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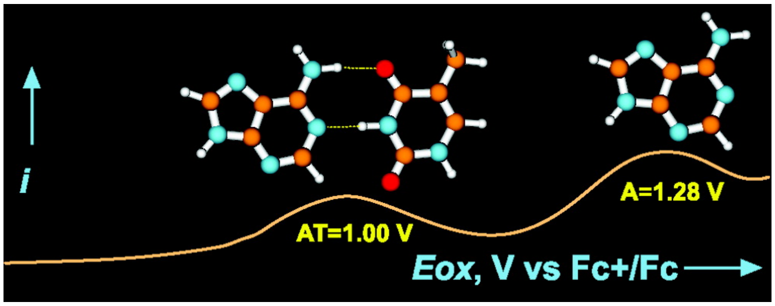
J. Am. Chem. Soc., **2007**, 129 (49), 15347-15353 • DOI: 10.1021/ja076181n



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The Oxidation Potential of Adenosine and Adenosine-Thymidine Base Pair in Chloroform Solution

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Abstract: The oxidation potential of 2′,3′-*O*-isopropylidene-5′-*O*-(*tert*-butyldimethylsilyl)adenosine and its change upon pairing with the complementary base thymidine have been studied in chloroform solution by voltammetric methods. Differential pulse and cyclic voltammetry of an equimolar solution of the two nucleosides show two well-resolved peaks, which have been assigned to the formation of a H-bonded complex between the two nucleosides and to the fraction of unassociated adenosine in solution. No signal for thymidine oxidation has been detected. Equilibrium constants and enthalpy and entropy changes for self- and hetero-association in solution have been determined by NMR spectra. Quantum mechanical computations show that Hoogsteen and Watson−Crick association modes are of comparable stability in solution, and exhibit very similar oxidation potential in chloroform.

Introduction

The redox properties of the DNA bases are of outstanding importance in the context of DNA damage caused by ionizing radiation and oxidizing agents.[1−3] One-electron oxidation of duplex DNA gives rise to a radical cation which easily migrates through the nucleobases; experimental evidence for long-range hole transfer over distance scales of 40−200 Å has been reported several times in the literature.[4−10]

Hole migration usually ends up at a guanine (G) site, because G is the nucleobase with the lowest oxidation potential,[11,12] thus acting as a trap, i.e. a lower-energy site, for hole transport in DNA. The knowledge of the redox properties of the single constituents of DNA is therefore important for understanding hole transport but still not sufficient; local environmental effects are also important. It is in fact well-known that in duplex DNA the redox properties of guanine (G) are significantly affected both by π-stacking interactions with other nucleobases and by the pairing with its complementary base cytosine (C).[13−17] Because of resonance, π-stacking effects are particular relevant for sites constituted by a sequence of identical nucleobases. Photocleavage experiments,[13] and photoelectron spectroscopy[12] have shown that π-stacking interactions between two or more consecutive G's give rise to sites with lower oxidation potentials, where damages caused by photoionization are preeminently observed. In particular, photoirradiation of suitable photosensitizers selectively generates labile sites at the 5′-G of a 5′-GG-3′ sequence. The recurrence of this 5′-GG-3′ specificity has been observed in several experiments with different photocleaving species, thus suggesting that the common pattern does not originate from a particular binding orientation of the photocleaving species but rather from an intrinsic, structural property of the 5′-GG-3′ sequence.

The effect of the complementary base pairing on the oxidation potential of G was first predicted by theoretical computations[18] and then experimentally observed for guanosine (Gs) and cytidine (Cd) derivatives in chloroform, a solvent in which the association constant for the formation of the Watson−Crick Gs:Cd H-bonded complex, the most stable one among all the possible H-bonded complexes,[19−21] is sufficiently high to allow its detection.[22] The change of the oxidation rate of Gs upon Cd pairing, determined via quenching of triplet absorption of suitable chromophores (*N*,*N*′-dibutylnaphthaldiimide and fullerene), suggested that oxidation of the Gs:Cd pair occurs at lower

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10.1021/ja076181n CCC: $37.00 © 2007 American Chemical Society

potential than that of unassociated or, more probably, self-associated Gs.[15,16] This finding was then confirmed by voltammetric measurements; the voltammogram of an equimolar solution of Gs and Cd is characterized by two well-resolved peaks, one falling at the same potential observed for solutions containing only Gs (0.91 V vs ferrocenium/ferrocene (Fc^+/Fc) half-couple used as internal reference) and the other (falling at lower potential, 0.57 V vs Fc^+/Fc) which has been attributed to the formation of the Watson−Crick H-bonded complex.[17]

Much less is known about the redox properties of adenine (A) in single-strand and duplex DNA, even though their knowledge can be important for a better comprehension both of the long-range hole-transfer process in DNA, and of the photoinduced oxidative damages in DNA oligomers which lack guanine.[1] In this paper we report the first voltammetric measurements of the effect of the complementary base pairing on the oxidation potential of adenine.

