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Free radicals in L-arginine · HCl · H_2O single crystals X-irradiated at 298 K-EPR, ENDOR and DFT studies

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HIGHLIGHTS

- Three distinct free radicals formed in X-ray irradiated L-arginine · HCl · H_2O single crystals at 298 K were identified.
- DFT modeling computations indicated the main-chain deamination radical has protonated carboxyl group.
- Two conformations of the radical dehydrogenated at C5 were detected. The conformational differences were analyzed with experimental and computational methods.
- The annealing experiments indicated these three radicals are stable radicals.

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ABSTRACT

Electron Paramagnetic Resonance (EPR), Electron-Nuclear DOuble Resonance (ENDOR) and ENDOR Induced EPR (EIE) results indicated at least three radicals produced in L-arginine · HCl · H_2O single crystals irradiated at 298 K. Radical RI dominated the central portion of the EPR spectra and was identified as the main-chain deamination radical, and Density Function Theory (DFT) calculations indicated that RI has protonated carboxyl group, $(H_2...OOC)\dot{C}H(CH_2)_3\ NHC(NH_2)_2^+$, and the COOH protons are transferred from the hydrogen bonded amino group and guanidyl group in two different neighboring molecules. Radicals RII and RIII were identified respectively as the radicals dehydrogenated at C5, $^-(OOC)CH(NH_3)^+(CH_2)_2\dot{C}HNHC(NH_2)_2^+$, and at C2, $^-(OOC)\dot{C}(NH_3)^+(CH_2)_3NHC(NH_2)_2^+$. Two conformations of RII were detected, denoted as RIIa and RIIb, and the conformational differences are mainly due to the different dihedral angles of the two β-protons bonded to C4, which were supported by the modeling calculations for RIIa and RIIb.

1. Introduction

When proteins and DNA interact, arginine (Chart 1) and lysine are the two amino acids most often in close contact with the DNA. Crystallographic study of nucleosome core found that direct interactions (not water-mediated) between histone proteins and DNA predominantly involve arginine and lysine (Davey et al., 2002). Also, arginine and lysine are two amino acids present in relatively large amounts (more than 20% of the amino acids) in the nuclear histone (Wolffe, 1992). In the more general context, as showed in a review of 129 crystallized DNA–protein complexes, arginine residues make the largest number of hydrogen-bond interactions with DNA, and especially, strongly favored in hydrogen bonding to guanine, which appeared to provide one of the specificities in the DNA base recognition (Luscombe et al., 2001).

The proteins in close proximity of DNA have an important influence on the processes leading to radiation damage of DNA in vivo. Many studies have focused on the radiation effects of the DNA–histone complexes as well as those of isolated purified DNA. Results indicate that histone proteins are radioprotective against hydroxyl radical attack on DNA (Lloyd and Peacocke, 1966; Lückle-Huhle et al., 1970; Mee and Adelstein, 1987), while EPR studies of irradiated chromatin found an increase yield of DNA-centered radicals from one-electron reduction in comparison to DNA alone, an indication that ionization-produced electrons transferred from histones to DNA and the oxidation centers (or holes) remained within the histones (Cullis et al., 1987; Faucitano et al., 1992; Weiland and Hütermann, 2000). In addition, the histones have been identified as the proteins predominantly involved in DNA–protein cross-links (Johansen et al., 2005; Morin and Cadet, 1995; Perrier et al., 2006; Xu et al., 2007).

The abundance of arginine residues in histone proteins as well as its frequently interacting with DNA makes arginine one of the key amino acids in influencing ionized-initiated DNA and the

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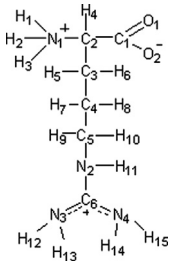

Chart 1. Zwitterionic and protonated arginine. As present in L-arginine · HCl · H_2O.

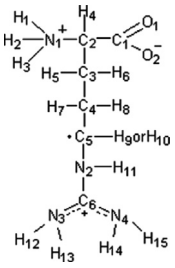

Chart 2. Radical from dehydrogenation at C5.

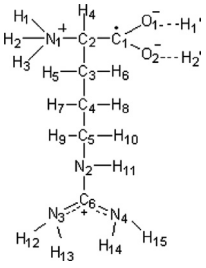

Chart 3. Carboxyl-centered radical from one-electron reduction.

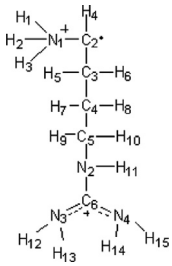

Chart 4. Decarboxylation radical.

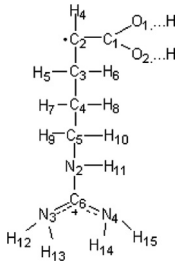

Chart 5. Main-chain deamination radical with two O–H dipolar protons.

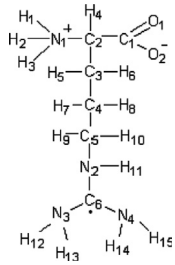

Chart 6. Guanidyl-centered radical from one-electron reduction.

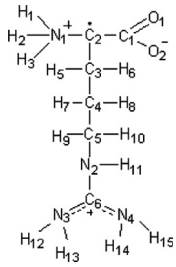

Chart 7. Radical from dehydrogenation at C2.

formation of DNA–protein cross-links. In order to figure out the role arginine might play in the proteins on these processes, we choose to investigate radical formation in X-irradiated L-arginine · HCl · H_2O (L-arg · HCl · H_2O) single crystals. There are very few previous studies on irradiated arginine in condensed media. Joshi and Johnsen (1976) examined the radical decay kinetics in crystalline amino acids under controlled warming from room to higher temperatures and proposed that the radical dehydrogenated at C5 (Chart 2) was the stable radical in L-arg · HCl · H_2O single crystal, but they did not definitely characterized it. Using EPR techniques, Aydin et al. (2008) also detected the radical dehydrogenated at C5 in γ-irradiated L-arginine powder at room temperature. More recently, Olsen (2008) studied L-arginine phosphate (LAP) single crystals using EPR/ENDOR techniques and DFT calculations. In his work, the radicals from electron trapping at the carboxyl (Chart 3), from decarboxylation (Chart 4) and from dehydrogenation at C5 were identified in LAP X-irradiated at 77 K, and the radicals from main-chain deamination (Chart 5) and from dehydrogenation at C5 were identified as the stable radicals in LAP irradiated at 295 K.

We previously reported the free radicals formed in L-arg · HCl · H_2O single crystals immediately after X-irradiation at 66 K (Zhou and Nelson, 2010b): the radicals from electron trapping at the carboxyl, from decarboxylation, from dehydrogenation at C5 and from electron captured by guanidyl group on the side chain (Chart 6). This work we continue to study the stable radicals formed from irradiation at 298 K using EPR/ENDOR spectroscopy supplemented with DFT-based calculations. In addition to main-chain deamination radical and the radical of dehydrogenation at C5, we identified the radical of dehydrogenation at C2 (Chart 7). The EPR taken from the crystal irradiated at 66 K and warmed to room temperature is same as that from the crystal irradiated at 298 K (Fig. S1 of the Support material). This result indicated that the stable radicals formed from irradiation at 66 K are same as those in the crystal irradiated at 298 K.

2. Experimental and computational methods

The normal and the partially deuterated single crystals of L-arg · HCl · H_2O were X-ray irradiated at 298 K and studied with K-band of EPR and ENDOR techniques at 298 K and at 66 K (by pumping the liquid nitrogen). The experimental results obtained at