Electron-induced damage of biotin studied in the gas phase and in the condensed phase at a single-molecule level

Adrian Keller\textsuperscript{1,2}, Janina Kopyra\textsuperscript{3}, Kurt V Gothelf\textsuperscript{2} and Ilko Bald\textsuperscript{2,4,5,6}

\textsuperscript{1} Institute of Ion Beam Physics and Materials Research, Helmholtz-Zentrum Dresden-Rossendorf, Bautzner Landstrasse 400, D-01328 Dresden, Germany
\textsuperscript{2} Interdisciplinary Nanoscience Center (iNANO) and Danish National Research Foundation: Centre for DNA Nanotechnology (CDNA), Aarhus University, DK-8000 Aarhus C, Denmark
\textsuperscript{3} Chemistry Department, Siedlce University, 3 Maja 54, 08-110 Siedlce, Poland
\textsuperscript{4} Department of Chemistry—Physical Chemistry, University of Potsdam, Karl-Liebknecht-Strasse 24-25, D-14476 Potsdam-Golm, Germany
\textsuperscript{5} BAM Federal Institute for Materials Research and Testing, Richard-Willstätter-Strasse 11, D-12489 Berlin, Germany
E-mail: bald@uni-potsdam.de

New Journal of Physics \textbf{15} (2013) 083045 (14pp)
Received 8 March 2013
Published 22 August 2013
Online at http://www.njp.org/
doi:10.1088/1367-2630/15/8/083045

Abstract. Biotin is an essential vitamin that is, on the one hand, relevant for the metabolism, gene expression and in the cellular response to DNA damage and, on the other hand, finds numerous applications in biotechnology. The functionality of biotin is due to two particular sub-structures, the ring structure and the side chain with carboxyl group. The heterocyclic ring structure results in the capability of biotin to form strong intermolecular hydrogen and van der Waals bonds with proteins such as streptavidin, whereas the carboxyl group can be employed to covalently bind biotin to other complex molecules. Dissociative electron attachment (DEA) to biotin results in a decomposition of the ring structure and the carboxyl group, respectively, within resonant features in the energy range 0–12 eV, thereby preventing the capability of biotin for

\textsuperscript{6} Author to whom any correspondence should be addressed.

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intermolecular binding and covalent coupling to other molecules. Specifically, the fragment anions (M–H)−, (M–O)−, C3N2O−, CH2O2−, OCN−, CN−, OH− and O− are observed, and exemplarily the DEA cross section of OCN− formation is determined to be 3 × 10−19 cm2. To study the response of biotin to electrons within a complex condensed environment, we use the DNA origami technique and determine a dissociation yield of (1.1 ± 0.2) × 10−14 cm2 at 18 eV electron energy, which represents the most relevant energy for biomolecular damage induced by secondary electrons. The present results thus have important implications for the use of biotin as a label in radiation experiments.

Contents

1. Introduction .............................. 2
2. Experimental methods ................. 4
   2.1. Gas-phase mass spectrometry ......... 4
   2.2. Condensed phase experiments on DNA origami templates .......... 4
3. Results and discussion .................. 5
   3.1. Dissociative electron attachment to gas-phase biotin ............... 5
   3.2. Characterization of electron-induced damage of biotin using the DNA origami technique .................. 8
4. Conclusions ............................ 11
Acknowledgments ......................... 11
References ................................ 11

1. Introduction

Biotin (Bt, figure 1) is a water-soluble B vitamin, which exhibits an exceptionally high binding affinity to the proteins avidin, streptavidin (SAv) and neutravidin. The Bt–SAv binding is among the strongest non-covalent interactions known with a dissociation constant of the order of 10−14–10−15 mol l−1 [1]. Biotinylation of biomolecules such as DNA and proteins has thus become a widely applied method in molecular biotechnology for purification, detection and immobilization purposes. A large variety of different SAv-modified probes, including gold nanoparticles [2], magnetic nanoparticles [3], fluorophores [4] and quantum dots [5], is nowadays commercially available and routinely used, for instance, in electron [2] and fluorescence microscopy of biological samples [5].

The high strength and specificity of Bt–SAv binding is also increasingly exploited in nanotechnology, e.g. in the controlled assembly of nanocrystal superstructures [6, 7] or the fabrication of nanoscale protein patterns [8]. Due to the broad availability of biotinylated DNA oligonucleotides, the field of structural DNA nanotechnology also makes more and more use of Bt–SAv binding, e.g. for the precise arrangement of functional entities on DNA nanostructures [9–11], or the visualization of single-molecule chemical reactions on DNA origami templates by atomic force microscopy (AFM) [12–14].

Despite its tremendous technological importance, the physiological roles of Bt are still not completely understood. As a B vitamin, Bt is essential for the metabolism, for instance, as a prosthetic group in carboxylase enzymes and it also plays a role in cell signaling.

