International Journal of Mass Spectrometry 277 (2008) 296–299

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Isotope effects in dissociative electron attachment to the DNA base thymine

S. Denifl[a], P. Sulzer[a], F. Zappa[a], S. Moser[b], B. Kräutler[b], O. Echt[c], D.K. Bohme[d], T.D. Märk[a], P. Scheier[a,*]

[a] Institut für Ionenphysik und Angewandte Physik and Center for Molecular Biosciences Innsbruck, Universität Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Austria
[b] Institut für Organische Chemie and Center for Molecular Biosciences Innsbruck, Universität Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria
[c] Department of Physics, University of New Hampshire, Durham, NH 03824-3568, USA
[d] Department of Chemistry, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3

ARTICLE INFO

Article history:
Received 31 March 2008
Received in revised form 4 June 2008
Accepted 9 June 2008
Available online 18 June 2008

Keywords:
Dissociative electron attachment
Thymine
Isotope effect

ABSTRACT

Dissociative electron attachment to thymine and two partially deuterated derivatives are studied with high electron energy resolution. For all fragment anions involving hydrogen loss, a dramatically reduced anion yield is measured for loss of D compared to loss of H. For the low-energy vibrational Feshbach resonances this isotope effect reaches a value of 40. Narrow features in the anion yields that previously were assigned to H loss from the N1 site of thymine and concomitant excitation of specific stretching modes are shifted to lower electron energies when deuterium is involved.

1. Introduction

The interaction of low energy electrons (LEE) with gas phase molecules is a fundamental process in technical and natural plasmas and has been studied for a large number of molecules. The attachment of a free electron to a molecule AB initially forms an unstable transient negative ion, AB^{-*}, with the same geometry as the neutral precursor (vertical transition). Often the attached electron occupies an antibonding orbital or the vertical transition ends up at the repulsive part of a (binding) potential energy curve that starts to separate parts of the transient negative ion. As long as the potential energy of the anionic complex is higher than that of the corresponding neutral complex with the same geometry the electron can be detached which competes with dissociative electron attachment (DEA) until the internuclear separation between the charged and neutral fragment(s) exceeds the intersection of the corresponding potential energy curves of the anionic and neutral system. The time to reach this point of no return towards DEA depends strongly on the mass of the lightest fragment and is the shortest if one of the fragments is a hydrogen atom. This may be one reason for the high probability of DEA to hydrogen containing molecules M including the nucleobases and other biomolecules that form a closed-shell anion $[M-H]^-$ upon loss of a neutral H radical [1–3]. While the replacement of hydrogen with deuterium in a molecule generally has only a small effect on its stability and chemical reactivity, a much larger isotope effect is expected for DEA. The separation of D requires substantially more time than H and thus autodetachment will be more effective. Indeed, a particularly large isotope effect (factor 200) is observed for the yield of H^- versus D^- upon DEA to H_2 and D_2, respectively [4].

The pioneering work of Boudaiffa et al. demonstrated that LEE have the potential to effectively induce strand breaks in plasmid DNA [5]. Their work has motivated a wealth of experimental and theoretical studies [6] of electron attachment to building blocks of DNA in the gas phase [7,8] and the condensed phase [9]. One of the most remarkable results from these studies of DEA to biomolecules is the unique possibility of LEE to selectively break specific bonds as a function of the electron energy. This has been demonstrated for neutral H loss from the nucleobases uracil, thymine and adenine [10,11] and the amino acid valine [12]. In this sub excitation energy regime energetic constrains can explain the suppression of some channels. In contrast, the site selectivity for H^- formation from thymine [13,14] and from simple organic acids [15] is not based on energetic limitations. Recently Bald et al. demonstrated the ability of LEE to selectively remove fragments containing a specific carbon atom in the ring of D-ribose [7]. This bond and site selectivity is also viable for hydrogen bridged clusters of DNA bases [16] and the nucleoside thymidine [17].

