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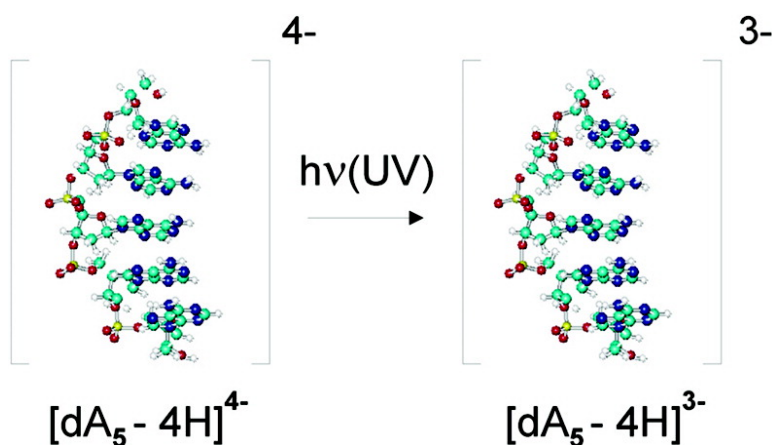
Photoelectron Spectroscopy of Isolated Multiply Negatively Charged Oligonucleotides

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Photoelectron Spectroscopy of Isolated Multiply Negatively Charged Oligonucleotides

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Abstract: Ultraviolet photoelectron spectroscopy in an ion beam was used to investigate the electronic properties of isolated DNA oligonucleotides $[dA_5\text{-}4H]^{4-}$ and $[dT_5\text{-}4H]^{4-}$ carrying four excess negative charges. We find the fourth adiabatic electron affinity to be slightly negative for $[dA_5\text{-}4H]^{4-}$ while it is positive for $[dT_5\text{-}4H]^{4-}$ This implies a significant influence of the base composition on energetics, which is in turn relevant for analytic applications and also for charge transport properties.

1. Introduction

The physics and chemistry of biologically relevant molecules in the gas phase have been growing areas of research in the past few years, and many groups have contributed to the effort of applying the know-how of gas-phase physical chemistry to these often large and complex systems. While mass spectrometry (MS), made feasible on a large scale by the development of matrix assisted laser desorption and ionization (MALDI)[1] and electrospray ionization (ESI),[2] has had the strongest impact, other techniques, such as ion mobility experiments[3−5] and studies involving the interaction of biomolecules with light, have begun to take root in this field.[6−10] Still, the intrinsic forces governing structural and, hence, functional aspects of polypeptides and oligonucleotides are largely uncharacterized in the absence of solvent molecules. Information on these properties is especially important for the quantitative understanding of structural data derived from mass spectrometry experiments. Specifically, gas-phase energetics is relevant for widely applied methods such as collision induced dissociation (CID),[11] electron

capture dissociation (ECD),[12] or infrared multiphoton dissociation (IRMPD).[13] In the case of DNA, related information on the energetics of isolated oligonucleotides is also valuable with respect to the charge transport properties of DNA[14] and to radiation damage caused by low energy electrons, fields which both have recently attracted much attention.[15,16]

One of the interesting effects observed in ESI-MS of large molecules is their propensity to take up more than just one excess charge, in analogy to their often multiply charged state in solution. Correspondingly, electrosprayed nucleotides and DNA readily form multiply charged anions (MCAs) by deprotonation of their phosphate groups.[17] Recent experiments on trapped DNA have observed sequential electron autodetachment from single-stranded multiply charged oligonucleotides and have measured their gas-phase kinetics.[18] In the past few years, the study of MCAs has become an emerging field of its own.[19,20] Combining photoelectron (PE) spectroscopy and MS of MCAs provides an opportunity to address questions pertaining to the electronic structure of these ions, such as their stability with respect to electron detachment, higher order electron affinities, effects of electron correlation and localization, dielectric screen-