New Journal of Physics 15 (2013) 083045 (http://www.njp.org/)
and in the expression of more than 2000 human genes [15]. Biotin can further affect the chromatin structure via biotinylation of histones and is thus believed to participate in gene silencing [16]. The same mechanism also seems to be associated with the cellular response to DNA damage [17–19] although the mechanisms and pathways are not yet known [16]. In human JAr choriocarcinoma cells for instance, biotinylation of histone H4 was observed to decrease in response to etoposide-induced double-strand breaks, which was interpreted as an early signaling event [18]. UV exposure of human T cells, on the other hand, was found to cause an increase in histone biotinylation, which might be a result of radiation-induced carboxylase degradation or a step during apoptosis [17].

Most of the radiation-induced damage to biomolecules is mediated via the radiolysis of water molecules which generates a large number of radicals and low-energy electrons (LEEs) along the radiation track [20]. In particular, reductive damage induced by secondary electrons with energies below 10 eV has recently been identified as the major source of DNA radiation damage [21]. Dissociative electron attachment (DEA) to DNA proceeds with high cross sections and can lead to the formation of single- and double-strand breaks [22–24]. LEE-induced bond breaking has thus received considerable attention and has been studied in a number of biologically relevant molecules, including amino acids [25–27], peptides [28, 29], vitamins [30], DNA nucleobases [31–33], model compounds for the DNA backbone [34–37] and complete DNA nucleotides [38]. Here, we study LEE-induced damage of Bt in the gas phase by negative ion mass spectrometry, as well as in the condensed phase at the single-molecule level using AFM of DNA origami nanostructures [14].

In the DNA origami technique a long single-stranded DNA scaffold (in most cases M13mp18 with 7249 nucleotides) is folded into specific two-dimensional (2D) and three-dimensional (3D) nanostructures simply by hybridization with a suitable set of short oligonucleotides [39, 40]. In this way it is not only possible to form complex structures such as 2D rectangles and triangles [39] but also 3D boxes [41], spheres [42] and many others [40]. The main advantage of the DNA origami technique for the study of DNA radiation damage is the simple functionalization with well-defined oligonucleotides, and the possibility to study LEE-induced DNA strand break yields at a single-molecule level using AFM as an analytical tool [14]. The DNA origami structures serve only as a template to arrange DNA target structures within well-defined ‘DNA nanoarrays’, which allows for the analysis of electron-induced damage to multiple target sequences in one irradiation experiment [14].

We attempt to gain a complete picture of Bt damage by considering a wide range of electron energies, different damage pathways and environments (gas phase versus condensed phase with Bt bound to DNA). We first consider damage pathways at low electron energies.
(<12 eV) that proceed via negative ion resonances. However, the probability function for LEE-induced damage processes, i.e. the cross sections for DEA, electronic excitation and ionization weighted by the energy distribution of secondary electrons in water has a global maximum right below 20 eV [43]. Consequently, we complement the DEA experiments with a characterization of electron-induced damage of Bt on DNA origami templates using 18 eV electrons.

2. Experimental methods

2.1. Gas-phase mass spectrometry

The DEA experiments on gas-phase Bt were performed in an ultrahigh vacuum (UHV) chamber (base pressure of 10\(^{-8}\) mbar) by means of a crossed electron-molecular beam. Briefly, the experimental setup consists of an electron source, an oven and a quadrupole mass analyzer. An incident electron beam of well-defined energy (full-width at half-maximum \(\approx 230\) meV, electron current \(\approx 10\) nA) generated from a trochoidal electron monochromator orthogonally intersects with an effusive molecular beam of the target molecule. Under ambient temperatures, the Bt sample is solid. Therefore, it was directly deposited into a vessel inside the vacuum system. During the experiments the overall system was heated up to temperatures of 463–473 K, which is sufficient to generate an effusive molecular beam of Bt with a pressure of 10\(^{-7}\) mbar as measured with an ionization gauge mounted at one of the flanges.

The negative ions are extracted from the collision area by a small electric field toward an entrance of the quadrupole mass analyzer and detected by single-pulse counting technique. The intensity of negative ions is recorded as a function of electron energy. The electron energy scale is calibrated by measuring the formation of SF\(_6^-\)/SF\(_6\) or Cl\(^-\)/CCl\(_4^-\) ions with a pronounced resonance feature near 0 eV. The measurements were performed in the absence of the calibration gas to avoid unwanted reactions between the target molecules and anions that arise from electron attachment to the calibration gas. The DEA cross section has been estimated by comparing the ion yields of the fragments generated from Bt with the ion yield of Cl\(^-\) from CCl\(_4\) at the 0.8 eV resonance. The sample of Bt was obtained from Sigma-Aldrich at a stated purity of \(\geq 99\%\) and used as delivered.

2.2. Condensed phase experiments on DNA origami templates

Triangular DNA origami structures have been prepared according to the procedure described by Rothemund [39]. Details on the DNA origami assembly can be found elsewhere [14]. Three of the staple strands have been modified at the 5’ end with Bt, and another three staple strands have been modified with a sequence containing a disulfide linker (S–S) and two T bases: 5’-Bt–TT–S–S. The DNA origami structures are exposed to LEEs, and the prolonged sequences constitute the target structure whose dissociation yield (DY) is determined. The DY of the disulfide containing sequence was determined previously [14] and in the present experiment it served as a reference sequence. The DNA origami structures have been adsorbed on SiO\(_2\)/Si substrates by incubation for 1 h in 10× TAE buffer containing 100 mM MgCl\(_2\) and subsequent rinsing with ethanol/water (1/1). The triangular shape of the DNA origami templates was chosen to minimize intermolecular interactions and thus clustering between the individual DNA origami structures. In this way, a rather homogeneous coverage of DNA origami templates on the SiO\(_2\)/Si substrates is obtained.