Except for sugar [18] and halogenated DNA bases [19], the most abundant product upon DEA to the building blocks of DNA is the dehydrogenated closed-shell anion $[M-H]^-$. The maximum cross section for $[M-H]^-$ is at around 1 eV for all DNA-bases and amino

---

* Corresponding author. Fax: +43 512 507 2932.
E-mail address: Paul.Scheier@uibk.ac.at (P. Scheier).

1387-3806/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2008.06.005

acids [2,8]. In addition, a series of sharp peaks in the dissociative electron attachment cross sections of uracil, thymine [20] and adenine [12] at energies below 3 eV are assigned to vibrational Feshbach resonances arising from coupling between the dipole bound state and the temporary anion state associated with occupation of the lowest σ^* orbital [21]. For uracil and thymine three distinct vibrational modes are identified as the stretch modes of N–H, C–H and C–N. Deuteration of the pyrimidine bases uracil and thymine will modify the N–H and C–H stretch modes and thus provides a method to test the explanation given by Burrow et al. [21] for the narrow features in the anion yields of the dehydrogenated closed-shell anions of thymine and uracil. In the present work the formation of $[M{-}H]^-$ or $[M{-}D]^-$ upon free electron attachment to the pyrimidine base thymine and two partially deuterated derivatives, T_{D4} (D replaces the four H atoms attached to carbon atoms) and T_{D2} (D replaces the two H atoms attached to the nitrogen atoms) are studied with high electron energy resolution.

2. Experimental

The molecular structures of thymine T, C-deuterated thymine T_{D4}, and N-deuterated thymine T_{D2} are shown in Fig. 1. T and T_{D4} were commercial products from Sigma Aldrich and the latter has a stated isotopic purity of >98%. T_{D2} was synthesized by dissolving normal T at room temperature several times in D_2O. The resulting isotopic purity was checked mass spectrometrically; it was better than 99%. In order to determine the isotopic purity of the samples we measured positive ion mass spectra with good signal to noise ratio for T_{D2} and T_{D4} at an electron energy of 70 eV (shown in Fig. 2). The peaks higher masses than the nominal parent ions can be assigned to isotopomers containing ^{13}C (1.07% natural abundance) ^{15}N (0.36%), ^{17}O (0.04%) and ^{18}O (0.2%) and match perfectly the calculated isotopic pattern of thymine. However, the peaks at lower masses than the nominal parent ions may originate from isotopic impurities where one or more of the deuterated sites are binding a hydrogen atom or from fragment cations that were formed via loss of one or more hydrogen atoms. For normal thymine the fragment $[T\text{-}H]^+$ and $[T\text{-}2H]^+$ have relative intensities of 0.5% and 1%, respectively. In the present case all these lower mass cations are about two orders of magnitude less abundant than the corresponding parent cation. Thus, even if we neglect the presence of fragment cations completely the isotopic purity of the samples is better than 99% for T_{D2} and about 98% for T_{D4}. Each sample was placed in an oven and vaporized at a temperature of about 450 K. The surfaces of the oven and a capillary attached to the oven were washed with D_2O prior to the filling with T_{D2} in order to minimize the fast exchange of D from the N-positions via reactions with water. The molecules leaving the oven formed an effusive beam that was crossed with an electron beam with an electron energy resolution better than 80 meV. The resulting anions were mass analyzed with a quadrupole mass filter and detected by a channel electron multiplier operated in the pulse counting mode. To compensate for differences in vapor pressure, all anion yields were normalized to the yield of the parent cation measured under identical conditions. The data shown in the upper panel of Fig. 3 are plotted as measured (without smoothening). More than 50,000 ions were collected for

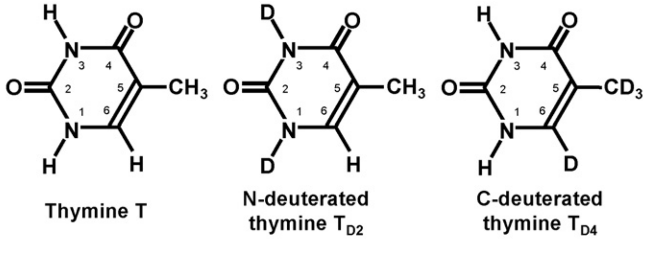

Fig. 1. Molecular structures of thymine T, nitrogen-deuterated thymine T_{D2} and carbon-deuterated thymine T_{D4}.

