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To cite this article: E Alizadeh et al 2015 J. Phys.: Conf. Ser. 635 012001

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Low-energy electron-induced dissociation in condensed-phase L-cysteine I: Desorption of anions from chemisorbed films

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Abstract. Among amino acids, cysteine has been widely studied, becoming a standard for molecular self-assembly experiments, because its mercapto group (–SH) allows the formation of self-assembled monolayers (SAMs) on metal surfaces. Dissociative electron attachment (DEA) on L-cysteine SAMs is investigated utilizing a time-of-flight mass spectrometer coupled with a low-energy electron gun. The results show that electrons with kinetic energies of 3 to 15 eV attach to L-cysteine producing anionic fragments of different masses (e.g., H¯, O¯, OH¯, S¯, SH¯) via dissociation of intermediate transient anions. The anion yield functions exhibited purely resonant behaviour with electron energies below 15 eV, indicating that the formation of transient anions is the predominant mechanism of production of anionic fragments from L-cysteine dissociation.

1. Introduction

Amino acids are the monomeric building blocks of living systems. They play a central role both as subunits of proteins and as intermediates in metabolism. Since histones and other chromosomal proteins are in close contact with DNA in the nucleus of living cells, reactive species resulting from the interactions of low-energy electrons (LEEs; < 30 eV) with amino acids may interact with nearby DNA, causing indirect damage [1]. Thus, there is considerable interest in studying the fragmentation of chromosomal proteins induced by LEEs. Unfortunately, the complexity of protein structure does not presently allow a direct detailed analysis of the products that underlie the fragmentation processes. Research has therefore been focused on the investigation of the action of LEEs on protein sub-units, more particularly on amino acids and their analogs [2].

In the recent years, there have been several investigations performed by means of soft ionization techniques [3,4] in gas phase to study the ionization and fragmentation of different amino acids and small peptides. Gas-phase investigations of LEE-induced damage to protein subunits have been reported for amino acids [5-7] as well as some small peptides such as dialanine [8]. For all the molecules studied, the anion yield functions exhibited maxima localized in energy below 15 eV indicating the formation of transient negative ions (TNIs). TNIs are resonant states formed usually below 20 eV by the capture of an incident electron; when their states are dissociative and possess a lifetime of the order or larger than a vibrational period, they can decay into a negative fragment ion and one or more neutral radical counterpart(s).
Despite the pertinence of extensive gas-phase experiments to reveal the fundamental interactions of LEEs with amino acids, such experiments do not necessarily correspond to those that would be observed in condensed phase, since the presence of neighboring molecules can change the electronic structure of amino acids, and thus can modify the energy, magnitude, lifetime and decay channels of the TNIs. For instance, in the gas phase, amino acids adopt a neutral conformation [9], which is a stable non-charge-separated structure, i.e., HS–CH₂–CH(NH₂)–(CO)OH in the case of cysteine; while in the solid phase or aqueous solution, they rather take a zwitterionic arrangement [10]: HS–CH₂–CH(NH₃)⁺–(CO)O⁻ configuration (in the case of cysteine). Therefore, molecular orbitals (MOs) differ between the neutral molecule and its zwitterionic form [11].

In the condensed phase, the electron stimulated desorption (ESD) of anions from multilayer films of molecular solids has attracted attention as a way to understand the LEE-induced dissociation of condensed molecules. In the case of physisorbed amino acids [2,12], the ESD of anions from thin films results in yielding H⁻ as the principal desorption fragment with lower signals of other fragments such as CH₃⁻, O⁻ and OH⁻. Similar results were obtained from self-assembled monolayers (SAMs) of Lys amides [13]. Among the dissociative processes involving LEEs, two are well-known that produce anions: dissociative electron attachment (DEA) and dipolar dissociation (DD). DEA involves temporary electron attachment to one of the valence orbitals of the molecule to form a TNI which leads to the rupture of some specific bonds. This process dominates for electron impact energies below ~15 eV. When the potential energy curve of the TNI state is repulsive in the Franck-Condon (FC) region and the lifetime of the TNI is sufficiently long to permit dissociation (> 10⁻¹⁴ s), DEA is then possible and results in the molecular dissociation into neutral and anionic fragments. DD dominates anion production with electron energies above a threshold of ~15 eV, and occurs when the incident electron creates an electronically excited state of the neutral molecule, which then dissociates into positive and negative charged fragments.

