

Simulations of the fragmentation of the $[V-H]^-$ anions as formed upon DEA to L-valine.

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Abstract. The dissociation of proteins due to radiation and charged particles is an important process which is not well understood. Measurements of the fragmentation of such large molecules using mass spectrometers can be difficult to describe and understand since for many of the masses observed there are more than one possible combination of atoms. We have simulated the metastable decay of the anions formed by dissociative electron attachment (DEA) to L-Valine to show that it is possible to recognize some of the observed mass to charge ratios by their further fragmentation. By using computer simulations of the classical dynamics of the ions and finding minimum energy paths for intermolecular atom transfer and reorganization, we have identified molecular combinations that can be assigned to the measured m/Z ratios. The atomic forces in these calculations are obtained from density functional theory using a gradient dependent functional.

1. Introduction

The damage to biologically relevant molecules due to high energy radiation is recognized to be the result of a complex process of sequential reactions. Though electro-magnetic radiation of sufficient energy can cause direct bond rupture the biological damage caused by high energy radiation traversing living organism is mainly caused by secondary species formed along the radiation track. These species may be ions, radicals or free electrons. It has long been accepted as a fact, that the radical species formed play the dominating role in initiating the chemistry that causes the actual damage. However, in recent years the attention has turned increasingly towards the role of low energy electrons in this process. These low energy electrons (<20 eV) are the most abundant species along the track [1]. Typically the yield is about 5×10^4 per MeV of the incident radiation [2].

Low energy electron attachment is a process that has been studied on a variety of molecular species throughout the last decades. The first step is the formation of a transient negative ion (TNI).



This TNI can then dissociate directly along the corresponding dissociative asymptote of the anion to form a negatively charged fragment and its neutral radical counterpart. The initial formation of the TNI may be described within the frame of the Born-Oppenheimer approximation as a vertical transition from the electronic ground state of the neutral molecule to the potential energy surface of the anion formed (Franck-Condon transition).

The dissociation of the anion formed can often be described within the framework of a diatomic dissociation. This is especially true when the molecule in question is fairly simple and the incident energy of the electron low; i.e.,



However, as the molecule becomes more complex more and more processes are observed that may only be described by considerable rearrange within the molecule.



Such pre-dissociation processes are commonly associated with an initial occupation of a π^* orbital as has for example been observed for the CN^{-} formation from the aliphatic amino acids at low electron energies [3-6]. This is a process where four bonds are broken and at least two new bonds must be formed to account for the energy needed.

Substantial work has been done on dissociative electron attachment to the individual building blocks of essential biological molecules such as the DNA, the RNA and proteins and peptides. This includes the nucleobases[7-12], ribose[13,14] and deoxyribose[15], phosphodiester[16] and a number of amino acids[3-6,17-19]. These experiments show clearly that low energy electrons can cause substantial damage to biological molecules. This has also been clearly demonstrated by low energy electron irradiation of plasmid DNA condensed on a surface. These experiments show that low energy electrons can cause both single- and double-strand breaks through a resonant attachment process[20].

Though it is clear in the meanwhile that low energy electrons can cause substantial damage to biologically relevant molecules it is less established how the damaging bond ruptures proceed. Even the assignment of m/Z ratios observed upon DEA to the smaller building blocks is often not unambiguous and high resolution mass spectrometry or isotope labeled molecules must be used to establish the actual reaction path[8,9,21-26]. Furthermore, it is often not trivial with the currently used experimental setups to distinguish between direct dissociation upon electron attachment and metastable decay processes, i.e., the further fragmentation of a negative ion fragment formed upon DEA. In the case of complex bio-molecules the large fragments initially formed through DEA can accommodate a substantial amount of internal energy in their vibrational degrees of freedom and further fragmentation of such pre-dissociated ions may take place a comparatively long time after the initial dissociation.

Here we present dynamic calculations on the further fragmentation of the dehydrogenated valine anion $[V-H]^{-}$ as formed by DEA. Dissociative electron attachment to L-valine was recently reported by Papp et al. showing that $[V-H]^{-}$ is the most abundant anionic fragment formed. This is also the case for the other aliphatic amino acids[3-6,19]. In addition to $[V-H]^{-}$ Papp et al. observed the formation of 6 other fragments which can partly not be unambiguously assigned to one molecular composition[6].