Following our previous investigation on redox properties of guanine-cytosine pair,[17] we have carried out electrochemical investigations of silylated adenosine (As) and thymidine (Td) derivatives in chloroform solution, a solvent in which, according to previous studies,[23] adenine and thymine mainly form H-bonded complexes, with the hetero-association equilibrium constant much larger than those of self-association. Equilibrium constants of self- and hetero-association have been determined by NMR titrations in the same high ionic strength conditions used in electrochemical measurements, and from their temperature dependence, association enthalpies and entropies have been obtained. H-bond hetero-association of As and Td is more complicated than that of Gs and Cd because the former pair can form several H-bond complexes, all of which were predicted to be of comparable stability in solution. We have therefore integrated the electrochemical measurements by a theoretical analysis mainly aimed to gain a better understanding of the structural aspects of the As-Td association equilibria in solution.

Materials and Methods

2′,3′-*O*-Isopropylideneadenosine and thymidine (Sigma-Aldrich) were derivativized with *tert*-butyldimethylsilyl groups on the ribose unit to yield 2′,3′-*O*-isopropylidene 5′-*O*-(*tert*-butyldimethylsilyl)-adenosine (As′) and 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)thymidine (Td′). A quantity of 0.54 g (3.6 mmol) of *tert*-butyldimethylsilyl chloride (TBDMSCl) was added to a solution of 2′,3′-*O*-isopropylideneadenosine (1.0 g, 2.4 mmol) in pyridine (10 mL). The reaction mixture was stirred at room temperature for 72 h and then evaporated to dryness. The residue dissolved in chloroform was washed with 1 M HCl (50 mL) and water (3 × 50 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by crystallization from hexane/acetate and characterized via ^1H NMR. The crystallization afforded As′ (89%). A quantity of 0.96 g (6.4 mmol) of TBDMSCl was added to a solution of thymidine (1.0 g, 2.1 mmol) in dimethylformamide (DMF) (10 mL) and pyridine (0.5 mL); after 48 h at room temperature, DMF was removed from the reaction mixture by washing several times with a mixture of HCl 0.1 M and chloroform, until complete removal of DMF from the crude yield. The organic layer was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by crystallization from hexane/acetate and characterized via ^1H NMR. The crystallization afforded Td′ (97%).

Voltammetric measurements were carried out by Metrohm 757 VA, by using both platinum and glassy carbon working electrodes. The supporting electrolyte, tetrabutylammonium perchlorate (TBAP), was selected because of its purity, solubility, and electrochemical stability in $CHCl_3$. The samples were accurately purged from dissolved oxygen by bubbling N_2 for 5 min. Ferrocenium/ferrocene half couple (Fc^+/Fc) was used as internal reference in all measurements.

The equilibrium constants for self-association of As′ and Td′, and of the heterodimer As′:Td′ have been evaluated in $CDCl_3$ solution containing 0.1 M TBAP, via H NMR titrations, by using the procedure outlined in ref 24.

All computations have been carried out by using Gaussian 03[25] and Spartan 04[26] packages. Ionization potentials of the two nucleosides and their pairs and association energies have been computed by using density functional theory, based on the hybrid B3LYP exchange correlation potential.[27] It has in fact been shown that, at least in the case of hydrogen-bonded nucleobase associations, this method gives results in very good agreement with those obtained by highly correlated methods,[28] especially for ionization potentials,[29] the property which we are mostly interested in. Use of more accurate ab initio methods, such as coupled cluster with single and double and perturbative triple excitations CCSD(T), already employed in theoretical studies of nucleobase associations,[30] is ruled out by the size of the two nucleosides used in this work. Electrostatic effects due to solvent polarization have been estimated by using the standard polarizable continuum model (PCM),[31] as implemented in Gaussian 03. For nucleobases the 6-311++G** triple-ζ basis set has been adopted both for geometry optimization and energy computations, because it represents the best compromise between reliability and computing time.[29] For nucleosides geometry optimizations started from the lowest-energy conformations obtained by molecular mechanics computations, carried out by using the MMFF force field,[32−34] implemented in Spartan 04. Because of their size, geometry optimizations have been carried out with the smaller 6-31G* double-ζ basis set, except for atoms engaged in H-bonds for which the 6-31++G** has been employed; computations of vibrational frequencies were also ruled out by the size of the systems. Accurate energies and ionization potentials have been evaluated by single-point computations with the same triple-ζ basis set used for nucleobases.

