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Degradation of *N*-Acetyl Tryptophan by Low-Energy (<12 eV) Electrons

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Abstract: Secondary low-energy electrons are abundantly created during the early moments following the deposition of energy by radiation into cells. Here we show the ability of slow (<12 eV) electrons to effectively decompose gas-phase *N*-acetyl tryptophan (NAT) which can model a simple protein. The fragmentation of NAT, initiated via a resonant electron−molecule interaction exclusively at the peptide bridge, produces a large variety of negative species. The present findings contribute to the molecular description of the initial step in the radiation-induced damage.

Introduction

One of the fundamental issues in radiation biology concerns the problem how eukaryotic genomes are altered. It is now well admitted that radiation is implicated in genomic diseases leading to mutations and cancers.[1,2] Paradoxically, the use of radiation is an alternative therapy to the ablative surgery for tumor treatments. However, cumulative radiation received by patients may increase the risk for the generation of secondary tumors.[3,4] Therefore, the understanding the details of the interaction of ionizing radiation with biological medium at the molecular level is a prerequisite for predicting long-term genotoxic effects via mathematical models[5] leading to a better comprehension of the effect of radiation.

Fast primary particles distribute energy along their tracks in the irradiated medium via the production of excited species, reactive free radicals, ions, and a large quantity of ballistic low-energy (<30 eV) electrons: ca. 5×10^4 per deposited MeV.[6,7] The former species are well-known to promote cytotoxic, mutagenic, and carcinogenic effects.[1] Since the interaction of the ballistic slow electrons with cells occurs during the very early moments (femtosecond to picosecond time scale) following the deposition of energy into the irradiated medium, their specific actions are not easy to investigate via the traditional techniques (e.g., pulse radiolysis). Only recently the ability of low-energy electrons to induce severe lesions to DNA by fragmenting the nucleic acid building blocks[8,9] has been demonstrated.[10] In addition to DNA, proteins are also the fundamental constituents of the genome, and they are involved in many biological functions (e.g., DNA methylation and gene silencing).[11,12] Therefore, their alteration may lead to dramatic instabilities in the genome.[13]

The present work investigates the alteration of protein by low-energy (<12 eV) electrons. Here we show the ability of slow electrons to decompose *N*α-acetyl tryptophan (NAT) as a simple model of a dipeptide. The experimental apparatus having a base pressure of 6×10^{-8} mbar consists of an electron monochromator generating an incident electron beam of well-defined energy (∼10 nA, fwhm ∼0.15 eV),[14] crossing at a right angle with an effusive beam of NAT. This latter emanates from an oven containing approximately 20 mg of 99% purity powder (Aldrich Ltd.) heated by two in vaccuo halogen bulbs. These lamps also prevent the powder from condensation on the surfaces (plates, chamber walls), which otherwise may lead to an undesirable change in contact potentials during measurements. The operating temperature of approximately 400−420 K measured by a platinum resistance directly at the oven is appreciably below the molecular decomposition temperature (480 K).[15] Therefore, the original structure of NAT produced in gas phase is likely to remain intact. Furthermore, it has been shown that thermal heating of the dipeptide *N*-acetyl tryptophan methyl amide at 420 K produces undecomposed molecules.[16] Furthermore, the presently recorded spectra are also indications of the production of nondecomposed NAT. Indeed, thermal decomposition of amino acids constituting proteins leads, for instance, to the formation of CO_2 via the cleavage of the