Dynamical simulations were performed on the $[V-H]^{-}$ system for three possible hydrogen abstraction sites; the hydroxyl group, the α -C and the amino group, here after denoted by subscript as $[V-H]_{O}^{-}$, $[V-H]_{\alpha C}^{-}$ and $[V-H]_{N}^{-}$, respectively. From our calculations we can see that the $[V-H]^{-}$ anionic fragments formed by DEA to the amino acid L-Valine at about 5 eV electron incident energy are metastable when the hydrogen extraction takes place from the amino group. In good agreement with previous experiments our calculations predict that the $[V-H]_{N}^{-}$ anion fragments further to form the anionic fragment $COOH^{-}$ and the neutral counterpart $(CH_3)_2CH-CH(NH_2)-$ if the hydrogen abstraction takes place from the amino group but is stable with respect to further decay if the hydrogen abstraction takes place from the hydroxyl group or the α -C. In addition to the fragmentation channel leading to the $COOH^{-}$ formation our dynamic calculations also reveal a stabilization mechanisms through hydrogen transfer from the hydroxyl to the amino group. The minimum energy reaction path for the hydrogen transfer is calculated using the elastic band method [27].

2. Methodology

The basic assumption in the calculations performed here is that the anion formed after electron attachment is long lived enough to reach the electronic ground state and that the excess energy brought in by the electron gets distributed evenly over the vibrational degrees of freedom of the anion. The calculations are used to explore what secondary fragmentation can occur if this state is reached.

We use density functional theory (DFT) with the generalized gradient approximation functional PW91 in combination with a plane wave basis set and ultrasoft pseudopotentials[28] for the calculations of the electronic degrees of freedom. The plane wave basis set includes kinetic energy up to 400 eV. The VASP code was used for these calculations [29].

The nuclear degrees of freedom were treated in two ways, first by carrying out classical dynamics, and then by finding minimum energy paths for transitions. In both cases the atomic forces were obtained from DFT calculations. The dynamical simulations were done in six steps:

- 1) The geometries of the charged fragments were optimized starting from an initial guess by minimizing the energy.
- 2) Internal energy of 1 eV was added, equally distributed over the vibrational degrees of freedom, to account for the temperature used in the experiments to sublime the valine powder (400 K).
- 3) The heated system is allowed to equilibrate in a canonical simulation for $T=400$ K over a period of 100 fs, giving it time for about 10 vibrations.
- 4) Additional 5 eV are added to the system to account for the kinetic energy of the attaching electron.
- 5) A constant energy, micro canonical ensemble is simulated over a time period of 500 fs.
- 6) At last, the charge density is analysed using Bader's method [30,31].

For each fragment 10 starting points were taken from step 3). By this procedure we have reached the state the anions are in some while after initial dissociation, already having distributed the internal energy it acquired and equilibrated itself via vibrations.

For the calculations of the minimum energy path of the hydrogen transfer we used the nudged elastic band method[27]. There, 19 images of the system were equally distributed between the minima on each side of the transfer, giving a discrete representation of the path. The initial state is $[V-H]_N^-$ and at the final state $[V-H]_O^-$ where the subscript indicates the site lacking the proton.

By using plane wave basis sets in the DFT calculations and only including valence electrons, the dynamics simulations and the minimum energy path calculations are quite straight forward and not so computationally demanding. They are feasible for even larger systems and increased fragmentation and fluctuations.

3. Results and discussion

The most significant m/Z ratio formed by direct dissociation of valine upon DEA is 116 amu, i.e., the $[V-H]^-$ anion formed by hydrogen loss from the initially formed transient negative ion. This ion is formed through two distinct resonances; one at 1.2 eV and another one at 5.3 eV[6]. The most probable sites for the hydrogen loss from the transient negative ion are the hydroxyl-group, the α -C and the amino-group. The theoretically estimated value at the B3LYP 6-311+G** level for the energy threshold for hydrogen abstraction from the hydroxyl group and the α -C is 1.04 eV and 2.20 eV, respectively[6]. At the same level of theory we calculate the threshold for the hydrogen abstraction from the amino group to be 2.85 eV. Hence, the O-H bond energy is significantly lower than the other two and at 1.2 eV electron energy this is the only hydrogen abstraction channel that is energetically available.

This is also reflected in recent experiments on metastable decay of deprotonated valine anions in MALDI as compared to the metastable decay of the dehydrogenated valine anion up on DEA and collision induced dissociation measurements. These experiments show that the two resonances are not the same anion. In these experiments, the low energy resonance at 1.2 eV does not show any

secondary fragmentation while the 5.3 eV resonance dissociates further by metastable decay to form the m/Z ratios 45 amu and 70 amu[26]. By considering the total energy, the lower resonance can only be due to loss of hydrogen from the hydroxyl group, but the second one is energetic enough for all of the hydrogen abstraction sites to be possible.