Results

Electrochemical Measurements. The differential pulse voltammograms of a 2 mM solutions of As′ and of Td′ in chloroform, and of an equimolar solution of As′ and Td′ in $CHCl_3$ containing 0.1 M TBAP are reported in Figure 1a and b, respectively. In $CHCl_3$ oxidation of As′ occurs at 1.28 V vs Fc^+/Fc, roughly 1.99 V vs NHE.[35,36] No signal attributable to Td′ oxidation was detected in the allowed potential window, in line with previous measurements of As and Td redox potentials in nonaqueous solution.[37]

The voltammogram of the solution containing an equimolar amount of As′ and Td′ shows two well-resolved peaks, one occurring at the same potential observed for solutions containing

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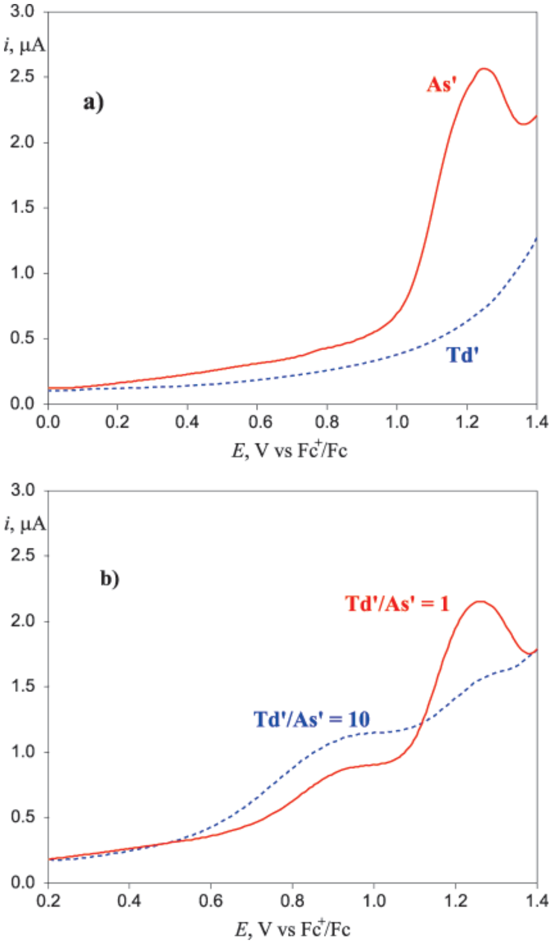

Figure 1. Differential pulse voltammograms of nucleoside derivatives in $CHCl_3$ at 298 K on glassy carbon electrode. (a) Solutions containing only As′ 2.0 mM (full red line), and only Td′ 2.0 mM (dashed blue line); (b) solutions containing As′ 2.0 mM and Td′ 2.0 mM (full red line) and As′ 2.0 mM and Td′ 20.0 mM (dashed blue line). Scan rate, 100 mV/s.

only the As′ nucleoside, which can therefore be assigned to the fraction of free As′ in solution, the other occurring at 1.0 V. The peak current of the oxidation process occurring at lower potential significantly increases upon increasing the concentration of Td′, cf. Figure 1b; thus, in strict parallelism with the case of guanosine and cytidine nucleosides,[17] the peak at lower potential is assigned to the 1:1 As′:Td′ H-bond complex. The pairing with the complementary base thus decreases the oxidation potential of As′ by ca. 0.28 V, the effect being slightly smaller than that observed for the guanosine-cytidine pair (0.34 V).[17]

The cyclic voltammetries of As′, and of an equimolar solution of As′ and Td′, see the Supporting Information, show that the oxidation of all the nucleosides in chloroform are irreversible processes, and therefore the potentials reported here cannot be considered as standard oxidation potentials, since they do not refer to equilibrium conditions.