A useful method to study the behavior of interfaces between organic molecules and metal surfaces in the condensed phase is the self-assembly technique, by which a well-ordered organic structured surface can be chemically bound to a substrate. SAM technique plays an important role in biological and chemical sensing, investigation of electron transfer in DNA and molecular electronics [14-16], and is of particular interest to anchor proteins via the thiol-containing amino acid cysteine to gold nanoparticles, thus imparting such particles with essential biochemical functions [17,18]. To further investigate biometallic interface functions, we characterized specifically the chemisorbed L-cysteine/Au(111) films (SAMs) by various spectroscopic techniques. In this present work, we investigate electron attachment to L-cysteine SAMs and the subsequent dissociation of the TNIs by measuring the anion yields desorbed from L-cysteine SAMs bombarded with LEEs of energies below 20 eV.

2. Experimental method

2.1. Preparation of L-cysteine/Au(111) SAMs

The gold substrates were prepared by vacuum evaporation of gold (99.99% purity) onto freshly cleaved mica sheets in an all-metal turbo molecular-pumped evaporation system, based on the protocol of DeRose et al. [19]. The condensed Au films (~200 nm) have predominantly a Au(111) structure owing to heating to 300°C before, during, and after evaporation of the metal. The L-cysteine (HS–CH₂–CH(NH₂)–COOH) was purchased from Sigma-Aldrich with a stated purity of 97% and used without further purification. The surface of Au films was cleaned with sulfochromic acid (chromium trioxide in concentrated H₂SO₄) to eliminate organic contaminants, then rinsed with copious amounts of ultrapure water (Milli-Q) and methanol, and then dried under a gentle flow of N₂. Cleared Au(111)/mica substrates were immediately immersed into 1 mM L-cysteine aqueous solution in methanol overnight at room temperature. The samples were rinsed afterwards with methanol and dried under a N₂ flow before analysis.
2.2. Electron Stimulated Desorption (ESD)
All the experimental data were obtained utilizing a high sensitivity time-of-flight (TOF) mass spectrometer (Kore-5000 Reflectron), which is housed in a UHV system reaching a base pressure of $5 \times 10^{-10}$ Torr. The SAM samples were mounted onto copper carrier plates and transferred into the load-lock coupled to the UHV chamber. The samples were degassed within the load-lock for at least 12 hours prior to their transfer into the main chamber, where they were bombarded with a pulsed electron beam from a Kimball Physics ELG-2 gun. The current of $\sim 2.6$ nA consisted of 800 ns pulses with a repetition of 5 kHz incident at an angle of 45° with respect to the sample normal. The electron beam spot size is 3 mm$^2$ and the electron energy can be varied from 0.1 to 20 eV. A negative potential pulse (-2.4 kV, pulse width of 2 ms) was applied to the substrate immediately after the end of an electron pulse to desorb the produced anions from the sample region into the entrance optics of the TOF mass analyzer, positioned along the surface normal, at 10 mm from the sample. By recording mass spectra at different incident electron energies, it is possible to investigate the variation of all anion signals with incident electron energy so as to obtain a yield function for each ion fragment. The yield functions were measured from 0 to 18 eV (nominal values prior to energy calibration) with electron energy increments of 0.5 eV and 50 000 electron pulses per point.

3. Results and discussion
Fig. 1 shows the energy-integrated mass spectra of negative ions desorbed from bare Au(111) and SAM of L-cysteine recorded with the TOF-MS at incident electron energies from 0 to 18 eV. As the yield functions of anions vary significantly in this incident electron energy range, all the mass spectra taken from 0 to 18 eV have been added to produce Fig. 1(a) and (b) to have an overview of the observable fragments during ESD without considering the energy dependence. As the experimental conditions were mainly the same for all measurements, the relative abundance of the emitted fragments are directly comparable. From Fig. 1 one can observe that anions are emitted from the bare Au substrate: H$^-$ (1 amu), O$^-$ (16 amu), OH$^-$ (17 amu), F$^-$ (19 amu), Cl$^-$ (35 and 37 amu), COOH$^-$ (45 amu), HCOOH$^-$ (46 amu), S$_2^-$/SO$_2^-$ (64 amu), SO$_3^-$ (80 amu), and SO$_4^-$ (96 amu). These masses can originate from atmospheric contamination before inserting the samples into the load-lock, adsorption of the residual gases in the UHV system, or contamination from the acid cleaning of the gold (especially for the case of anions at 64, 80 and 96 amu). When L-cysteine is grafted onto the Au substrate, some masses increase or even appear in the mass spectra. The most striking features are: an increase of the O$^-$, OH$^-$ and HCOOH$^-$ signals, and the detection of new masses at 32 (S$^-$) and 33 amu (SH$^-$). H$^-$ is the most abundant anion emitted from LEE-irradiated L-cysteine SAM, that is similar to previous condensed-phase study (physisorbed cysteine on a Pt substrate) and in contrast with other amino acids like glycine and alanine [12]. While in the case of gaseous amino acids, the most intense product was the dehydrogenated parent anion and H$^+$ was not detected [7].