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ing of the excess charges by the molecular frame, and, in comparison with information gained in condensed phase studies, their interplay with solvent molecules. The interaction potential between an excess electron and a negatively charged molecule is governed by a repulsive Coulomb barrier (RCB) that interfaces the long-range Coulomb repulsion and the short-range attractive interaction of the excess electron and its anionic "host". In cases where tunneling can be neglected, the presence of the RCB modifies the condition for photoemission of an electron from a MCA, as the photon energy has to be greater than the sum of the binding energy of the electron and the RCB height.
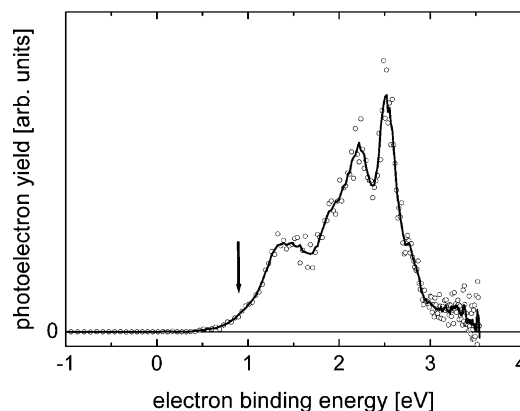
In this work, we present PE spectra and temperature-dependent MS results of single-stranded oligonucleotides consisting of either five adenosine (A) or five thymidine (T) units, joined by four phosphate groups, each of which is carrying one excess charge, resulting in tetra-anions $[dA_5\text{-}4H]^{4-}$ and $[dT_5\text{-}4H]^{4-}$ For brevity, we will denote them dA_5 and dT_5, respectively. These are, to our knowledge, the first such measurements on DNA oligonucleotides.

2. Experimental Section

Our experimental setup has been previously used in PE spectroscopy of fullerene dianions[21] and tetra-anions of metal organic compounds.[22] Briefly, oligonucleotide tetra-anions were produced in a commercial micro-ESI source (Analytica of Branford) from a 0.2 mM solution of the corresponding tetra-sodium salt (purchased from Jena Bioscience and used without further purification) in a mixture of water, methanol, and trifluoroethanol (7:2:1), with N_2 at room temperature (unless noted otherwise) as drying and spraying gas. Ions were accumulated for $^1/_{30}$ s in a hexapole ion trap. These ions were then mass selected using a relectron time-of-flight MS with a mass resolution of (m/z): $\Delta(m/z) =$ 1200. After mass selection, the ions entered the detachment zone of a "magnetic bottle" type time-of-flight PE spectrometer[23] and interacted with the third or fourth harmonic of a pulsed Nd:YAG (laser pulse duration 5−6 ns, typical fluence 30 and 15 mJ/cm^2 at 3.49 and 4.66 eV photon energy, respectively). Prior to photodetachment, the ions were decelerated by a high voltage pulse in order to enhance energy resolution by reducing the kinematic Doppler effect inherent in this spectrometer type. Typical energy resolution is around $\Delta E/E = 5\%$ at 1 eV electron kinetic energy. The PE spectra shown in Figures 1 and 2 are the results of several 10^5 laser shots, and the background generated without ions was measured and subtracted for every second laser shot. The spectra were calibrated using the known PE spectra of I$^-$ produced by ESI from a 1 mM solution of CsI in a methanol/water mixture (9:1) at photon energies of 3.49 and 4.66 eV, respectively.

3. Results and Discussion

3.1. Photoelectron Spectra. The PE spectrum of dT_5 shown in Figure 1 has been taken at 4.66 eV photon energy. It shows a broad peak at 1.43(15) eV and more strongly bound features around 2.2 and 2.5 eV observed toward higher binding energies. Linear extrapolation of the low binding energy slope onto the energy axis yields an estimated fourth adiabatic electron affinity (i.e., the binding energy of the fourth excess electron, AEA$_4$) of 0.90(15) eV. It was not possible to observe photoelectrons using 3.49 eV photons. This is most likely caused by the repulsive Coulomb barrier (RCB), the height of which we can