New Journal of Physics 15 (2013) 083045 (http://www.njp.org/)
Figure 2. Ion yield curves of four different fragment ions, which are formed by dissociative electron attachment to isolated (gas-phase) biotin.

The dry samples were introduced into the UHV chamber of a commercial time-of-flight secondary-ion-mass-spectrometry instrument, and irradiated with LEEs by means of a flood gun operated at 20 V acceleration voltage. The charging of the SiO$_2$ surface was determined to be $\approx 1.7$ eV by recording the shift of the electron current onset measured on the aluminum sample holder and the SiO$_2$/Si substrates. Thus, the effective energy of electrons arriving at the sample was $\approx 18$ eV. The electron fluence $\Phi$ was determined from the measured electron current $I$, the irradiation time $t$ and the irradiated area $A$: $\Phi = I \cdot t / A$. Five samples were irradiated at a fluence of $\approx 3 \times 10^{12}$ cm$^{-2}$, and two samples were used as a control. The irradiated samples were removed from the UHV chamber and rinsed with 4 mL ethanol/water (1/1) to remove fragmentation products. Subsequently, the samples were incubated for 5 min with a solution of 50 nM SAv and again rinsed with an ethanol/water mixture. AFM imaging of the dried samples was performed in air using a Bruker MultiMode 8 microscope operated in ScanAsyst-HR mode and SCANASYST-AIR-HR probes (Bruker). The DNA origami technique allows for the direct comparison of electron-induced damage to different target structures in one irradiation experiment. Consequently, a determination of the fluence dependence of Bt damage is not required [14]. Instead, the DY can be determined by comparing the damage of Bt to the number of strand breaks ($N_{SB}$) in the reference sequence (SSTT) and to the corresponding DY: 

$$D_{Y_{Bt}} = \frac{N_{SB}(Bt, \Phi) - N_{SB}(Bt, 0)}{N_{SB}(SSTT, \Phi) - N_{SB}(SSTT, 0)} D_{Y_{SSTT}}.$$ 

(1)

3. Results and discussion

3.1. Dissociative electron attachment to gas-phase biotin

LEE impact on gas-phase Bt produces several fragment anions that have been detected at $m/z$ 243, 228, 80, 46, 42, 26, 17 and 16. From the stoichiometry, they are assigned to the (M–H)$^-$, (M–O)$^-$, $C_3N_2O^-$, $CH_2O_2^-$, OCN$^-$, CN$^-$, OH$^-$ and O$^-$ ions, respectively. The corresponding ion yield curves are shown in figures 2 and 3. In general, the fragment anions are generated

New Journal of Physics 15 (2013) 083045 (http://www.njp.org/)
Figure 3. Ion yield curves of the fragment anions $[M-O]^-$, $CH_2O_2^-$, $OH^-$ and $O^-$, which are formed by dissociative electron attachment to gas-phase biotin.

by electron attachment to the molecule to form a transient negative ion (TNI). The TNIs are unstable and dissociate into a fragment anion and one or more neutral counterparts.

Figure 2 shows the yield of the dehydrogenated parent ion $[M-H]^-$, which is formed within a broad resonance centered at 1.3 eV and with lower intensity at 0 eV. This fragment ion is most likely generated by hydrogen loss from the carboxyl group of Bt and represents a stable closed shell anion. Similar to other organic acids such as formic acid \cite{44} and halogenated organic acids \cite{45, 46}, the resonance at 1.3 eV is assigned to a $\pi^*$ shape resonance, i.e. the incoming electron occupies a formerly empty $\pi^*$ orbital located on the COOH group. For the dissociation of the O–H bond the $\pi^*$ shape resonance has to couple to the $\sigma^*$($O$–$H$) orbital. Recently, it was suggested that the $\sigma^*$($O$–$H$) orbital can be directly accessed at low energies due to the large width of the $\sigma^*$ shape resonance \cite{47, 48}. Nevertheless, we cannot completely exclude the generation of $[M-H]^-$ due to the loss of an H atom from the N site in a similar way that has been previously reported from thymine \cite{49}. The origin of the lower energy signal is not clear, but it may be attributed to hot band transitions, i.e. transitions involving vibrationally excited molecules. Due to the reciprocal energy dependence of the electron attachment cross section, the intensity of the signal at threshold can be high despite a moderate population of higher vibrational states.

In most biotechnological applications Bt is bound to other molecules via the carboxyl group, usually with an amide bond. From the esters of simple organic acids and amino acids it is well-known that the electron-induced formation of the carboxylate ions observed in free acids is preserved in DEA to the corresponding esters \cite{50, 51}. Hence, also in a situation where Bt is covalently bound within a more complex environment it is likely that the carboxylate ion is formed by electron attachment at 0.5–2.5 eV to the Bt unit and subsequent cleavage of a C–O bond.