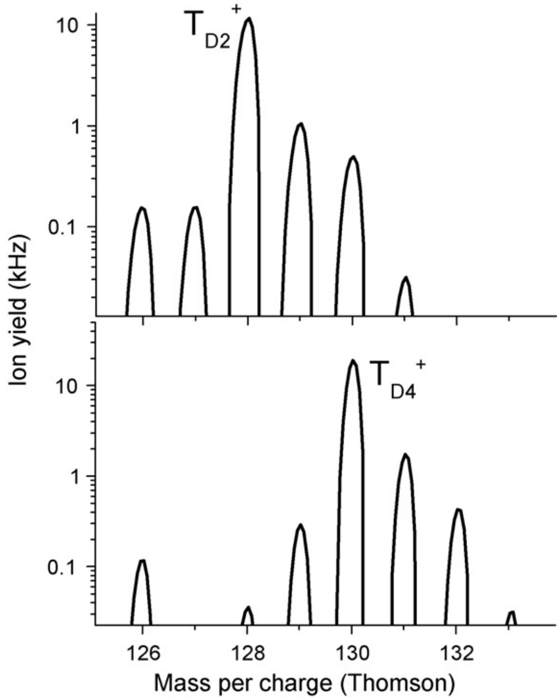

Fig. 2. Electron impact ionization mass spectrum of T_{D2} (upper panel) and T_{D4} (lower panel) recorded at the electron energy of 70 eV (see text).

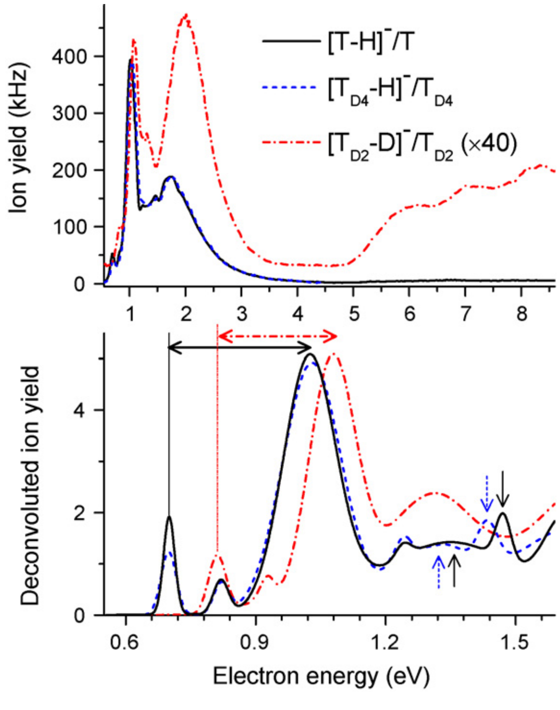

Fig. 3. The upper graph shows the anion yields of $[T\text{-}H]^-$ (solid line), $[T_{D4}\text{-}H]^-$ (dashed line) and $[T_{D2}\text{-}D]^-$ (dash-dotted line) formed upon DEA to the corresponding gas phase molecules. The anion yields in the lower graph were deduced by deconvolution of the anion yields (from the upper panel) with the electron energy distribution, deduced from the measured anion yield of Cl^- formed via s-wave attachment to CCl_4.

each data point at the maximum of the anion yields. In the case of [T_{D2}-D]$^-$, the investigations lasted for more than two weeks, including a frequent cleaning of the monochromator – the moisture (D_2O) in the T_{D2} sample quickly contaminated the surfaces of the electron monochromator and required a complete cleaning of the instrument every second day. The sample was always completely exchanged after a cleaning cycle. For the 2 eV resonance in the (T_{D2}-H)$^-$ anion yield we observed about 50 counts/sec which is far above noise level of about 0.1 count/sec. Drifts in the electron energy calibration caused by surface charging of lenses or instabilities of the power supplies were corrected by frequent calibration measurements of the production of Cl^- from CCl_4. The electron energy resolution for the [T-H]$^-$ curve was 60 meV. [T_{D4}-H]$^-$ and [T_{D2}-D]$^-$ were measured with a resolution of 70 meV.