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Figure 1. PE spectrum of $[dT_5\text{-}4H]^{4-}$ at 4.66 eV photon energy. The arrow marks the estimated value of AEA$_4$. The open circles are raw data points, and the solid line is a five-point averaged curve to guide the eye.
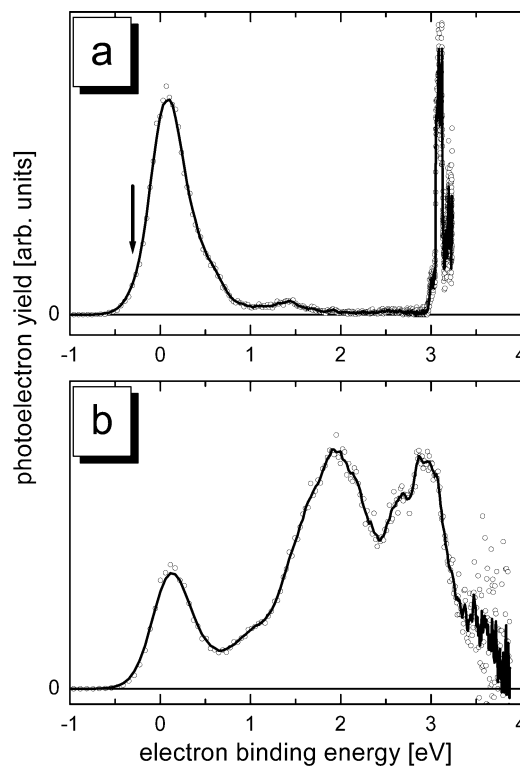
Figure 2. PE spectra of $[dA_5\text{-}4H]^{4-}$ at 3.49 eV (a) and 4.66 eV (b) photon energy. The arrow marks the estimated value of AEA$_4$ (see text). The open circles are raw data points, and the solid lines are five-point averaged curves to guide the eye.
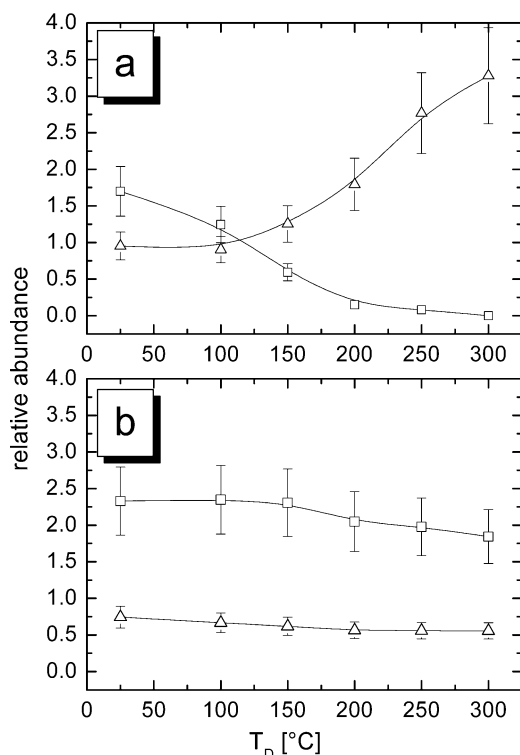
estimate to be 2.0(2) eV (as measured from the asymptotic value for infinite electron-anion distances) from evaluation of the cutoff of the spectrum at 4.66 eV photon energy.

The spectrum of dA_5 at 3.49 eV photon energy (Figure 2a) is dominated by a peak at 0.11(15) eV binding energy, while AEA$_4$ is estimated to be −0.30(15) eV. Aside from a partially resolved state around 0.5 eV, only a very weak feature around 1.4 eV is observed. The sharp peak at 3.1 eV is probably due to a two-photon effect, as its relative intensity decreases at lower laser fluence. The spectrum taken at 4.66 eV photon energy (Figure 2b) yields the same value for AEA$_4$. In contrast to the 3.49 eV spectrum, one observes several congested states at higher electron binding energies. The height of the repulsive Coulomb barrier (RCB) can be estimated from comparing the two PE spectra. The absence of PE yield at binding energies

Figure 3. Drying gas temperature dependence of the relative abundances of $[dR_5\text{-}4H]^{4-}$ (□) and the sum of the unresolved ion signals of $[dR_5\text{-}4H]^{3-}$ and $[dR_5\text{-}3H]^{3-}$ (△), each normalized to $[dR_5\text{-}4H+Na]^{3-}$ to account for spray efficiency for R = A (a) and R = T (b). The lines are spline interpolations to guide the eye, and errors are conservatively estimated to be 15% due to the statistics of different experimental runs.

greater than 1.7 eV in Figure 2a, together with the observation of a large PE yield at higher photon energies (Figure 2b), suggests an RCB of 1.8(2) eV.

Although the lowest energy peaks in the PE spectra could be attributed to the fourth vertical detachment energies of the oligonucleotides, this assignment would be based on the assumption that a single final state is responsible for this peak in each spectrum. It is possible, however, that these features are due to several unresolved electronic states;[24] hence we caution that this assignment could be problematic.