From the equilibrium association constants, determined in $CDCl_3$ solution containing 0.1 M TBAP (see infra), when equal volumes of 1×10^{-3} M solutions of As′ and Td′ are mixed at 25 °C, in the electrochemical cell under operational conditions 5.3% of each dissolved substance form the heterodimer, 0.5% of Td′ and 0.3% of As′ form the homodimers, and 93.6% of Td′ and 94.1% of As′ are left unassociated. The above equilibrium concentrations of associated and free As′ fit well with the relative height of the peak current in the voltammograms of Figure 1.

Table 1. Thermodynamic Properties for Nucleoside Hetero- and Self-Association

	K_{ass} (M^{-1})	ΔH_{ass} (kcal/mol)	ΔS_{ass} (eu)
As:Td	60 (130^a, $60-73^b$, 74^c 33^d 70^e)	−5.9 (-6.2^f)	−11.6 (-11.8^f)
Td:Td	6.0 (3.2^a, $2.2-3.2^b$, 6.8^g, 3.5^d, 12.7^h)	−4.2 (-4.3^f)	−10.7 (-10.7^f)
As:As	3.1 (3.1^f, 1.4^g, 2.4^d, 2.3^e)	−4.0 (-4.0^f)	−11.3 (-11.4^f)

[a] Reference 23. [b] Reference 38. [c] Reference 40. [d] Reference 41. [e] Reference 42. [f] Reference 39. [g] Reference 43. [h] Reference 44.

Equilibrium Constants. There are several studies in the literature dealing with the self- and hetero-association of A and thymine (T) derivatives in chloroform solution.[23,38−44] From IR spectra it was inferred that A and T forms H-bonded heterodimers much stronger than homodimers:[23,43,44] the equilibrium association constant for the 1:1 H-bonded complex between 9-ethyladenine and 1-cyclohexylthymine (K_{AT}) was estimated to be 130 M^{-1}, much larger than the two self-association constant $K_{AA} = 3.1$ M^{-1} and $K_{TT} = 3.2$ M^{-1}. NMR spectroscopy gave a lower value for K_{AT} (60−73 M^{-1}),[38] still significantly higher than self-association constants, and a similar value (70 M^{-1}) has been obtained recently for the association of substituted 2′-deoxyuridine and 2′-deoxyadenosine in $CDClF_2/CDF_3$ solution.[42]

In order to determine the effects of the high ionic strength conditions used in electrochemical measurements upon the association constants, the equilibrium constants for self-association of As′ and Td′, and for As′:Td′ heterodimer have been reevaluated in $CDCl_3$ solution containing 0.1 M tetrabutylammonium perchlorate via ^1H NMR titrations. From the temperature dependence of ΔG^0_{ass} around 298 K, the enthalpy and entropy changes for the formation of the H-bonded complex have also been obtained. All the results are reported in Table 1, together with the values reported previously in the literature for nucleobases and similar nucleosides. The equilibrium association constant for the As′:Td′ complex is 60 M^{-1}, very similar to those obtained by NMR spectroscopy for substituted nucleobases and nucleosides. The self-association equilibrium constants are both 1 order of magnitude lower than hetero-association, in line with those obtained previously for nucleobases. Thus, the high ionic strength conditions do not alter significantly the association equilibria, as already found previously for the guanosine-cytidine pair.[17]

Contrarily to guanosine-cytidine H-bonded pair, for which the Watson−Crick association form is much more stable than all other possible H-bonded complexes,[19,21] adenosine and thymidine can form at least four cyclic structures, shown in Figure 2, depending on which of the two ring nitrogens of adenine and of the two carbonylic oxygens of thymine are used as proton acceptor sites in the H-bond complex. All of the association modes are expected, both from experimental and computational evidence, to be of comparable stability.[30,41]