3. Results and discussion

The upper graph of Fig. 3 shows the anion yields of [T-H]$^-$, [T_{D4}-H]$^-$ and [T_{D2}-D]$^-$ from DEA to thymine and carbon- and nitrogen-deuterated thymine, respectively. In case of an isotopically pure sample the loss of H from T_{D4} and the loss of D from T_{D2} must occur from a nitrogen site. We also observe (not shown) loss of two mass units from T_{D4} and loss of one mass unit from T_{D2}, which might suggest hydrogen loss (D and H, respectively) from a carbon site (see the molecular structures in Fig. 1). However, the anion yield for these reactions is more than two orders of magnitude smaller and, as argued in our earlier study of T_{D4} [22], can instead be assigned to isotopic impurities that lose H from a nitrogen site. This is also in perfect agreement with the isotopic purity of the samples probed via the positive mass spectra shown in Fig. 2.

The anion yields of [T-H]$^-$ and [T_{D4}-H]$^-$ are almost identical. This indicates that both are formed by loss of H from a nitrogen site. In contrast, the [T_{D2}-D]$^-$ yield is dramatically reduced (note the factor 40 applied to the data in Fig. 3). This observation can be explained by a lower tunneling probability for D in comparison to the lighter H atom that will result in increased autodetachment for the TNI of T_{D2}. This isotope effect has a very large value of 40 and is among the largest ever measured [4,23] in the gas phase. In addition, the high-energy features of the anion yield of [T_{D2}-D]$^-$ are enhanced compared to [T-H]$^-$ and [T_{D4}–H]$^-$. The relative abundance of the broad asymmetric peak with a threshold at 1.4 eV is increased by a factor of two. Experiments with thymine methylated at the N1 site demonstrated that this resonance corresponds to the H loss from the N3 site [10], where mixing of the second lowest π^* resonance with the second lowest σ^* state occurs [21]. This repulsive σ^* state can be reached more directly than the competing H loss from N1 where tunneling is involved. Thus, for D loss from the N3 site the cross section is only reduced by a factor of 20 compared to H loss from the undeuterated molecule. The resonances at electron energies higher than 5 eV can be assigned to sequential H loss [22]. As neutral hydrogen can be emitted there is no enhanced competition of autodetachment that explains the high relative abundance of this channel for T_{D2}.

All three anion yields in the upper graph of Fig. 3 show a series of narrow overlapping resonances from 0.5 eV to about 2 eV. A deconvolution procedure described previously [21] was used to remove broadening of the resonances due to the finite electron energy resolution. Thereby the deconvolution procedure included (i) multiple Gaussian fits of the resonance structure, (ii) deconvolution of the obtained Gaussian peaks and (iii) superposition of the deconvoluted peaks to obtain the final anion yield. The reliability of this procedure was tested by a convolution of the obtained spectrum with the electron energy distribution and comparison with the measured anion yield. The deconvoluted anion yields are shown in the lower graph of Fig. 3. Recent experiments with thymine and uracil, methylated at the N1 and N3 site (see the molecular structures in Fig. 1), respectively, demonstrated that all narrow features between 0.5 eV and 2 eV originate from H loss from the N1 site [10]. A recent model by Scheer et al. [24] and an upgraded scheme by Burrow et al. [21] explain this series of narrow resonances as vibrational Feshbach-resonances and tunneling of the H-atom from the N1 site through a potential barrier formed by an avoided curve crossing of the dipole bound state and the antibonding (N1-H) σ^* state.

For [T-H]$^-$ (solid line) and [T_{D4}-H]$^-$ (dashed line) the first four resonances are almost identical. This agrees well with the assignment of these peaks to vibrational levels of the N1-H and N1-C6 stretching modes that are expected to be hardly affected by deuteration at the carbon sites. In contrast, the next two resonances (indicated with vertical arrows in the lower panel of Fig. 3) are clearly shifted to lower electron energies for T_{D4}. This observation fits perfectly well with the excitation of C6-D stretching modes.