3.2. Temperature Dependence. A negative value for AEA_4 implies that the corresponding tetra-anions are thermodynamically metastable with respect to electron loss, similar to other known MCAs, such as $PtCl_4^{2-}$ [25,26] or metal phthalocyanine tetrasulfonate tetra-anions ($MPc(SO_3)_4^{4-}$),[22,27] which can have autodetachment lifetimes of many seconds. This result is corroborated by the dependence of the observed dA_5 ion signals on the ESI drying gas temperature T_D (Figure 3a). One can clearly see that the relative abundance of the tetra-anion decreases monotonically with increasing T_D, while at 100 °C $\leq T_D \leq 200$ °C the abundance of the trianion increases. Our observation suggests that the lifetime of $[dA_5\text{-}4H]^{4-}$ decreases with increasing temperature, similar to the case of $PtCl_4^{2-}$ [26] and also measurements on other multiply negatively charged gas-phase oligonucleotides.[18] The time associated with the

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experimental cycle in our experiment suggests an autodetachment lifetime at room temperature on the order of at least 0.1 s. Measurements of electron autodetachment as described in ref 18 have recently been performed for dA_5^{4-} and dT_5^{4-} at 45 °C. The measured detachment rates were $k(dA_5^{4-}) = 0.105 \pm 0.009$ s^{-1} and $k(dT_5^{4-}) = 0.033 \pm 0.003$ s^{-1} which are consistent with the results shown in Figure 3.

In contrast to the case of dA_5, the relative abundance of dT_5 tetra-anions is essentially independent of drying gas temperature in our time window (see Figure 3b), consistent with the observed differences in electron binding energy.

The difference in electronic stability between dA_5 and dT_5 is quite remarkable. It is also consistent with measurements[18] of the autodetachment lifetimes of heptanucleotide trianions in a Paul trap over the range 102 °C to 150 °C, in which dA_7 was found to have a decay rate exceeding that of dT_7 by more than a factor of 5. To shed light on the difference in behavior for different base compositions, we will first introduce an empirical model to describe electron binding energies and their interplay with structure. Subsequently, we look more closely at the localization of the highest occupied molecular orbital (HOMO), phosphate and base energetics, and structural issues.

3.3. Empirical Model for Binding Energy Estimates. An approach to estimate AEA_4 values has been introduced as an empirical model by Wang and co-workers,[28] and we will briefly present this model in a slightly modified version, which has been previously used to describe fullerene dianions.[21] In this model, the total binding energy $E(q)$ of excess charges for a charge state q can be calculated as

$$E(q) = q \cdot E_B - s \cdot \frac{1}{2} \sum_{i,j=1, j \neq i}^{q} \frac{e^2}{4\pi\epsilon_0 r_{ij}} \tag{1}$$

The first term represents the binding energy at the excess charge binding sites (here, the phosphate groups), while the second term describes the Coulomb repulsion between the excess charges, weighted by an empirical factor s that accounts for effects of dielectric screening, delocalization, and spatial correlation of the excess charges.[21] E_B is the binding energy of an excess charge to a phosphate group, e, the elementary charge, ϵ_0, the vacuum dielectric constant, and r_{ij}, the average distance between the charges on positions i and j, counted from the 3′ end to the 5′ end of the oligonucleotide. Dielectric shielding and spatial correlation both tend to decrease the value of s, while excess charge delocalization will increase it. Assuming localized point charges on the phosphate groups, effects of correlation and delocalization can be neglected, and the value of s will be ≤ 1 due to dielectric screening by the ribose part of the molecule.

Note that the weighted Coulomb term in eq 1 depends on the structure of the molecule through both the value of s (describing the effect of the molecular frame between the excess charges) and the distances between the excess charges (determined by the relative positions of the phosphate groups). However, the base composition is not explicitly taken into account. Consequently, any influence of the bases is implicit through the backbone structure and dielectric properties, which will be discussed in section 3.5.