Fig. 2 shows the yield functions of the most intense anion signals produced from L-cysteine SAMs irradiated by 0-18 eV electrons. Each point corresponds to the mean value of the yields from four SAMs prepared under identical conditions; the error bars denote the standard deviation of the experiments. Peaks or maxima are clearly visible in these curves, at incident electron energies above $\sim 4$ eV. Each peak indicates the formation of a TNI prior to fragmentation. According to the interpretation of Abdoul-Carime and Sanche on the LEE-induced degradation of protein constituents [12], these TNIs are core-excited resonances consisting of two excited Rydberg electrons bound to a positive ion core, that is, a one-hole two-electron state. Anion emission occurs via crossing of the potential energy curves of these Rydberg transient anions with those of valence transient anions of the molecule. The broad width of the structure can be interpreted as due to the superposition of several overlapping resonances with maxima between 6 and 12 eV. Above $\sim 13$ eV, in most of the yield functions, a continuously rising signal attributed to DD is recorded on which resonances are superimposed.

The H$^-$ yield function exhibits a resonance at $\sim 9.0$ eV for both Au and cysteine SAM samples. In the case of cysteine, this signal can only originate from the carboxyl, amino or hydrocarbon sites via a
simple bond cleavage reaction involving the formation of a dissociative TNI. ESD studies from organic and biological molecules [13,20-22] have shown H⁺ emission from carboxyl, amino and hydrocarbon sites have resonance energies in the ranges 6.8-7.7 eV, 5-8 eV, and 8.5-10.5 eV, respectively. These suggest that H⁺ in Fig. 2 desorbs principally from the hydrocarbon sites in cysteine, but not exclusively, as Bertin et al. observed a resonance at 9 eV in H⁺ yield functions from condensed deuterated CD₃OOH [23]. The similar resonance energy of H⁺ for L-cysteine SAM and Au substrate is caused by the chemical nature of the molecules from which H⁺ desorbs. It has been demonstrated [24] that organic contamination still remains on the Au substrate after acid cleaning. This contamination are principally hydrocarbon molecules that exhibit a resonance for H⁺ at around 9 eV, which is also the resonance energy of H⁺ in the case of cysteine.

**Figure 1.** Energy-integrated mass spectra of (a) Au deposited on mica and (b) L-cysteine SAM.

Similar yield functions are observed for 16 amu anion for both Au and SAM (Fig. 2). As the majority of the detected anions during low-energy ESD are produced near of the vacuum-film interface [25], the chemical origin of this anion is similar for both samples and, in the case of the Au substrate, it originates from organic contamination that still remains on the surface after cleaning [24]. The 16 amu negative fragment can be attributed, a priori, to O⁻ or NH₂⁻. The higher positive electron affinity of O (1.46 eV) compared to that of NH₂ (0.78 eV) [26] may favor the formation of O⁻ rather than NH₂⁻. Moreover, in previous experiments on electron impact on molecules containing amino groups, no C–N bond rupture to produce NH₂⁻ was reported [27,28], which strongly suggests that the 16 amu fragment can be mainly attributed to O⁻. The peak located at 8.6 eV in O⁻ yield function is indicative of the formation of a core-excited resonance, prior to dissociation of an electronically excited state of cysteine (π* and/or σ*). Indeed, the O⁻ anion could result from the cleavage of the strong C=O or C-O⁻ bonds (which can be produced after deprotonation of the COOH group by some LEE-induced reaction) [7]. The emission of O⁻ could also originate from the zwitterionic form of cysteine in the SAM film, thus permitting the emission of O⁻. Dodero et al. [29] have found by XPS that L-cysteine is mainly present in the zwitterionic state while chemisorbed on gold; the transfer of the proton form the carboxyl group to the amine could permit the emission of O⁻ without LEE-induced loss of H. Abdoul-Carime et al. [7] suggested that O⁻ could also be produced by the transformation of the pristine carboxylic group of L-cysteine to aldehyde via DEA (at around 6 eV):
\[
e^- + R-C(O)OH \rightarrow (R-C(O)OH)^- \rightarrow R-C(O) + H + O^-
\]  \hspace{1cm} (1)