In the case of [T_{D2}-D]$^-$ (dash-dotted line) the first three resonances are shifted towards higher electron energies and the distance between the third peak and the first two peaks (indicated with the horizontal double headed arrows) is reduced. According to the model of Burrow et al. [21] deuteration at the N sites will reduce the distance between the first and the third resonance since these result from vibrational excitation of the N1-H stretching mode of thymine. For the non-deuterated molecule these peaks were assigned to the $v = 2$ and $v = 3$ level. Since these levels will be downshifted for T_{D2} we propose that the next higher levels, i.e., $v = 3$ and $v = 4$, have to be excited to enable tunneling through the potential barrier between the dipolar bound state to the repulsive σ^* state of the anion. The next three resonances between 1.2 and 1.5 eV are not resolved and thus form a broad single peak for [T_{D2}-D]$^-$. Only for [T-H]$^-$ the statistics and isotopic purity are high enough to deduce individual narrow resonances above 1.5 eV. The shift of the resonances agree qualitatively very well with calculated vibrational energies for both deuterated derivatives of thymine and this observation supports the findings in [24] and the model of Burrow et al. [21].

Distinct differences are also observed for other fragment anions formed upon DEA to the three isotopomers, such as the hydride anion H^- and NCO^- (see Fig. 4). The anion yield of H^- from thymine shows four resonances. In recent work carbon deuterated thymine T_{D4} (see also present data) and pyrimidine bases methylated at the nitrogen sites were utilized to distinguish between the loss of either H [10] or H^- [13], from N1 and N3. The yields of H^- and D^- upon DEA to T_{D2} observed here confirm the earlier result, i.e., the two low energy resonances correspond to the nitrogen sites and the two high-energy resonances to the carbon sites. The yields for D^- (symbols) are about 50% less than the corresponding H^- anion yields (lines). This observation again can be explained by an increased competition by autodetachment, resulting from the longer time required for the separation of D^- compared to H^- from the respective TNI.

The anion with a mass of 42 Dalton is the most abundant anion at electron energies above 4 eV for all three isotopic forms of thymine. We can exclude any significant contribution of other (deuterated) anions like C_2DO^- on this mass since in the case of thymine the yield of C_2HO^- or $C_2NH_3{}^-$ is more than a factor of 20 lower than that for NCO^-. For formation of NCO^- at least three bonds of the thymine molecule have to be broken. The NCO^- anion yield from N-deuterated thymine T_{D2} (dash-dotted line in Fig. 4, upper panel) is reduced (25% less) at electron energies below 8 eV compared to the two other isotopomers. The position of this resonance matches perfectly the second resonance of H^- which has been assigned to hydride formation from the N3 site [14]. This is confirmed in the present study as for the D^- anion yield from T_{D2} this energy range corresponds to loss from the nitrogen sites. Hence for all three