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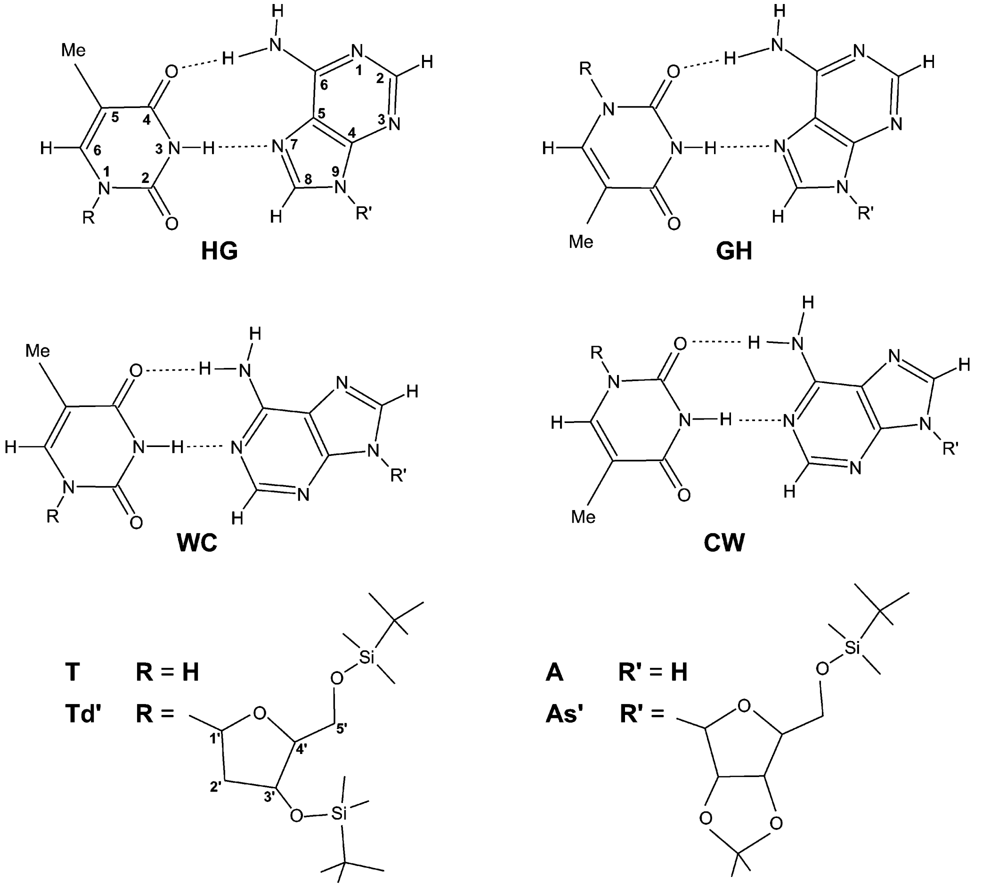

Figure 2. Thymidine-adenosine H-bonded cyclic complexes. HG = Hoogsteen; GH = reversed Hoogsteen; WC = Watson and Crick; CW = reversed Watson and Crick. Substituents and standard numbering.

The Watson−Crick complex is the usual association form in DNA, whereas both the Hoogsteen (HG) and the reversed Hoogsteen (GH) configurations are more commonly found in crystals of A-T, and more rarely in biological systems.[45] The crystal structure of 9-methyladenine and 1-methylthymine showed that the two nucleobases are associated in a Hoogsteen-type configuration,[46] and the same form of association was found in the solid state for the d(ATATAT) oligonucleotide, but when crystals were dissolved in water the oligomer showed a standard B-form conformation, in which all bases form Watson−Crick pairs throughout the duplex.[47] Quantum mechanics computations employing highly correlated ab initio methods have shown that in $CHCl_3$ 1-methylthymine and 9-methyladenine form an H-bond complex, whose minimum energy nuclear configurations is a slightly nonplanar, reversed Hoogsteen-type configuration, slightly more stable than all the others.[20]

For unsubstituted nucleobases the situation is further complicated by the presence of other two acidic protons; noteworthy, IR and UV spectra gave indication that in the gas-phase neither the Watson−Crick nor the Hoogsteen-type of association are observed, but rather A and T form an H-bonded complex involving just those protons which in nucleosides are substituted by ribose rings.[48]

In chloroform solution, neither IR nor NMR spectroscopy have been able to discriminate between the four association forms of Figure 2 at room temperature, because of their fast interconversion rates. The 2D NOE spectrum of an equimolar solution of substituted 2′-deoxyuridine and 2′-deoxyadenosine recorded in freon mixture showed that the Watson−Crick association forms are predominant in solution at low temperature (125 K), but at higher temperature ^{15}N NMR spectra gave evidence that the two Hoogsteen forms are more abundant in solution.[42] Our own 2D NOE spectrum of a mixture of As′ with Td′ at 298 K in $CDCl_3$ with a 400 ms mixing time reported in Figure 3 shows a cross-peak of imino signal at 11.84 ppm with the two adenosine amino resonances at 5.91 ppm. No exchange cross-peaks for the N3 proton of Td′ and the H2 and H8 adenosine protons are observed. These features can be interpreted in terms of a rapid interconversion between WC and HG forms occurring without disrupting the stronger N−H···O hydrogen bond. Thus, on the basis of the experimental evidence discussed above, the oxidative process observed at lower potential for solution of As′ and Td′ cannot be assigned to a specific association form but rather to a mixture of Watson−Crick and Hoogsteen association modes.