where R represents H(NH₂)CH₂SH. From a thermodynamic point of view, molecular rearrangement is possible, as the transformation of the COOH group to aldehyde with the emission of O⁻ has an estimated energy threshold of 2.5 eV [7], and the threshold of emission of O⁻ in Fig. 2 is at ~4 eV. Assuming the necessary kinetic energy of ~1 eV to overcome the induced polarization potential produced by the presence of the TNI [30], reaction (1) could be considered, but strong resonance located at ~8.6 eV in the O⁻ yield function suggests that other reaction processes are involved in the production of this anion, as reported in gas-phase studies on L-cysteine [7] and methanol [31], unless that the zwitterionic state is involved in such a way that this could modify the energies of dissociative states of L-cysteine. Another possible process for formation of O⁻ via a cascade process, i.e., by hydrogen loss from OH⁻, has been suggested by Wang et al. using theoretical calculations of resonant electron attachments to cysteine [32]. In this process, the authors hypothesize the production of OH⁻ in a dissociative state after the DEA process that could dissociate into O⁻ and H in vacuum, which it seems improbable; for this reason, the cascade process is not proposed as a pathway for the production of O⁻. Production of several radical fragments during DEA allows suggesting multiple fragmentation reactions for the production of O⁻. Indeed, another way for O⁻ desorption is the complete dissociation of the alcohol part in the carboxylic group, which has been reported for gas-phase methanol [31]:

\[
e^- + R-C(O)OH \rightarrow (R-C(O)OH)^- \rightarrow R-C(O) + H + O^-
\]  \hspace{1cm} (2)

Figure 2. ESD yield functions of the most intense anions desorbed from L-cysteine SAMs (blue ▼) and acid-washed Au substrate (yellow ■). Each point corresponds to the mean value of the yields from four SAMs and two Au samples, prepared under identical conditions; the error bars denote the standard deviation of the experiments.
The OH\(^{-}\) fragment could come from two distinctive processes: by the direct dissociation of the carboxyl group (pathway D in Fig. 3), or by reactive scattering during emission of O\(^{-}\) (i.e., O\(^{-}\) + H \rightarrow OH\(^{-}\)). As the threshold energy of OH\(^{-}\) desorption is about 2 eV lower than that of O\(^{-}\), it is unlikely that OH\(^{-}\) desorption derives from reactive scattering of O\(^{-}\), and this second process is not further considered as a significant process of emission of OH\(^{-}\). Possible dissociation pathways for formation of H\(^{-}\) (via A), O\(^{-}\) (via B and C) and OH\(^{-}\) (via D) after LEE attachment to L-cysteine are represented in Fig. 3.

In contrast to 16 amu anion, which has been detected in studied amino acids in gas phase [5,6,8] and physisorbed cysteine [12], no 15 amu fragment attributed to CH\(_3\)\(^{-}\) or NH\(_2\)\(^{-}\) has been observed. Similarly, in this present work, 15 amu anion is desorbed from both clean Au and L-cysteine SAM at very low intensities (Fig. 1), which means that no significant CH\(_3\)\(^{-}\)/NH\(_2\)\(^{-}\) signal comes from L-cysteine. It should be noted that production of NH\(^{-}\) anion from L-cysteine requires the scission of C–N and N–H bonds, which is much less probable than production of some other fragments such as NH\(_2\)\(^{-}\), produced by the breaking of only C–N bonds. As previously explained in the case of NH\(_2\)\(^{-}\), no C–N bond rupture was already reported [27,28]. Thus, detection of no significant NH\(^{-}\) signal is an evidence that the emission of 16 amu anion can be attributed to O\(^{-}\) instead of NH\(_2\)\(^{-}\).

Fig 2 also exhibits the yield functions of anions recorded at 31-34, 46 and 64 amu from SAM films of L-cysteine and acid-washed bare Au. According to the natural abundance of the isotopes of sulfur, i.e. \(^{32}\)S (94.9 %), \(^{33}\)S (0.8 %) and \(^{34}\)S (4.3 %), the two peaks appearing on 32 and 34 amu in Fig. 1(b) are due to desorption of \(^{32}\)S\(^{-}\) and \(^{34}\)S\(^{-}\), respectively. From further consideration of the isotopic ratios, \(^{33}\)S\(^{-}\) is expected to make only a small contribution to the 33 amu signal. Thus, the peak located at 6 eV in 33 amu ion yield seems to be attributed to SH\(^{-}\).