Here, we use DFT calculations to study from which site the hydrogen abstraction through the high energy resonance at 5.3 eV takes place. The dynamical simulations performed on the system were done on hydrogen loss from all three sites, i.e., starting with the $[V-H]_O^-$, $[V-H]_N^-$ and $[V-H]_{\alpha C}^-$ anions. Of the ten simulations done on each of those DEA fragments, none of the $[V-H]_O^-$ nor $[V-H]_{\alpha C}^-$ show any further fragmentation. $[V-H]_N^-$ on the other hand fragments into $COOH^-$ and $[V-COOH]$ in 7 cases out of ten and the other three show a hydrogen transfer from O to N.

Figure 1 shows four snapshots from the simulations on $[V-H]_N^-$ (a) where further fragmentation of $[V-H]_N^-$ leads to the formation of $COOH^-$ and the neutral counterpart $[V-COOH]$, and (b) where stabilization is achieved through hydrogen transfer from the hydroxyl group to the amino group to form the stable $[V-H]_O^-$ anion.

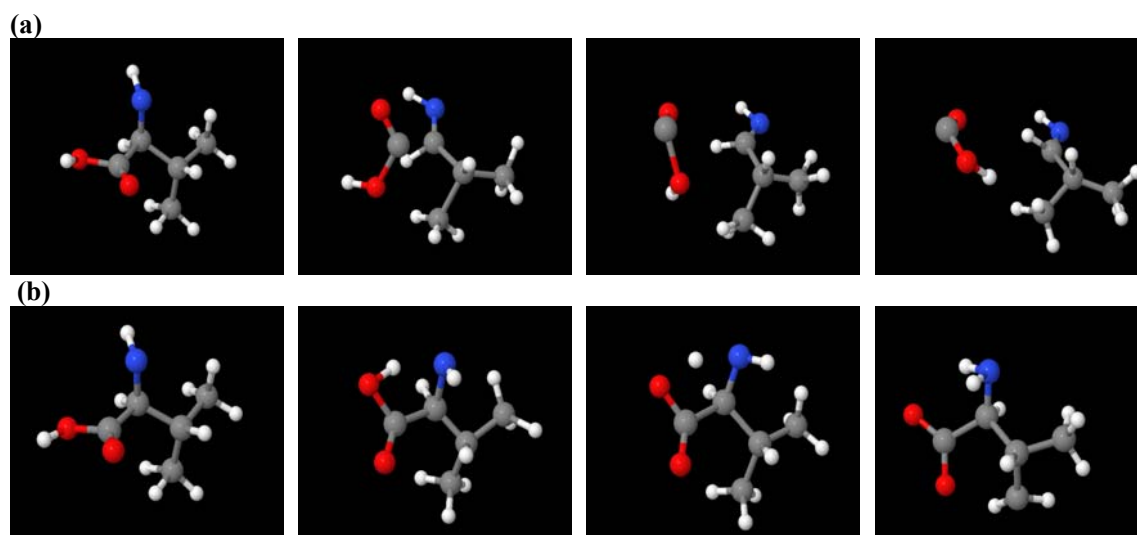


Figure 1. Simulated dynamics of $[V-H]_N^-$. (a) Formation of $COOH^-$ ($m/Z = 45$ amu) and the complementary neutral fragment $[V-COOH]$. (b) Proton transfer from the hydroxyl group to the amino group. The $[V-H]_O^-$ ion is stable and did not dissociate during the time interval simulated.

As is demonstrated in Fig. 1, our dynamic calculations are capable of reproducing the dissociation as observed from metastable decay of $[V-H]_N^-$ formed by hydrogen abstraction in DEA as well as through deprotonation in MALDI. In both cases the fragment $m/Z = 45$ amu is one of the two dominating fragments, which is in good agreement with our observation of the $COOH^-$ formation. The predicting power of the dynamic calculations is also supported by the absence of any further fragmentation of $[V-H]_O^-$ and $[V-H]_{\alpha C}^-$ as is to be expected from the experimental results.