The most intense fragment anion was detected at $m/z$ 80 and is visible within a sharp resonance located at 0.3 eV (figure 2). Since the resonance is located well below the $\pi^*$ shape resonance of the carboxyl group, it is assigned to a shape resonance located on the Bt ring structure. The composition of this ion is not clear, but it can be assigned to the sum formula
C$_3$N$_2$O$^-$, which is accompanied by a complete decomposition of the Bt ring. Further fragment anions that are associated with a decomposition of the ring structure are the OCN$^-$ and CN$^-$ ions.

The OCN$^-$ fragment ion is formed within two resonant features centered at 5.2 and 7.8 eV, respectively. The corresponding ion yield curve is shown in figure 2. The OCN$^-$ ion can be excised from the Bt ring by simultaneous dissociation of three chemical bonds. The cleavage of two C–N bonds and one N–H bond requires approximately 6.2 and 4.0 eV (taking the bond dissociation energies of (CH$_3$)$_2$–NCOH$_5$ and H–N(CH$_3$)$_2$)$_2$ [52], which is compensated by the electron affinity of the OCN radical (3.609 eV) [53]. Taking into account the above numbers the thermodynamic threshold for OCN$^-$ formation would be 6.6 eV. In fact we observe the resonance at lower energy at 5.2 eV. Hence, the thermodynamic threshold has to be lowered by the formation of a C≡N double bond within the OCN$^-$ ion in order that DEA still becomes energetically accessible. The formation of the TNI at higher electron energies, i.e. at 5.2 and 7.8 eV must be associated with electronic excitation in the parent molecule and the corresponding resonances are referred to as core-excited resonances.

The CN$^-$ anion was exclusively observed from a low-energy resonance located at 1.7 eV, which is in striking contrast to the observation of the OCN$^-$ ion. The only possible way for its generation is the expulsion of the CN fragment from the Bt ring, which is most likely accompanied by a complete degradation of the ring. The CN$^-$ ion formation requires a complex reaction, since five covalent bonds need to be broken prior to its formation (N–H, two C–N bonds and a C≡O double bond). Nevertheless CN$^-$ is often observed after electron attachment to various molecules such as amino acids [51, 54], trifluoroalanine [46], N-acetyl-glycine [55], hexafluoroacetone azine [56], formamide [57] and various amide derivatives [58] even at very low energies (0–3 eV). The low-energy thresholds for CN$^-$ formation are usually explained by the high electron affinity of the CN radical (3.8 eV), the additional two bonds that are formed within CN$^-$, and a number of new bonds that must be formed in byproducts in the course of the reaction.

In general, the fragment ions are observed with very low count rates indicating a small DEA cross section. As an example we determined the DEA cross section for OCN$^-$ formation at 7.8 eV to be 3 × 10$^{-19}$ cm$^2$ using the Cl$^-$ signal from CCl$_4$ as a reference. The damage of Bt by DEA is thus much less effective (about two orders of magnitude) than the damage to DNA. OCN$^-$ is a common fragment anion observed in DEA to DNA nucleobases such as e.g. uracil. However, from uracil OCN$^-$ is formed with cross sections up to 1.5 × 10$^{-17}$ cm$^2$ [59]. Recently, the cross section of the most intense fragmentation channel in DEA to the DNA nucleobase thymine (T)—i.e. loss of H from the parent ion resulting in [T–H]$^-$—was accurately determined to be 7.9 × 10$^{-17}$ cm$^2$ [60].

Some additional fragment anions are observed in DEA to Bt that can be formed by cleavage of a single or a double bond, i.e. [M–O]$^-$, OH$^-$ and O$^-$ (figure 3). The released oxygen atom can either originate from the Bt ring or the carboxyl group. However, [M–O]$^-$ was previously not observed in DEA to organic acids, therefore it is likely to be formed from the Bt ring by cleavage of the C=O double bond. Since [M–O]$^-$ is generated close to threshold the energy that is required for the cleavage of the double bond must be compensated by a large electron affinity of the neutral [M–O] molecule. This fragment ion could also be assigned to [M–NH$_2$]$^-$, which also occurs in DEA to amino acids [54]. However, in contrast to the situation in amino acids the formation of [M–NH$_2$]$^-$ from Bt would require the cleavage of three bonds and the formation of at least one new bond.
Figure 4. Characterization of electron-induced damage of biotin using the DNA origami technique. (A) Scheme of the DNA origami design showing the positions of 5′-Bt-TTSS and 5′-Bt modifications protruding from the DNA origami template. (B) AFM images of single DNA origami templates after incubation with streptavidin without (left) and after (right) irradiation. (C) 1 µm² AFM image from a non-irradiated control sample. (D) 1 µm² AFM image from a sample irradiated with 18 eV electrons at a fluence of $3.1 \times 10^{12}$ cm⁻².

A ubiquitous reaction in DEA to organic acids is the formation of OH⁻. It is generated from a single C–O bond cleavage, and within a larger molecular network this reaction would correspond to a dissociation of the Bt unit. In simple organic acids, OH⁻ was only observed from core-excited resonances at 8–12 eV [61, 62]. On the other hand, at low energies (<1 eV) the OH⁻ anion was observed from more complex molecules such as N-acetyl-glycine [55] and the sugar d-ribose [63], indicating that rearrangement reactions within the neutral fragments are required to decrease the thermodynamic threshold.