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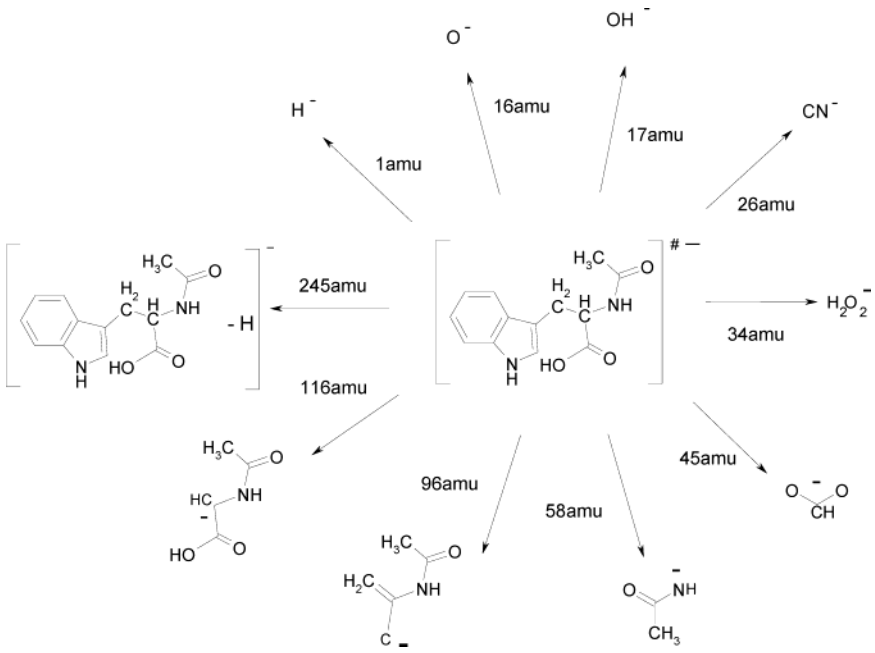

Figure 1. Structure of *N*α-acetyl tryptophan (bracket) with all the detected negative species produced from fragmentation of the transitory negative ion.

carboxylic COOH group.[17] Therefore, the yield function of the O^- anion would exhibit a strong characteristic signature of DEA to CO_2.[18] The negative ions are extracted from the reaction volume by a small electric field (<0.5 V cm^{-1}) toward a quadrupole mass analyzer and are detected by single pulse counting techniques. Finally, calibration of the energy scale is performed by means of SF_6, in a sequential procedure, avoiding the presence of SF_6 while recording spectra from NAT. Indeed, SF_6^- generated in the collision region can induce dissociative electron-transfer reactions in the target molecules, leading to dehydrogenated anions.[19]

Low-energy (<12 eV) electron impact on gaseous NAT produces various negatively charged species (*m*/*e*), as shown in Figure 1. They are tentatively assigned by stoichiometry to $(NAT - H)^-$ (245 amu), $H_6C_4NO_3^-$ (116 amu), $H_6C_5NO^-$ (96 amu), $H_4C_2NO^-$ (58 amu), HCO_2^- (45 amu), $H_2O_2^-$ (34 amu), CN^- (26 amu), OH^- (17 amu), O^- (16 amu), and H^- (1 amu). Figures 2 and 3 exhibit the recorded anion yield functions. The most dominant species ($H_6C_4NO_3^-$) results from the cleavage of the methylen-indole (MI) ring from NAT, with the excess charge located on the peptide bridge (Figure 3f). This observation agrees with the very recent investigation on the impact of slow electrons to gas-phase tryptophan, which does not show the negatively charged MI ring moiety[20] but the glycil-yl anion counterpart. While the most dominant reaction decomposes NAT into two large subunits, i.e., the amino acid residue (MI ring) and the peptide bridge, the other abundant species (H^- and $HCOO^-$) arise from fragmentation processes occurring directly on the peptide bridge (Figures 3a and 2d).

The ion yield curves (Figures 2,3) show pronounced resonance profiles reminiscent of dissociative electron attachment (DEA). The incoming electron is captured by NAT forming a