Furthermore, our dynamic simulations also reveal channels that can not be experimentally observed, i.e., the hydrogen transfer from the hydroxyl group to the dehydrogenated amino-group. This quite interesting channel shows that the ion created is not necessarily the ion observed even though the m/Z ratio is unchanged. In good agreement with our observations for the precursor $[V-H]_O^-$ and $[V-H]_N^-$, this structural change makes the ion more stable and no further fragmentation is observed.

The rate of such a process is largely governed by the energy barriers along the minimum energy path. Assuming that the vibrations of the ion have thermally equilibrated and that the harmonic approximation is accurate enough, the activation energy (AE) is given by the highest energy along the

minimum energy path. Figure 2 shows the minimum energy reaction path for the hydrogen transfer calculated by using the nudged elastic band method [27].

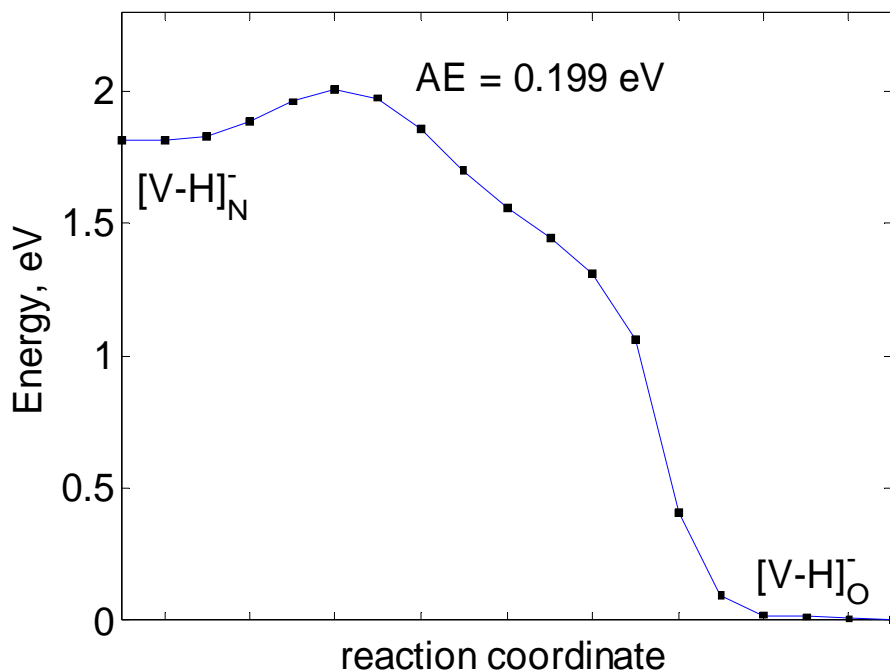


Figure 2. The energy along the minimum energy path for the hydrogen transfer. To the left is the $[V-H]_N^-$ (1.809 eV) and to the right is the $[V-H]_O^-$ (set as 0.000 eV). The data points show the energy of intermediate configurations used in the calculation. They are connected by a line for clarity.

The minimum energy path for the hydrogen atom transfer is calculated from the $[V-H]_N^-$ initial state to the $[V-H]_O^-$ final state, which is 1.809 eV lower in energy. The path (shown in Fig 2) was represented by 19 intermediate configurations and the results of the optimization are shown in Fig. 2. The maximum energy along the reaction coordinate was found to be at 2.008 eV compared to the energy of $[V-H]_O^-$. This results in an activation energy of 0.199 eV. Such a low energy barrier will be overcome quite readily given the large vibrational energy in the molecule. Figure 2 shows that the minimum energy path has a shoulder to the right of the maximum. This shoulder is because of a stabilization of the anion that occurs when the oxygen and the nitrogen share the hydrogen and a strained hydrogen bond is formed.

4. Conclusion

From these calculations we can see that some of the anionic fragments of the amino acid L-Valine are metastable, and have their specific and characteristic secondary fragmentation products. In good agreement with experiment we find that from the fragments $[V-H]_O^-$, $[V-H]_{\alpha C^-}$ and $[V-H]_N^-$ which all have the same m/Z ratio only $[V-H]_N^-$ fragments further. With our simulations we observe the characteristic decay of this ion, suggesting that our theoretical approach has the potential of becoming a valuable tool to aid the interpretation of DEA data by helping to identify the primary fragments through their characteristic secondary fragmentation. Furthermore, we also observe stabilization of the $[V-H]_N^-$ through hydrogen transfer from the hydroxyl group showing the potential this approach has as a tool to identify reaction channels that can not be observed with current experimental setups.

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