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If we want to establish a lower limit for AEA_4, we neglect dielectric screening by the bases and ribose parts of the molecule and consequently choose s to be 1. AEA_4 is then given by the difference ΔE_{34} between the tetra-anion and the lowest energy charge isomer of the trianion:

$$\Delta E_{34} = E_4 - E_3 = E_B - s \cdot \frac{1}{2}(E_{C,4} - E_{C,3}) \qquad (2)$$

where $E_{C,q}$ is the Coulomb repulsion term of charge state q, as derived from the sum in eq 1. E_B can be estimated to be 4.57 eV from PE spectra of $H_2PO_4^-$,[29] and we approximate the distances between the charges as the distances between the P atoms using the known bulk phase crystal structure of Z-DNA as taken from the database of the HyperChem Pro 6 computer program.[30] For $(i - j) = 1$ (i.e., neighboring phosphate groups), the average distance r_{ij} amounts to 5.88 Å, while, for $(i - j) = 2$, $r_{ij} = 9.94$ Å, and, for $(i - j) = 3$, $r_{ij} = 15.24$ Å. We stress the fact that this structure is not to be taken as the correct gas-phase structure, as the high charge state of the ions studied here will probably lead to a more elongated conformation,[31] which would tend to increase AEA_4. Nevertheless, in the spirit of a lower limit estimate for an elongated conformer, this structure seems appropriate. With these assumptions, the lower limit on AEA_4 amounts to -1.78 eV. The corresponding values for A- and B-DNA are -1.65 eV and -1.07 eV, respectively, somewhat higher than those for the Z-structure, as A- and B-conformers are less compact than the Z-conformer.

If we use the experimentally estimated values of AEA_4 for ΔE_{34}, extracting the values of the empirical scaling factor s (assuming identical backbone structures and, hence, Coulomb repulsion terms for the two pentanucleotides) from eq 2 yields $s = 0.58$ and $s = 0.77$ for dT_5 and dA_5, respectively.

3.4. HOMO Localization, Energetics of Phosphate and Bases. One of the interesting questions associated with the charge transport properties of DNA is concerned with the degree of HOMO localization. Calculations by Lewis and co-workers suggest that the LUMO of static periodic *neutral* DNA duplexes is distributed in Bloch-like states along the backbone (i.e., the phosphate groups and ribose part of the molecule),[32] but an injected charge in a dynamical duplex will be resident in localized states on specific phosphate groups due to off-diagonal disorder induced by structural fluctuations. For hole transport in condensed phase DNA duplexes, it is known that, upon site selective charge injection, holes tend to become preferentially localized on guanine.[16] Analogous results for site-selective electron injection have not been reported so far.

As both experimental AEA_4 values lie above the lower limit of -1.78 eV derived in section 3.3, our values are consistent with HOMO localization on the phosphate groups. Note that the empirical model described above includes no base specificity and is therefore unable to explain the observed base dependence of the electron binding energies, if the structures of dT_5 and dA_5 were the same (see section 3.5).

Further evidence toward judging whether the HOMO is localized on the phosphate groups or the bases might be gleaned

by comparing our PE spectra both with each other and with those of $H_2PO_4^-$.[29] dT_5 and dA_5 measurements differ not only in their absolute electron binding energy values but also in their energetic structure, suggesting a different electronic structure. This might imply that the HOMO is not localized on the phosphate groups, at least not in *both* dT_5 and dA_5. If we compare the PE spectra of the oligonucleotides studied here with those of $H_2PO_4^-$,[29] however, the situation becomes less clear. The spectrum of $H_2PO_4^-$ taken at 7.90 eV photon energy bears some resemblance to that of dT_5, while the spectrum taken at 6.42 eV is reminiscent of that of dA_5. Due to the spectral congestion and the lack of clear congruence (even among the individual spectra for $H_2PO_4^-$ taken at different photon energies), we judge that comparison with the electronic structure of $H_2PO_4^-$ does not allow assignment of the highest lying electronic states to detachment from the phosphate groups, nor can it be completely ruled out on the grounds of this comparison alone.

Let us briefly explore an alternative hypothesis: that of HOMO localization on one or more bases. In contrast to the very small difference in the gas-phase AEAs of adenine and thymine, which are due to electrostatically dipole bound anionic states,[33] gas-phase ionization potentials differ significantly (8.44(3) eV for adenine and 9.14(3) eV for thymine, respectively[34]). If the HOMO is localized on a base, it is obvious that the differences in base electronic structure translate into a difference in the PE energetics, even if the backbone structures of dT_5 and dA_5 were identical. Such a scenario, where the HOMO is not coincident with the excess charge orbitals, has been previously found for $CuPc(SO_3)_4$,[4-22,27] although with a planar "charge corral" molecular topology different from that encountered here. A similar change in the ordering of orbitals has been proposed by calculations concerning the electron binding energies in guanine containing oligonucleotides in aqueous solution.[24,35,36]

Another explanation involving the bases could be that deprotonation does not take place on all the phosphate groups, but that one of the nucleotides is deprotonated instead. Deprotonation of the NH group between the keto groups on thymine is only 61 kJ/mol less favorable than deprotonation of phosphoric acid,[37,38] and the Coulomb repulsion in the system could favor deprotonation at this site rather than at the backbone phosphate. This would result in dT_5 being more stable than dA_5, explaining the trend of the experimental results. A comparison with the pentanucleotides containing the other bases could serve to answer this question.