The fragment anion at $m/z$ 46 ($\text{CH}_2\text{O}_2^-$) is ascribed to the excision of the complete carboxyl group accompanied by hydrogen transfer and localization of the negative charge on the carboxyl group. It is formed close to threshold in a similar way as in DEA to acetic acid [61], propanoic acid [62], several amino acids [51] and chlorodifluoroacetic acid [45]. The structure of this fragment is presently not known. In principle, it can be assigned to the formic acid anion (HCOOH⁻); however, this anion only exists as a short-lived scattering state and hence cannot bind the extra electron to form a thermodynamically stable anion.

3.2. Characterization of electron-induced damage of biotin using the DNA origami technique

The most relevant energy regime for secondary electron damage is around 18 eV [43]. However, at this energy no negative ions have been observed in DEA to Bt, indicating that other processes such as electronic excitation and ionization may become relevant. In the following, we consider electron-induced Bt damage using the DNA origami technique.

Figure 4(a) shows a scheme of the DNA origami design used in the present study. The pure Bt modifications of the staple strands are placed on the right side of the trapezoids.
Figure 5. Histograms showing the number of specifically bound streptavidin (SAv) on non-irradiated DNA origami templates (control), and on DNA origami templates irradiated with 18 eV electrons at a fluence of $3.1 \times 10^{12}$ cm$^{-2}$. As the positions of different modifications can be distinguished in the AFM images, the damage to the different structures can be analyzed separately.

of the DNA origami structures, and the protruding 5′-Bt–TT–SS sequences are placed into the centers of the trapezoids. The AFM images in figures 4(b)–(d) have been recorded after incubation with SAv, and they demonstrate that the positions of the different modifications on the triangular DNA origami structures can be clearly distinguished. Electron irradiation of the DNA origami structures can lead to damage in both the double-stranded DNA origami template and the protruding modifications. Damage of the protruding modifications can prevent the subsequent binding of SAv to Bt, and thus a missing SAv is interpreted as a dissociation or strand break, respectively. Damage of the DNA origami template does not result in dissociation of the protruding strand and is therefore not detected. In figure 4(c) an image of a non-irradiated control sample is shown, and the image in figure 4(d) was obtained from a sample irradiated with 18 eV electrons at a fluence of $3.1 \times 10^{12}$ cm$^{-2}$ (magnified images in figure 4(b)).

A total number of 5439 molecules have been analyzed (1860 molecules from control samples and 3579 molecules from irradiated samples), and the corresponding histograms are shown in figure 5.

In the samples irradiated with 18 eV electrons at a fluence of $3.1 \times 10^{12}$ cm$^{-2}$, the number of bound SAv is markedly reduced. In the histogram for the positions of the SSTT sequence,
the maximum of the number of bound SAv is shifted to two. In the case of pure Bt the counts of the lower numbers of bound SAv are also increased, although most of the DNA origami templates still carry three SAv. The reduced number of bound SAv is due to the electron-induced damage to the protruding species (i.e. the Bt moiety at the positions of pure Bt, and the SSTT sequence including the Bt label at the other positions). The histograms clearly indicate that the electron-induced damage of the SSTT sequence is considerably higher than that of the pure Bt label. Thus, most of the SSTT damage is ascribed to an electron-induced strand breakage of the disulfide bond, which was previously determined to be $7.1 \times 10^{-14}$ cm$^2$ [14]. Using equation (1), a DY for the Bt label at 18 eV electron energy is determined to be $(1.1 \pm 0.2) \times 10^{-14}$ cm$^2$.

The above-mentioned DEA experiments indicate that at 18 eV no direct electron attachment to Bt is possible. The electron impact at 18 eV can either lead to ionization or electronic excitation of Bt followed by dissociation of the Bt moiety, thereby preventing subsequent SAv binding. An alternative pathway is the generation of low-energy secondary electrons from the Si substrate followed by electron attachment to the Bt unit. The electron ionization cross sections of DNA bases at 18 eV are in the order of $10^{-16}$ cm$^2$ [64, 65], and it can be assumed that the ionization cross section for Bt is of the same order. In comparison, the DEA cross sections determined in the present study are of the order of $10^{-19}$–$10^{-18}$ cm$^2$. Since the secondary electron yield of oxidized Si surfaces at 18 eV primary energy is only around 0.5 [66], the dominating process at 18 eV electron energy is most likely initiated by ionization of Bt.

The histograms of the control samples in figure 5 show two additional features that are worth mentioning:

1. In the control samples, a considerable number of DNA origami structures carry less SAv than three, i.e. the maximum number of labels. This is due to a limited purity of the prolonged staple strands, i.e. the number of strand breaks without irradiation simply corresponds to the number of staples that do not carry a Bt marker due to errors in the oligonucleotide synthesis. This error is larger for the SSTT sequences than for the oligonucleotides that carry only the Bt marker.