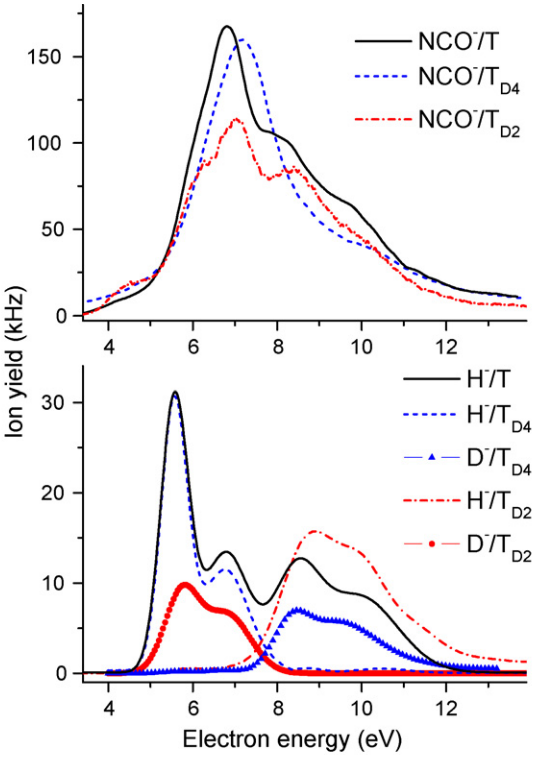

Fig. 4. The upper panel shows the measured anion yields of NCO$^-$ formed upon DEA to thymine, carbon-deuterated thymine T_{D4} and nitrogen-deuterated thymine T_{D2}. The lower panel shows the anion yields of H$^-$ formed upon DEA to gas phase molecules of T, T_{D4} and T_{D2}. H$^-$ and D$^-$ were measured with an electron energy resolution of about 0.1 eV. The yield of all anion yields shown in Figs. 3 and 4 can be directly compared (see text).

anions, i.e., the closed-shell dehydrogenated parent anion, H$^-$ and NCO$^-$, the reduced yield for the deuterium labeled species can be explained by an enhanced probability for autodetachment, resulting from a slower decomposition process, when a deuterium atom is removed. For T_{D4} the NCO$^-$ anion yield is clearly reduced at electron energies higher than 8 eV. This is the energy range where D$^-$ is formed from the carbon sites and again the reduced anion yield of NCO$^-$ can be assigned to an increased competition of autodetachment. In a recent work we investigated electron attachment to thymine embedded in He droplets [25]. Rapid cooling of the anions by the surrounding He clearly demonstrated that NCO$^-$ formation is a two step process which starts with the loss of a hydrogen atom and is followed by a subsequent decomposition of the ring. The second step is completely quenched in the He droplets and instead of NCO$^-$ the closed shell anion [T-H]$^-$ is formed. The first step of NCO$^-$ formation, i.e., the loss of a neutral hydrogen atom is in competition with autodetachment and thus deuteration of a given site will lead to a reduced anion yield of NCO$^-$ at the electron energy where the bond of this D atom is broken.

In conclusion, DEA to thymine and two partially deuterated forms of thymine, where hydrogen is replaced by deuterium either at all the nitrogen or carbon sites, was studied with high electron energy resolution. For the dehydrogenated closed-shell anions [T-H]$^-$, [T_{D4}-H]$^-$ and [T_{D2}-D]$^-$ deuteration shows the expected shifts of the vibrational Feshbach resonances in which stretching modes of deuterium are excited. This confirms the model developed by Burrow et al. explaining the narrow resonance structures in the anion yields of [T-H]$^-$ and [U-H]$^-$. We observe an isotope effect for all anions that lose deuterium. In the case of neutral D loss from the N1 site for which tunneling through a potential barrier is required, the anion yield of [T_{D2}-D]$^-$ is 40 times less compared to H loss from the same site of the other two isotopic forms of thymine. D loss from the N3 site where the repulsive σ^* state can be reached more directly the cross section is only reduced by a factor of 20 compared to the corresponding H loss. For all energetically higher-lying resonances that lead to the formation of H$^-$ and NCO$^-$, the effect of deuteration is much less pronounced but still measurable. The larger mass of D implies a reduced speed of dissociation of the excited TNI, leading to an increased competition of autodetachment and thus a lower anion yield.

Acknowledgements

This work was supported in part by the FWF, Wien, and the European Commission through the networks ITS-LEIF, COST-P9, EIPAM and RADAM. S.D. gratefully acknowledges an APART grant from the Austrian Academy of Sciences. The authors thank Eugen Illenberger for an outstanding, long-term collaboration and wish him all the best for the future.