Theoretical Analysis. For better assessing this point, we have resorted to theoretical computations. Indeed, in cases such as this one, where several species could be involved and measurement assignments can pose problems, theoretical computations can be of help for trying to disentangle the skein and hopefully for gaining hints for designing new experiments.

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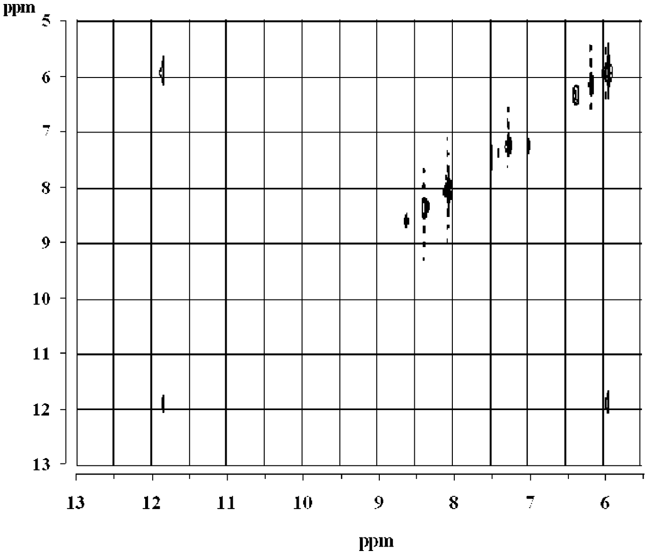

Figure 3. Portion of the NOESY spectrum of a mixture of As′ (35 mM) with Td′ (5 mM) at 298 K in $CDCl_3$, 400 ms mixing time, showing the cross-peak of imino signal at 11.84 ppm with the two adenine amino resonances at 5.91 ppm.

There are several theoretical papers which have addressed the problem of adenine-thymine association in the gas phase and in solution, but unfortunately only a few of them dealt with ionization potentials,[18,49−56] and no one, to the best of our knowledge, considered the effect of the complementary base pairing on the ionization potential of adenosine in chloroform. We have therefore carried out theoretical computations of the oxidation potential of As′ and Td′ and of their Hoogsteen and Watson−Crick association forms in $CHCl_3$. Because many experimental data are available only for nucleobases, and because the size of the two nucleosides prevents computations of vibrational frequencies, we have also performed computations on nucleobases—for which the vibrational frequencies of the four H-bonded complexes can be computed at reasonable costs—both for having a direct comparison between computed and observed quantities, an important point for judging the reliability of the adopted computational procedure and for obtaining an estimate of the vibrational contributions to nucleoside association energies.

The optimized geometries of the two nucleosides in chloroform are shown in Figure 4. For both nucleosides the silylated groups are sufficiently far from the nucleoside aromatic ring to prevent a direct interaction between them. The optimized geometries of the four H-bonded complexes are reported in the Supporting Information.

Both the Hoogsteen and the Watson−Crick association modes have been found as minimum energy points by standard geometry optimizations. The computed ionization potentials, both adiabatic and vertical, and the binding energies for both neutral and one-electron oxidized H-bonded complexes together with the available experimental results are reported in Table 2 and in Table 3, for nucleosides and nucleobases, respectively.