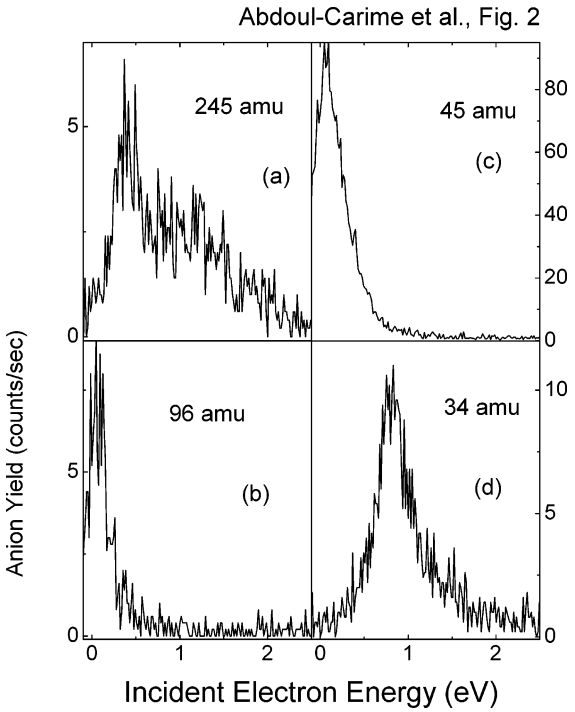

Figure 2. Incident electron energy dependence of (a) $(NAT\text{-}H)^-$ (245 amu), (b) $H_6C_5NO^-$ (96 amu), (c) $(HCO_2)^-$ (45 amu), and (d) $H_2O_2^-$ (34 amu) anion produced from electron impact on gaseous *N*α-acetyl tryptophan.

transitory negative ion ($NAT^{\#-}$), which subsequently undergoes dissociation into one negatively charged fragment and one (or more) neutral fragment. At incident electron energies below the first electronic excitation (∼4−5 eV),[21] the $NAT^{\#-}$ can be assigned to a *shape resonance* characterized by the accommodation of the excess electron into one of the virtual molecular orbitals (MOs), in the present case most likely π^*-type MOs.[22] Hence, shape resonances in NAT are the precursors for the

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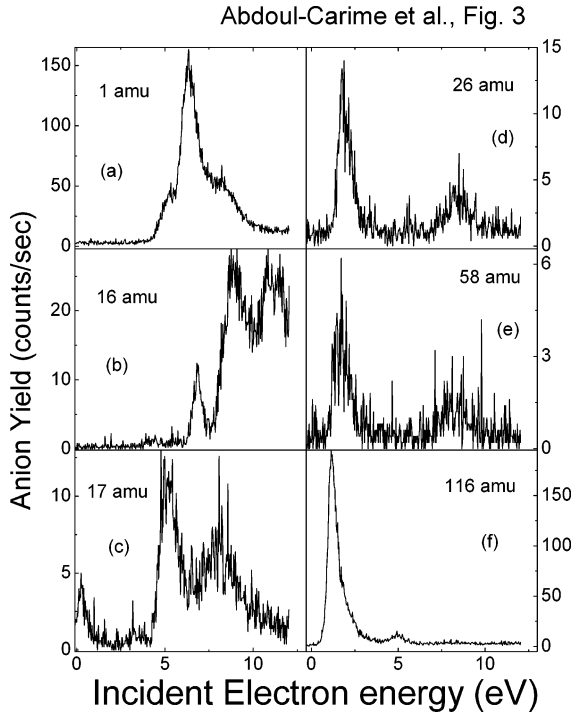

Figure 3. Incident electron energy dependence of (a) H^- (1 amu), (b) O^- (16 amu), (c) HO^- (17 amu), (d) CN^- (26 amu), (e) H_4C_2NO (58 amu), and (f) $H_6C_4NO_3^-$ (116 amu) anion produced from electron impact on gaseous *N*α-acetyl tryptophan.

dominant channels $H_6C_4NO_3^-$ (1.2 eV) and HCO_2^- (0.1 eV) but also for the less intense fragments $H_2O_2^-$ (0.8 eV), $H_6C_5NO^-$ (0.1 eV, 1.6 eV), OH^- (3.3 eV), CN^- (1.8 eV), $H_4C_2NO^-$ (1.6 eV) and the dehydrogenated NAT anion, $(NAT\text{-}H)^-$ (0.4, 1.1 eV). The peak observed near 0 eV in the OH^- yield function (Figure 3c) is unexpected. Thermodynamically, this ion can only be formed at appreciably higher electron energies. Therefore, the origin of this peak is still unknown. Temporary negative ions created at energies in the vicinity of electronically excited states of the neutral molecule can be characterized as *core-excited resonances*, consisting of two electrons in normally unoccupied MOs, moving in the field of the positive core. Thus, the structures observed for H^- (5.2 eV, 6.4 eV, 8.3 eV), O^- (6.8 eV, 8.9 eV, 11.0 eV), OH^- (5.2 eV, 8.1 eV), CN^- (8.3 eV), $H_4C_2NO^-$ (8.3 eV), and $H_6C_4NO_3^-$ (4.9 eV) are signatures of core-excited resonances associated with $\pi \rightarrow \pi^*$ or $\sigma \rightarrow \sigma^*$ electron transitions.[21,23]