References

[1] P. Papp, J. Urban, S. Matejcik, M. Stano, O. Ingolfsson, J. Chem. Phys. 125 (2006) 204301 (and references therein).
[2] S. Gohlke-S, E. Illenberger, Europhys. News 33 (2002) 207.
[3] S. Denifl, S. Ptasinska, B. Sonnweber, P. Scheier, D. Liu, F. Hagelberg, J. Mack, L.T. Scott, T.D. Märk, J. Chem Phys. 123 (2005) 104308.
[4] G.J. Schultz, R.K. Asundi, Phys. Rev. 158 (1967) 25.
[5] B. Boudaïffa, P. Cloutier, D. Hunting, M.A. Huels, L. Sanche, Science 287 (2000) 1658.
[6] I. Anusiewicz, J. Berdys, M. Sobczyk, P. Skurski, J. Simons, J. Phys. Chem. A 108 (2004) 11381 (and references therein).
[7] I. Bald, J. Kopyra, E. Illenberger, Angew. Chem. Int. Ed. 45 (2006) 4851.
[8] S. Denifl, S. Ptasinska, M. Cingel, S. Matejcik, P. Scheier, T.D. Märk, Chem. Phys. Lett. 377 (2003) 74.
[9] S. Ptasinska, L. Sanche, J. Chem. Phys. 125 (2006) 144713.
[10] S. Ptasinska, S. Denifl, P. Scheier, E. Illenberger, T.D. Märk, Angew. Chem. Int. Ed. 44 (2005) 6941.
[11] S. Denifl, P. Sulzer, D. Huber, F. Zappa, M. Probst, T.D. Märk, P. Scheier, N. Injan, J. Limtrakul, R. Abouaf, H. Dunet, Angew. Chem. Int. Ed. 46 (2007) 5238.
[12] H.D. Flosadottir, S. Denifl, F. Zappa, N. Wendt, A. Mauracher, A. Bacher, H. Jonsson, T.D. Märk, P. Scheier, O. Ingolfsson, Angew. Chem. Int. Ed. 46 (2007) 8057–8059.
[13] S. Ptasinska, S. Denifl, V. Grill, T. Märk, P. Scheier, S. Gohlke, M. Huels, E. Illenberger, Angew. Chem. Int. Ed. 44 (2005) 1647.
[14] S. Ptasinska, S. Denifl, V. Grill, T.D. Märk, E. Illenberger, P. Scheier, Phys. Rev. Lett. 95 (2005) 093201.
[15] V.S. Prabhudesai, A.H. Kelkar, D. Nandi, E. Krishnakumar, Phys. Rev. Lett. 95 (2005) 143202.
[16] S. Denifl, F. Zappa, I. Mähr, J. Lecointre, M. Probst, T.D. Märk, P. Scheier, Phys. Rev. Lett. 97 (2006) 043201.
[17] S. Ptasinska, S. Denifl, S. Gohlke, P. Scheier, E. Illenberger, T.D. Märk, Angew. Chem. Int. Ed. 45 (2006) 1893.
[18] S. Ptasinska, S. Denifl, P. Scheier, T.D. Märk, J. Chem. Phys. 120 (2004) 8505.
[19] S. Denifl, S. Matejcik, S. Ptasinska, B. Gstir, M. Probst, P. Scheier, E. Illenberger, T.D. Märk, J. Chem. Phys. 120 (2004) 704.
[20] S. Denifl, S. Ptasinska, M. Probst, J. Hrusak, P. Scheier, T.D. Märk, J. Phys. Chem. A 108 (2004) 6562.
[21] P.D. Burrow, G.A. Gallup, A.M. Scheer, S. Denifl, S. Ptasinska, T. Märk, P. Scheier, J. Chem. Phys. 124 (2006) 124310.
[22] S. Ptasinska, S. Denifl, B. Mroz, M. Probst, V. Grill, E. Illenberger, P. Scheier, T.D. Märk, J. Chem. Phys. 123 (2005) 124302.
[23] M.H.V. Huynh, T.J. Meyer, PNAS 101 (2004) 13138.
[24] A.M. Scheer, K. Aflatooni, G.A. Gallup, P.D. Burrow, Phys. Rev. Lett. 92 (2004) 068102.
[25] S. Denifl, F. Zappa, A. Mauracher, F. Ferreira da Silva, A. Bacher, O. Echt, T.D. Märk, D.K. Bohme, P. Scheier, Chem. Phys. Chem 9 (2008) 1387.