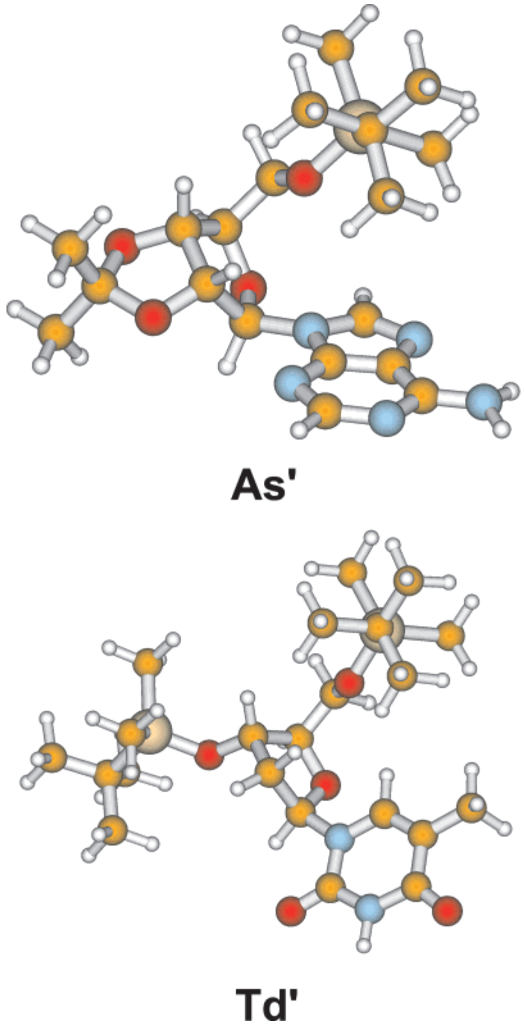

Figure 4. Optimized geometries of As′ and Td′ in chloroform.

Table 2. Computed Ionization Potentials (IP, eV) and Association Energies (kcal/mol) of As′:Td′ Watson and Crick and Hoogsteen-Type H-bonded Complexes

	gas phase			$CHCl_3$		
	ΔE_{ass}^{TA}	ΔE_{ass}^{TA+}	IP $_{adiab(vert)}$	ΔE_{ass}^{TA}	ΔE_{ass}^{TA+}	IP $_{adiab(vert)}$
As′	–	–	7.53 (7.87)	–	–	6.35 (6.60)
Td′	–	–	7.84 (8.10)	–	–	6.72 (7.04)
WC	−12.89	−23.36	7.07 (7.25)	−5.72	−7.24	6.28 (6.50)
CW	−12.07	−21.60	7.11 (7.27)	−5.62	−6.42	6.31 (6.52)
HG	−14.25	−23.75	7.12 (7.32)	−5.03	−7.07	6.26 (6.49)
GH	−14.00	−22.68	7.15 (7.33)	−4.42	−6.28	6.27 (6.53)

As also found in previous theoretical works, the computed ionization potentials of nucleobases in the gas phase are in very good agreement with the experimental results, both as concerns adiabatic and vertical ionization potentials, the largest difference between predicted and observed values being 0.15 eV. The association enthalpy of A and T in the gas phase is also well predicted by computations; the experimental association enthalpy in the gas phase is −13.0 kcal/mol at 323 K,[57] whereas

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Table 3. Computed Ionization Potentials (IP, eV), Association Energies (kcal/mol), and Enthalpies (kcal/mol) at 323 K for Nucleobase Watson and Crick and Hoogsteen-Type H-bonded Complexes

		gas phase			CHCl$_3$		
	[a]$\Delta H^{TA}_{ass,323K}$	ΔE^{TA}_{ass}	ΔE^{TA+}_{ass}	[b]IP $_{adiab(vert)}$	ΔE^{TA}_{ass}	ΔE^{TA+}_{ass}	IP $_{adiab(vert)}$
A	−	−	−	8.11 (8.30)	−	−	6.42 (6.61)
T	−	−	−	8.81 (9.05)	−	−	6.94 (7.18)
WC	−11.24	−12.65	−21.96	7.70 (7.86)	−4.76	−6.72	6.34 (6.57)
CW	−10.93	−12.32	−21.14	7.73 (7.87)	−4.51	−6.05	6.36 (6.58)
HG	−11.94	−13.39	−23.06	7.69 (7.86)	−5.27	−7.55	6.32 (6.54)
GH	−11.74	−13.19	−22.21	7.72 (7.88)	−5.18	−6.89	6.35 (6.56)

[a] Experimental value for 9-methyladenine:1-methylthymine is 13.0 kcal/mol at 323 K, ref 57. [b] Experimental IPs for the gas-phase free nucleobases are A: 8.26 (8.44) eV; T: 8.87 (9.14) eV; ref 61.

computations, including zero point energy correction and thermal contribution, predict −11.9 and −11.7 kcal/mol for the HG and the GH association modes, respectively. The Watson−Crick and reversed Watson−Crick association modes are predicted to be slightly at higher energy, ca. 1 kcal/mol. Thus, both the association energy and ionization potentials are slightly underestimated, but on the whole, there is a good agreement between the computed and the available experimental results, which testifies to the reliability of the computational procedure adopted here.