3.5. Structural Arguments. The gas-phase structures of single strands of dA_5 and dT_5 are not known. As mentioned above, it is clear that they will strongly differ from those of individual strands in solution phase or from A-, B-, or Z-DNA. Ion mobility studies on single strands by Clemmer and co-workers[31] and on double strands by Bowers and co-workers[5,39,40] show that there are several structural motifs such as globular

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and elongated conformers, as well as various degrees of folding, all dependent on bases, sequences, and charge states. Based on the results shown in ref 31, we argue that the fully deprotonated dT_5 is most likely elongated, as the Coulomb repulsion between the excess charges stretches the molecule. On the same grounds, one could argue that both dT_5 and dA_5 populate elongated rather than globular conformers in the high charge states studied here. Under this assumption, dielectric screening should be similar for both species as well, resulting in roughly the same value of the scaling factor s for both species (see eqs 1 and 2). However, differences in intramolecular hydrogen bonding behavior and (to a lesser degree) base stacking interaction can serve to make dA_5 more compact than dT_5, changing the Coulomb repulsion terms. For equal scaling factors s, the structure of dT_5 would have to result in about 30% longer average distances between the excess charges than for dA_5. Alternatively, dA_5 could preferentially populate partially folded conformers, while dT_5 is elongated, again resulting in a more compact structure and larger Coulomb repulsion.

4. Summary and Conclusions

We have shown that the photoelectron spectra of isolated pentanucleotide ions possess a marked base specificity regarding the electron binding energies. The observed differences in energetics can be approached by two different models: "base electron emission" and "backbone electron emission".

In the "base electron emission" approach, the HOMO of at least one of the two pentanucleotides studied here involves the bases being spatially separated from the excess charges, which are located on the phosphate groups, and photoemission therefore occurs from the bases. Consequently, the differences in energetics are due to the electronic properties of the bases. The mutual dissimilarity of the PE spectra of dT_5 and dA_5 together with the difference in base energetics are the arguments in favor of this approach.

In the "backbone electron emission" approach, both the HOMO and the excess charges reside on the phosphate groups, and the charge removed by photoemission therefore stems from the phosphate groups. An estimated lower limit of AEA_4 of an elongated conformer is compatible with our observed results. Structural differences account for the different energetics in this approach. The empirical model used to describe the binding energies in the "backbone electron emission" approach is certainly not sufficiently sophisticated to accurately describe

the situation, but it shows in an intuitive way the interplay among structure, dielectric properties, and energetics. More detailed calculations on the HOMO localization in dynamical duplexes also favor the "backbone electron emission" scenario.

We cannot prove or rule out either approach on the grounds of our experimental observations, although the large variety of possible structural conformations and the available theoretical data lend somewhat more credibility to the latter.

PE spectroscopy of other single- and double-stranded oligonucleotides may serve to develop a clearer picture of the interplay of structure, base composition, and energetics, and experiments to this end are under way in our laboratory. Temperature-dependent PE spectroscopy measurements could be used to study structural changes. Clearly, more theoretical work and experimental approaches (e.g., ion mobility measurements) on oligonucleotides are needed for a better understanding of their gas-phase properties.

Our results have some relevance to related DNA research. It is uncertain if the structures of short gas-phase oligonucleotide ions resemble those in solution, as counterions and solvent molecules will shield the Coulomb repulsion between excess charges to some extent. On the other hand, DNA duplex structures with as few as 8 base pairs have been shown to be elongated (and possibly helical) in the gas phase as well as in solution.[40,41] Moreover, DNA ion energetics may not only be base specific in the sense shown here but may depend on the exact base ordering as well, which could provide a new tool for sequence analysis. Ion yields and reaction pathways in MS techniques such as ECD or CID depend on the energetics of the ions in question, and therefore these should be known for better understanding and interpretation of MS experiments. Similarly, the aspect of charge localization is relevant for the charge transport properties of DNA, which can be better understood if the intrinsic properties of this type of molecular wire are known.

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