2. A considerable number of DNA origami templates do not carry a single SAv at the position of the pure Bt target (‘Zero counts’). This effect is particularly obvious for the control samples, where the number of zero counts for the SSTT sequence is close to zero, but is close to 150 for the pure Bt sequence. The number of DNA origami structures without SAv is for the pure Bt position almost as high as for 1 SAv molecule per DNA origami structure. This observation can be ascribed to the adsorption geometry of the flat triangles on the Si substrate. It was shown previously that about 17% of the triangular DNA origami structures adsorb ‘face-down’ [14], i.e. with the protruding strands pointing toward the Si surface. The protruding SSTT strands are long enough to expose the Bt linker in a way that SAv can in most cases still bind to Bt in this configuration. However, the Bt linker without protruding strand is apparently too short, so that Bt is buried underneath the DNA, thereby blocking the binding to SAv. Since the DY is determined from the difference of decomposed molecules from the control sample and the irradiated sample, this adsorption geometry is not expected to influence the DY considerably.
4. Conclusions

The electron-induced damage to Bt was characterized with two complementary methods, i.e. in the gas phase with a crossed-beam setup and in the condensed phase with the DNA origami technique. The DEA experiments revealed the formation of a series of fragment anions that are either associated with decomposition of the carboxyl group or the Bt ring structure. The deterioration of the ring structure destroys the capability of Bt to bind to proteins such as SAv via intermolecular hydrogen bonding. A decomposition of the carboxyl group leads to a desorption of the Bt unit when it is bound to other molecules. In general, the DEA cross sections are rather small, and exemplarily we determined the cross section for OCN$^-$ formation at 7.8 eV to be $3 \times 10^{-19}$ cm$^2$. With the DNA origami technique, we studied electron-induced damage of Bt at 18 eV electron energy and determined a DY of $(1.1 \pm 0.2) \times 10^{-14}$ cm$^2$. In previous experiments the DYS of 5'-Bt–TT–SS and 5'-Bt–TT sequences have been determined to be $7.1 \times 10^{-14}$ and $1.7 \times 10^{-14}$ cm$^2$, respectively [14]. Along with the present results, we can conclude that the cleavage of a TT sequence (without Bt) proceeds with a yield of $0.6 \times 10^{-14}$ cm$^2$, whereas the contribution that can be ascribed to a cleavage of the disulfide bond (S–S) is $5.4 \times 10^{-14}$ cm$^2$.

These experiments indicate that Bt damage has to be considered when using Bt as a label in single-molecule experiments using the DNA origami technique for the determination of DNA strand break yields. This concerns, in particular, not only the energy regime where ionization takes place (>10 eV) but also the energy range where DEA is most effective, i.e. below 1 eV.

Acknowledgments

This work was supported by the Polish Ministry of Science and Higher Education, and by grants from the Danish National Research Foundation and the Danish Research Agency. IB and AK acknowledge financial support from the Deutsche Forschungsgemeinschaft and the Alexander von Humboldt foundation, respectively.

References

[1] Green N M, Anfinsen C B, Edsall J T and Frederic M R 1975 Advances in Protein Chemistry (New York: Academic) pp 85–133
[2] Sun X J, Tolbert L P and Hildebrand J G 1995 Using laser-scanning confocal microscopy as a guide for electron-microscopic study—a simple method for correlation of light and electron-microscopy J. Histochem. Cytochem. 43 329–35
[3] Wang J, Xu D K, Kawde A N and Polsky R 2001 Metal nanoparticle-based electrochemical stripping potentiometric detection of DNA hybridization Anal. Chem. 73 5576–81
[4] Willard D M, Carillo L L, Jung J and Van Orden A 2001 CdSe–ZnS quantum dots as resonance energy transfer donors in a model protein–protein binding assay Nano Lett. 1 469–74
[5] Leduc C, Ruhnow F, Howard J and Diez S 2007 Detection of fractional steps in cargo movement by the collective operation of kinesin-1 motors Proc. Natl Acad. Sci. USA 104 10847–52
[6] Connolly S and Fitzmaurice D 1999 Programmed assembly of gold nanocrystals in aqueous solution Adv. Mater. 11 1202–5
[7] Lee J, Govorov A O, Dulka J and Kotov N A 2004 Bioconjugates of CdTe nanowires and Au nanoparticles: plasmon–exciton interactions, luminescence enhancement and collective effects Nano Lett. 4 2323–30
[8] Hoff J D, Cheng L J, Meyhofer E, Guo L J and Hunt A J 2004 Nanoscale protein patterning by imprint lithography Nano Lett. 4 853–7

New Journal of Physics 15 (2013) 083045 (http://www.njp.org/)
Bui H, Onodera C, Kidwell C, Tan Y, Graugnard E, Kuang W, Lee J, Knowlton W B, Yurke B and Hughes W L 2010 Programmable periodicity of quantum dot arrays with DNA origami nanotubes Nano Lett. 10 3367–72

Ko S H, Gallatin G M and Liddle J A 2012 Nanomanufacturing with DNA origami: factors affecting the kinetics and yield of quantum dot binding Adv. Funct. Mater. 22 1015–23

Eskelinen A-P, Kuzyk A, Kaltiaisenaho T K, Timmermans M Y, Nasibulin A G, Kauppinen E I and Torma P 2011 Assembly of single-walled carbon nanotubes on DNA-origami templates through streptavidin–biotin interaction Small 7 746–50