The computation of the association enthalpy in chloroform is of course a much more demanding task.[58] However, the results reported in Table 3 show that in the gas phase nuclear contributions to association enthalpies are small, about 1 kcal/mol, and can be neglected in first approximation. On the basis of that approximation, the computed association energies in chloroform are again in good agreement with experimental results, cf. Tables 1 and 2. The same also holds for nucleosides; the association enthalpy obtained by NMR measurements in a temperature range going from 233 to 323 K amounts to −5.9 kcal/mol and compares well with the computed association energies in chloroform, reported in Table 2. In chloroform, as already observed in the gas phase, the association energies of the four H-bonded complexes are all within an energy range of about 1 kcal/mol, both for nucleobases and for nucleosides, but the relative order of stability is reversed; the Watson−Crick association mode comes out to be the most stable one for nucleosides, in line with experimental results which show that at very low temperature this association mode predominates.[42] Finding a rationale for that requires a very accurate analysis of the different contributions to the association energies,[59,60] which goes far beyond the scopes of the present work.

In summary, computations as well as experimental results suggest that at room temperature all four association modes can be present in solution. We have specifically looked for different association modes, e.g., a twisted H-bond association mode in which only the H-bond between the exocyclic amino group of As′ and a carbonylic oxygen of Td′ is retained, as the 2D NOE spectrum discussed above would suggest. Although molecular mechanics computations predict that such an association mode is a relative minimum in the gas phase, at the DFT level of computation, in solution, this association mode comes out to be at an energy maximum of the potential energy hypersurface, which easily relaxes into the nearest Hoogsteen or Watson−Crick minimum-energy configurations.

As concerns the ionization potential of nucleosides, noteworthy B3LYP computations predict that both As′ and Td′ have lower oxidation potential than their nucleobases, the effect being more pronounced for the latter, for which the lowering of the ionization potential amount to 0.97 eV in the gas-phase and 0.22 eV in chloroform, than for the former (0.58 and 0.07 eV, respectively). This finding is in accord with the available experimental results; the oxidation potential of adenine is higher than that of adenosine by 0.11 V in acetonitrile,[37] very close to the computational prediction (0.07 V) in chloroform. To our knowledge there are no experimental data for thymine and thymidine in organic solvent, since the oxidation potentials of both species fall outside the potential windows allowed by the most suitable supporting electrolytes.

From data reported in Table 2, the lowering of the oxidation potential of As′ upon Td′ pairing can be easily calculated as the difference between the association energy of the neutral and positively charged H-bonded complex. The computed shifts amounts to ca. 0.1 V, for all the association forms, a value which is again slightly underestimated with respect to the observed value. The oxidation potentials of the four association forms come out to be very similar to each other, so that the hypothesis that several association modes can contribute to the voltammetric signal observed at lower potential is confirmed by computations.

Conclusions

The effect of the complementary base pairing upon the oxidation potential of a substituted adenosine has been determined by direct voltammetric measurements in chloroform solution. Such nonphysiological conditions are dictated by the fact that the formation of H-bonded complexes between adenine and thymine only takes place in aprotic, scarcely polar solvents. Both NMR spectra and theoretical computations provide evidence that all four possible association forms, Hoogsteen or Watson−Crick type, are present in solution at room temperature. Theoretical computations also predict that in chloroform the Watson−Crick association mode is slightly more stable, in line with experimental results which show that at very low-temperature this association mode predominates in solution.[42] Providing an explanation of this theoretical and experimental finding is a big challenge to theoreticians, since it requires a very accurate analysis of the intermolecular energies at short distance; work is in progress along this line.

Although the observed value cannot be unequivocally assigned to a specific Hoogsteen or Watson−Crick association

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mode, theoretical computations suggest that all the association forms have similar oxidation potentials in chloroform. Therefore, the values reported in this paper, together with those previously obtained for the guanosine-cytidine pair,[17] can be safely used for representing the lowering of nucleobase oxidation potentials upon complementary base pairing in duplex DNA. The redox potentials reported here thus provide the first tassels (the unperturbed energies) necessary for building up the complex mosaic constituted by the low-lying electronic states in oxidized single-strand and duplex DNA. Many other pieces of information are still missing. For instance, stacking interactions will probably play an important role, and their effects on the energies of a hole site in DNA still need to be quantified; further work is therefore necessary for obtaining realistic models for hole transport in DNA.

Acknowledgment. The financial support of the University of Salerno is gratefully acknowledged.

Supporting Information Available: Cyclic voltammograms of As′, and of an equimolar solution of As′ and Td′; optimized geometries (Cartesian coordinates) of Td′:As′ (PCM/$CHCl_3$) in the four association modes of Figure 2; complete reference 25. This material is available free of charge via the Internet at http://pubs.acs.org.

JA076181N