Dissociative electron attachment requires that one of the fragments at least has a positive electron affinity for electron attachment. Thus, the formation of $H_4C_2NO^-$ (116 amu) and its neutral MI complement, arising from a simple C−C bond cleavage, suggests that the electron affinity of the peptide bridge radical must be sufficiently high to drive the reaction. In contrast, formation of the formate anion HCO_2^- is due to a more complex reaction involving substantial molecular rearrangements. The simplest reaction would involve cleavage of the C−C bond in the peptide bridge and concomitantly a rearrangement from $(COOH)^-$ to the formate anion $HCOO^-$. The electron affinity of the HCOO radical is sufficiently high (3.50 eV[24]) to enable such a reaction. The production of H^- naturally arises from a single bond rupture. Since this ion only appears above 4.5 eV, its formation is energetically possible from the cleavage of the hydrogen atom at any of the N or C sites. Finally, the production of the $(H_2O_2)^-$ negative fragment can be initiated by the formation of the so-called two-center, three-electron (2c,3e) bonds systems,[25] $OH\therefore OH^-$, during the lifetime of the transient parent anion. Such a system, involving two σ bonding and one σ^* antibonding electrons, has been reported for sulfide systems[26] and dihalogen ions (e.g., F_2^- [27]). However, in the particular case of $(H_2O_2)^-$, the three-electron bonded isomer is not stable.[25] It may thus undergo rearrangement into a more stable hydrogen-bonded $(HO\cdots HO)^-$ configuration.[28,29]

The absolute cross section for HCO_2^- formation at 0.1 eV can be estimated by comparing the measured ion signals to that of the calibration gas, SF_6^-, for which the absolute cross section is well-known (4.5×10^{-14} cm^2 [30]). Assuming then the same detection efficiencies,[31] for SF_6^- and HCO_2^- the cross section for the production of the negative fragment is estimated to be 1×10^{-16} cm^2. The total decomposition cross section of NAT averaged over the 0−12 eV energy range is found to be 6×10^{-17} cm^2, with an accuracy of 1 order of magnitude. This value, leading to an average rate constant of 1×10^{12} s^{-1} M^{-1}, can be compared to that from the thermal rate for the reaction of hydroxyl radicals with different amino acids which reach values up to 3×10^{10} s^{-1} M^{-1}.[32] These findings indicate the higher efficacy of low-energy electrons to damage proteins compared to that of radicals. Here we compare rates from gas-phase experiments (electrons) with those from the condensed phase (OH radicals). Extensive studies on DEA reactions have in fact shown that the DEA cross sections can considerably be enhanced in a condensed phase environment.[33] Furthermore, in condensed medium, reaction of electrons with *N*-acetylpeptides can lead to additional decomposition channels. For instance, electron spin resonance spectroscopy experiments on *N*-acetylpeptides have shown the following alteration sequence: anion formation, deamination, and hydrogen abstraction to form an α-carbon radical.[34,35]

The present results clearly demonstrate the ability of secondary low-energy (<12 eV) electrons to deteriorate *N*α-acetyl tryptophan, modeling a simple protein. The dominant decomposition reactions can be ascribed to the decomposition into the neutral MI and the negatively charged peptide bridge and also fragmentation of the peptide bridge. In contrast, radicals also produced by high energy radiation attack proteins almost exclusively at the side chain (e.g., aromatic ring) of the amino acids due to their steric hindrances.[36] However, it has been

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shown that the side chain of some amino acid residues can also be damaged by slow electrons:[37,38] e.g., via conversion of cystein to alanine.[38] Finally, the fragments initiated by DEA may additionally react with the surrounding proteins and/or DNA, leading to further damage to the genome.

It is noteworthy that in a more realistic biological medium, where proteins are bound to other proteins, to nucleic acids and/or water, the interaction of ballistic low-energy electrons with the corresponding target may be modified to some extent. Nevertheless, the fundamentals of such interactions will usually persist regardless of the composition of the system or its state of aggregation.[39] Thus, the present findings from a simple model peptide may be transferred to more complex proteins.

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