Voigt N V et al 2010 Single-molecule chemical reactions on DNA origami Nature Nanotechnol. 5 200–3

Keller A, Bald I, Rotaru A, Cauet E, Gothelf K V and Besenbacher F 2012 Probing electron-induced bond cleavage at the single-molecule level using DNA origami templates ACS Nano 6 4392–9

Zempleni J 2005 Uptake, localization, and noncarboxylase roles of biotin Annu. Rev. Nutr. 25 175–96

Hassan Y I and Zempleni J 2006 Epigenetic regulation of chromatin structure and gene function by biotin J. Nutr. 136 1763–5

Peters D M, Griffin J B, Beck M M and Zempleni J 2002 Exposure to UV light causes increased biotinylation of histones in Jurkat cells Am. J. Physiol.—Cell Physiol. 283 C878–84

Rodriguez-Melendez R, Griffin J B and Zempleni J 2004 Biotin supplementation increases expression of the cytochrome P4501B1 gene in Jurkat cells, increasing the occurrence of single-stranded DNA breaks J. Nutr. 134 2222–8

Kothapalli N, Sarath G and Zempleni J 2005 Biotinylation of K12 in histone H4 decreases in response to DNA double-strand breaks in human JAr choriocarcinoma cells J. Nutr. 135 2337–42

Baccarelli I, Bald I, Gianturco F A, Illenberger E and Kopyra J 2011 Electron-induced damage of DNA and of its components: experiments and theoretical modellings Phys. Rep. 508 1–44

Nguyen J, Ma Y, Luo T, Bristow R G, Jaffray D A and Lu Q-B 2011 Direct observation of ultrafast-electron-transfer reactions unravels high effectiveness of reductive DNA damage Proc. Natl Acad. Sci. USA 108 11778–83

Boudaiffa B, Cloutier P, Hunting D, Sanche L 2000 Resonant formation of DNA strand breaks by low-energy (3–20 eV) electrons Science 287 1658–60

Martin F, Burrow P D, Cai Z L, Cloutier P, Hunting D and Sanche L 2004 DNA strand breaks induced by 0–4 eV electrons: the role of shape resonances Phys. Rev. Lett. 93 068101

Huels M A, Boudaiffa B, Cloutier P, Hunting D and Sanche L 2003 Single, double and multiple double strand breaks induced in DNA by 3–100 eV electrons J. Am. Chem. Soc. 125 4467–77

Abdoul-Carime H and Sanche L 2004 Alteration of protein constituents induced by low-energy (<40 eV) electrons: III. The aliphatic amino acids J. Phys. Chem. B 108 457–64

Abdoul-Carime H, Gohlke S and Illenberger E 2004 Conversion of amino-acids by electrons at subexcitation energies Phys. Chem. Chem. Phys. 6 161–4

Kopyra J, Szamraj I, Abdoul-Carime H, Farizon B and Farizon M 2012 Decomposition of methionine by low energy electrons Phys. Chem. Chem. Phys. 14 8000–4

Alizadeh E et al 2011 Bond dissociation of the dipeptide dialanine and its derivative alanine anhydride induced by low energy electrons J. Chem. Phys. 134 054305

Gschliesser D, Vizzaino V, Probst M, Scheier P and Denil S 2012 Formation and decay of the dehydrogenated parent anion upon electron attachment to dialanine Chem. Eur. J. 18 4613–9

Abdoul-Carime H and Illenberger E 2004 Degradation of vitamin C by low-energy electrons Chem. Phys. Lett. 390 481–4

Abdoul-Carime H, Gohlke S and Illenberger E 2004 Site-specific dissociation of DNA bases by slow electrons at early stages of irradiation Phys. Rev. Lett. 92 168103

New Journal of Physics 15 (2013) 083045 (http://www.njp.org/)
Ptasinska S, Denifl S, Grill V, Maerk T D, Scheier P, Gohlke S, Huels M A and Illenberger E 2005 Bond-selective H-ion abstraction from thymine Angew. Chem. Int. Edn Engl. 44 1647–50

Denifl S et al 2007 Influence of functional groups on the site-selective dissociation of adenine upon low-energy electron attachment Angew. Chem. Int. Edn Engl. 46 5238–41

Bald I, Dabkowska I and Illenberger E 2008 Probing biomolecules by laser-induced acoustic desorption: electrons at near zero electron volts trigger sugar-phosphate cleavage Angew. Chem. Int. Edn Engl. 47 8518–20

Bald I, Kopyra J, Dabkowska I, Antonsson E and Illenberger E 2007 Selective excision of C5 from D-ribose in the gas phase by low-energy electrons (0–1 eV): implications for the mechanism of DNA damage Angew. Chem. Int. Edn Engl. 45 4851–5

Kopyra J 2012 Low energy electron attachment to the nucleotide deoxycytidine monophosphate: direct evidence for the molecular mechanisms of electron-induced DNA strand breaks Phys. Chem. Chem. Phys. 14 8287–9

Rothemund P W K 2006 Folding DNA to create nanoscale shapes and patterns Nature 440 297–302

