with that of the hybrid duplexes in the range from −0.05 to about −1.1 V; the peak of the hybrid

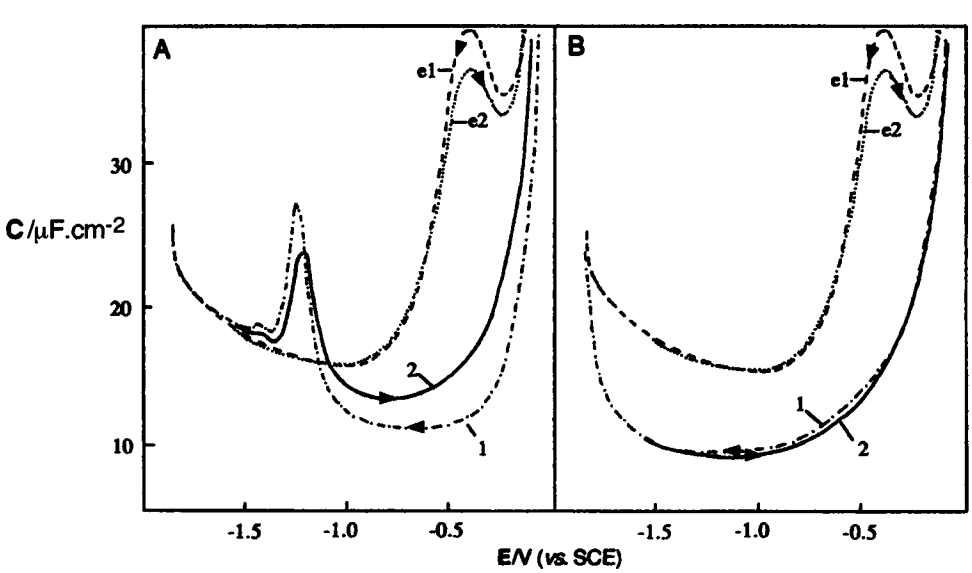

FIGURE 7 Influence of direction of the potential scan on the AdT C–E curves of HMDE modified with DNA2 (*A*) and PNA2 (*B*). Before the measurements, HMDE was fully covered with DNA or PNA. Immediately after closing of the circuit, the potential of the electrode was scanned either from −0.05 V to −1.9 V (*curves marked 1*) or from −1.55 V to −0.05 V (*curves marked 2*). *e1*, *e2*, Background electrolyte measured in the respective direction. Other conditions were as in Fig. 2.

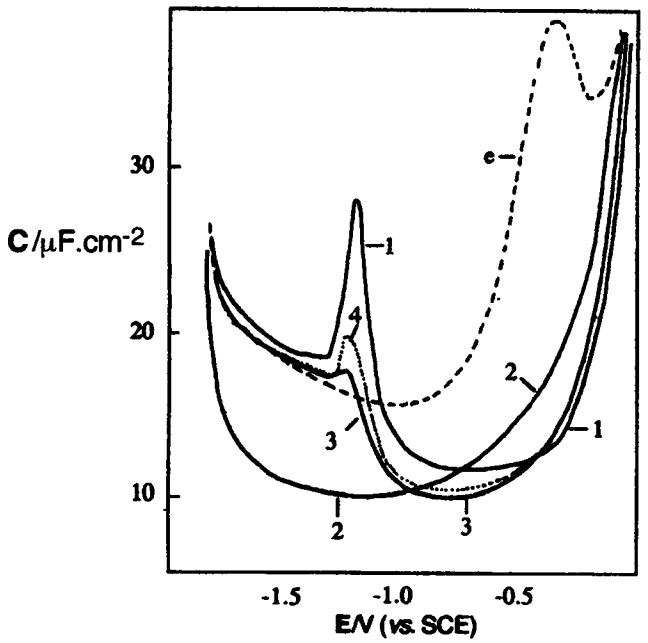

FIGURE 9 AdT *C–E* curves of DNA2-DNA1 (*1*), PNA2-PNA1 (*2*), and DNA1-PNA2 (*3*) duplexes. The duplexes (20 μg/ml) were adsorbed from 5 mM sodium phosphate (pH 7) for t_A = 120 s. During the adsorption, the sample solutions were cooled (see Materials and Methods). *4*, The *C–E* curve of a mixture of PNA2 and DNA2 (both at a concentration of 10 μg/ml) was measured under the same conditions. Other conditions were as in Fig. 2.

DNA-PNA (E_p = −1.24 V) is, however, substantially smaller than that produced by the mixture of the noncomplementary PNA and DNA decamers (Fig. 9). The marked difference in the adsorption behavior of the hybrid as compared to PNA and DNA alone represents an interesting possibility of selective separation of the hybrid molecules at the surface. Apparently the PNA moiety facilitates the binding of the hybrid to the surface.

CONCLUSIONS

Electrostatic interactions of DNA with positively charged surfaces are frequently considered in the literature (Clemmer and Beebe, 1992; Hansma et al., 1996). DNA can also be adsorbed at a negatively charged surface, as demonstrated earlier with chromosomal DNA (Brabec and Paleček, 1972; Paleček, 1983) and with DNA decamers in this paper. The adsorption of DNA and PNA is due to strong hydrophobic interactions of bases with the negatively charged mercury surface. At highly negative potentials DNA molecules are slowly removed from the surface (Figs. 7 and 8). In contrast to the negatively charged DNA, molecules of the PNA remain firmly adsorbed at the negatively charged surface (under the conditions where DNA is desorbed) (Figs. 2 and 4 and Table 1). This strong adsorption is due not only to the strong hydrophobic interactions of PNA (which, in contrast to DNA, is not electrostatically repelled from the negatively charged surface) with the surface, but also to the strong lateral interactions between the neighboring PNA molecules (Figs. 3 *C* and 5). Strong interactions of the DNA or PNA bases adversely affect the efficiency of hybridization at the negatively charged mercury surfaces (Cai et al., 1997). On the other hand, positively charged carbon surfaces are suitable for the DNA hybridization sensors (Mikkelsen, 1996; Wang et al., 1996; Cai et al., 1997).

Recently we have proposed a potentiometric sensor for DNA hybridization that is capable of differentiating between fully matched and single base mismatched PNA-DNA hybrids, showing properties superior to those observed earlier with voltammetric DNA sensors (Wang et al., 1996). In this sensor PNA immobilized at the carbon electrode was hybridized with oligonucleotides in solution. Experiments with mixtures of large pieces of DNA used in practical hybridization analysis have not been yet performed. It may be expected that large pieces of DNA nonspecifically attached to the electrode may interfere with the analysis. This paper suggests that exposing the electrode to negative potentials might result in the removal of this DNA, but not of the PNA-DNA hybrid from the surface.

It has been shown that the adsorption behavior of single-stranded chromosomal (Brabec and Paleček, 1972; Paleček, 1983) and plasmid DNAs (Paleček, 1996) differs strongly from that of double-stranded DNA. The *C–E* curves of single-stranded and double-stranded decamers (Fig. 9) do not, however, substantially differ from each other. This result can be attributed to a strong effect of the duplex ends (in a relatively short decamer molecule), which might be more open (or even denatured) at the surface than in solution.

In this paper we have described the AdTZ technique, which is capable of measuring submicrogram samples. This technique offers new possibilities for the analysis of the interfacial behavior of a number of samples that are not available in large quantities, including nucleic acid–protein complexes and chemically modified DNAs and RNAs.

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