\(^{32}\)S\(^{-}\) and \(^{32}\)SH\(^{-}\) have been previously observed in ESD experiments on gaseous cysteine. However, this has been restricted to resonances below 4 eV and lower intensity resonance at 6 eV [7]. In fact, the fragments detected at 32 amu and 33 amu can in principle also be ascribed to O\(_2\)\(^{-}\) and HO\(_2\)\(^{-}\) anions, respectively, arising from the dissociation of the carboxyl group, but these species have not been detected in previous electron impact studies on various amino acids [2,5]. Therefore, O\(_2\)\(^{-}\) and HO\(_2\)\(^{-}\) fragments are not likely to be produced here. The possible dissociation pathways for production of S\(^{-}\) and SH\(^{-}\) are represented in Fig. 3 via reactions E and F, respectively. In contrast to gas-phase conditions [7], the yield functions of S\(^{-}\) and SH\(^{-}\) exhibits two structures in the energy range of 6-11 eV, which differ to observed resonances for physisorbed films [12], as dissociative core-excited resonances are usually involved at these energies. The production of these fragments could come from the dissociation of the Au-thiolate and/or C–S bonds, as observed with alkane SAMs irradiated by 0-50 eV electrons [33,34], producing S\(^{-}\) fragments which some of them can make reactive scattering to form SH\(^{-}\). Another possible source for emission of these products can come from the realistic structure of the SAM film. Indeed, Cavalleri et al. [35] have studied L-cysteine adsorbed on Au(111) from solution at pH 5.7 by XPS and concluded that the film is organized as a double layer which consists to a first layer chemisorbed on gold via the thiol group and a second layer physisorbed by intermolecular hydrogen bonding between zwitterionic functional groups. The presence of such second layer could explain the detection of sulfur fragments during ESD, as considering a partial overlayer during the fabrication of the cysteine SAM.

Production of COOH\(^{-}\) (45 amu) have been reported from LEEs impact with gaseous amino acids [5,6,8]. In this work, no significant signal from this mass was detected. However, two resonances (8.5 and 13 eV) can be identified in anion yield of 46 amu anion (Fig. 2) which may be assigned to HCOOH\(^{-}\). Thus, we suppose that the carboxylic anion can react with a hydrogen atom before desorption to form HCOOH\(^{-}\) anion, as the form of the yield function of mass 46 is similar to those observed in LEE experiments with gas-phase amino acids, that is a multi-resonance yield function with a peak separation near to one observed in Fig. 2 (4.5 eV vs \(~3\) eV in gas phase). Reactive scattering has been observed in studies involving LEE impact [36-38]; for this reason, one may expect that HCOOH\(^{-}\) could come from reactive scattering (via G in Fig. 3).
Some anions observed in Fig. 1 arise from impurities or from contaminants in the SAM sample and Au substrate: mass 19, assigned to F\(^-\), and two peaks at 35 and 37 amu with a ratio of 3:1 in intensity, which are associated with \(^{35}\text{Cl}\) (75.78\%) and \(^{37}\text{Cl}\) (24.22\%). Signals at 64, 80 and 96 amu, which have almost the same intensities and exactly the same resonances in both SAM and bare Au, may be attributed to \(\text{S}_2\^-\), \(\text{SO}_3\^-\) and \(\text{SO}_4\^-\) products, respectively, that could arise from acid solution used in cleaning process of gold substrate.

\[ \text{Figure 3.} \] Possible dissociation pathways for formation of \(\text{H}^-\) (A), \(\text{O}^-\) (B and C), \(\text{OH}^-\) (D), \(\text{S}^-\) (E), \(\text{SH}^-\) (F) and \(\text{COOH}^-/\text{HCOOH}^-\) (G) after LEE attachment to L-cysteine and producing a TNI.

4. Summary
Anion efficiency curves were reported for more pronounced produced fragments from L-cysteine by measuring the anion yields as a function of the incident electron energy from 0 to 18 eV. ESD measurement on L-cysteine SAMs have shown that LEEs (< 20 eV) are able to efficiently decompose this amino acid via DEA and DD when the molecule is chemisorbed via the SH group to a Au surface. The results presented here are similar to those observed from physisorbed films, as light anion species (with mass lower than 35 amu) have been detected. In addition, we also measured heavier fragments desorbed from the SAM.

Due to the strong influence of the electronic states on the function of biometallic systems, we need to study the interface behavior, and how they may be modified and controlled. Therefore, the present study should be extended to those occupied electronic states below the Fermi level and unoccupied orbitals lying below the vacuum level of the L-cysteine/Au(111) interface, which are feasible by employing other complementary spectroscopic techniques, i.e., ultraviolet photoelectron, metastable impact electron, and two-photon photoemission spectroscopies.

5. Acknowledgments
The work was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Institutes of Health Research (CIHR Grant No. MOP 81356). The authors would like to thank K. Koczur for help with the XPS measurements. We are also indebted to A. D. Bass for his useful comments and P. Cloutier for technical support.
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