Tørring T, Voigt N V, Nangreave J, Yan H and Gothelf K V 2011 Chem. Soc. Rev. 40 5636–46

Andersen E S et al 2009 Self-assembly of a nanoscale DNA box with a controllable lid Nature 459 73–6

Han D, Pal S, Yang Y, Jiang S, Nangreave J, Liu Y and Yan H 2013 DNA Gridiron nanostructures based on four-arm junctions Science 339 1412–5

Alizadeh E and Sanche L 2012 Precursors of solvated electrons in radiobiological physics and chemistry Chem. Rev. 112 5578–602

Martin I, Skalicky T, Langer J, Abdoul-Carine H, Karwasz G, Illenberger E, Stano M and Matejcik S 2005 Low energy electron driven reactions in single formic acid molecules (HCOOH) and their homogeneous clusters Phys. Chem. Chem. Phys. 7 2212–6

Kopyra J, Konig-Lehmann C and Illenberger E 2011 Low energy (0–10 eV) electron driven reactions in the halogenated organic acids CCl3COOH, CClF2COOH and CF3CHNH2COOH (trifluoroalanine) J. Chem. Phys. 135 124307

Scheer A M, Mozejko P, Gallup G A and Burrow P D 2007 Total dissociative electron attachment cross sections of selected amino acids J. Chem. Phys. 126 174301

Gallup G A, Burrow P D and Fabrikant I I 2009 Electron-induced bond breaking at low energies in HCOOH and glycine: the role of very short-lived sigma(+) anion states Phys. Rev. A 79 042701

Ptasinska S, Denifl S, Mroz B, Probst M, Grill V, Illenberger E, Scheier P and Mark T D 2005 Bond selective dissociative electron attachment to thymine J. Chem. Phys. 123 124302

Martin I, Langer J and Illenberger E 2008 Reactions in fluorinated acetic acid esters triggered by slow electrons: bond cleavages, hydrogen transfer reactions and loss of halocarbons Z. Phys. Chem. 222 1185–96

Vasil’ev Y V, Figard B J, Voinov V G, Barofsky D F and Deinzer M L 2006 Resonant electron capture by some amino acids and their methyl esters J. Am. Chem. Soc. 128 5506–15

McMillen D F and Golden D M 1982 Hydrocarbon bond-dissociation energies Annu. Rev. Phys. Chem. 33 493–532

Lide D (ed) 2002 CRC Handbook of Chemistry and Physics (Boca Raton, FL: CRC)

Muftakhov M V and Shchukin P V 2010 Resonant dissociative electron capture by the simplest amino acids and dipeptides Russ. Chem. Bull. 59 896–911

New Journal of Physics 15 (2013) 083045 (http://www.njp.org/)
Kopyra J, Konig-Lehmann C and Illenberger E 2012 Low energy electron attachment to N-acetylglycine Chem. Phys. Lett. 550 47–51
Bald I, Dabkowska I, Illenberger E and Ingolfsson O 2007 Energy selective excision of CN—following electron attachment to hexafluoroacetone azine ((CF3)(2)C≡N−N≡C(CF3)(2)) Phys. Chem. Chem. Phys. 9 2983–90
Hamann T, Edtbauer A, Ferreira da Silva F, Denifl S, Scheier P and Swiderek P 2011 Dissociative electron attachment to gas-phase formamide Phys. Chem. Chem. Phys. 13 12305
Koenig-Lehmann C, Kopyra J, Dabkowska I, Kocisek J and Illenberger E 2008 Excision of CN—and OCN—from acetamide and some amide derivatives triggered by low energy electrons Phys. Chem. Chem. Phys. 10 6954–61
Ptasinska S, Denifl S, Grill V, Mark T D, Illenberger E and Scheier P 2005 Bond—and site-selective loss of H—from pyrimidine bases Phys. Rev. Lett. 95 093201
Kopyra J, Koenig-Lehmann C and Illenberger E 2009 On the absolute value for the cross section of dissociative electron attachment (DEA) to the DNA base thymine Int. J. Mass Spectrom. 281 89–91
Sailer W, Pelc A, Probst M, Limtrakul J, Scheier P, Illenberger E and Mark T D 2003 Dissociative electron attachment to acetic acid (CH3COOH) Chem. Phys. Lett. 378 250–6
Pelc A, Sailer W, Scheier P, Mark T D and Illenberger E 2004 Fragmentation of propanoic acid by subexcitation electrons Chem. Phys. Lett. 392 465–9
Baccarelli I, Gianturco F A, Grandi A, Sanna N, Lucchese R R, Bald I, Kopyra J and Illenberger E 2007 Selective bond breaking in b-d-ribose by gas-phase electron attachment around 8 eV J. Am. Chem. Soc. 129 6269–77
Bernhardt P and Paretzke H G 2003 Calculation of electron impact ionization cross sections of DNA using the Deutsch–Mark and binary-encounter-Bethe formalisms Int. J. Mass Spectrom. 223 599–611
Mozejko P and Sanche L 2003 Cross section calculations for electron scattering from DNA and RNA bases Rad. Environ. Biophys. 42 201–11
Dionne G F 1975 Origin of secondary-electron-emission yield-curve parameters J. Appl. Phys. 46 3347–51