International Journal of Mass Spectrometry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

H-atom loss and migration in hydrogen-rich peptide cation radicals: The role of chemical environment

Vanessa Riffet[a,1], Denis Jacquemin[b,c], Gilles Frison[a,*]

[a] Laboratoire de Chimie Moléculaire, Ecole polytechnique and CNRS, 91128 Palaiseau cedex, France
[b] Laboratoire CEISAM, UMR CNRS 6230, Université de Nantes, 2 rue de la Houssinière – BP 92208, 44322 Nantes cedex 3, France
[c] Institut Universitaire de France, 103 bd Saint-Michel, F-75005 Paris cedex 05, France

ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form 1 August 2015
Accepted 4 August 2015
Available online xxx

Keywords:
Electron capture dissociation
Electron transfer dissociation
Hydrogen bond
Density function theory calculations
Proton coupled electron transfer
DFT benchmarks

ABSTRACT

Hydrogen-atom loss and intramolecular hydrogen-atom migration in peptide radical and cation radical were investigated by quantum chemical calculations for models that include zero to three hydrogen bonds with the ammonium groups. Five different DFT functionals have been checked to study the geometry relaxation and fragmentation processes after electron attachment to peptide cations. The structural changes associated with the geometry relaxation are shown to be dependent on the level of calculation. Furthermore, comparison with CCSD(T) calculations indicates that an adequate description of the electronic state of the reduced protonated peptide is not a sufficient prerequisite to provide accurate energy barrier. Calculating fragmentation reactions of hydrogen-rich peptide cation radicals is pointed out to be a challenging task as none of the DFT functionals are fully effective to describe the whole process. M06-2X is an adequate choice if it provides the correct ground state, otherwise LC-BLYP should be chosen.

The electron attachment site and the electron affinity value are highly sensitive to the number of hydrogen bonds with the ammonium group. An ammonium cation with zero, one, and, to a smaller extent, two hydrogen bonds easily captures an incoming electron and subsequently undergoes an H-atom loss process rather than a proton coupled electron transfer. This suggests an explanation for the low amount of *c* and *z* fragments observed upon ECD for small protonated peptides, where the ammonium groups cannot be highly intramolecularly "solvated".

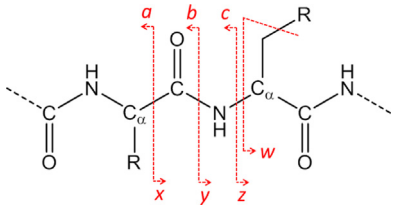
1. Introduction

Non-covalent interactions, and in particular hydrogen bonding interactions [1–3], are well known to play a key role in the secondary and tertiary structures of proteins [4–6]. Indeed, intramolecular and intermolecular hydrogen bonds, occurring between the amino acids residues and the surrounding water molecules, are the primary stabilizing forces in static structures in proteins [7]. They also have a prominent impact in their solvation dynamics [7,8] and folding [9], as well as in their biological reactions [10]. For instance, among several thousands of examples, one can illustrate this statement with the reaction mechanisms of the water oxidation by photosystem II [11] and of the hydration of carbon dioxide by carbonic anhydrase [12] which both implies a proton transfer step requiring a hydrogen-bond network around the active site. Similar implications of hydrogen bonds in the structure of gas phase ions [13–15] and in the related fragmentation mechanism [16,17] have been reported in the literature.

Electron capture dissociation (ECD) and electron transfer dissociation (ETD) are mass spectrometry fragmentation techniques that have shown an important potential for the analysis of peptides and proteins [18–21]. Their key characteristics include preferential formation of *c* and *z* type fragments, which result from cleavage along the backbone N—C_α bond (Scheme 1). ECD and ETD thus provide complementary structural information in comparison with more conventional ion dissociation methods, such as collision-induced dissociation (CID), which typically yields *b* and *y* type fragments due to amide-backbone N—CO bond cleavage. The precise mechanism used in these techniques is still a matter of debate but implies that multiply charged cations are partially reduced by receiving one electron, and are consequently converted from even-electron compounds to intermediate cation radicals which undergo further fragmentation [22,23]. It has been suggested that the conformation of the multiply charged peptides,

* Corresponding author. Tel.: +33 01 69 33 48 34; fax: +33 01 69 33 48 03.
E-mail address: gilles.frison@polytechnique.edu (G. Frison).
[1] Present address: Laboratoire de Chimie Théorique, Université Pierre et Marie Curie and CNRS, F-75005 Paris, France.

http://dx.doi.org/10.1016/j.ijms.2015.08.001
1387-3806/



Scheme 1. Peptide fragment ion nomenclature.

and therefore their intramolecular hydrogen-bond networks, influence the nature and the relative abundance of the fragment ions [24–26]. Hydrogen bonds also allow intramolecular hydrogen or proton transfer together with the backbone cleavage [27,28], or intermolecular hydrogen or proton transfer in an intermediate ion-molecule complex [29], enabling fragment isomerization [30].

Numerous studies using quantum chemical calculation techniques, among other methods, have been performed to gain insight into the ECD/ETD mechanism [31–73]. The capture of one electron by diprotonated peptides is associated with a molecular geometry relaxation leading to a local minimum of the peptide radical cation. It has been shown that this reorganization is either small, i.e. without conformational change or bond breakage or formation, as for the doubly protonated AlaAlaHisAlaLys pentapeptide [49], or large, i.e. related to a barrierless intramolecular migration of one ammonium proton, possibly coupled with electron transfer, to a neighboring site of the peptide [47,57]. This initial reorganization step induces all the following structural rearrangements determining the overall fragmentation process. To reach a better understanding of the reaction mechanism of this process, it is of fundamental importance to obtain a clearer view of the factors influencing these initial transfers. Nevertheless, up till now, no full rationalization has been proposed. In particular, the effects of the amino acid sequence and of the conformational structure of multiprotonated peptides on these initial transfers are still not understood.

We have recently shown that the electronic structure of hydrogen-rich peptide radicals is strongly related to the number of hydrogen bonds on their charged ammonium groups [74]. This confirms that the nature of the hydrogen-bond network around the ammonium groups, and consequently the conformation of the multiprotonated peptide, is of importance to determine the electron attachment site, the initial transfer step and the ion fragments. In this work, we explore this hypothesis based on DFT calculations performed for models that include zero to three hydrogen bonds with the ammonium groups. Minima and transition states for both H-atom loss and migration processes have been computed. Furthermore, we study here the impact of the selected computational level on the results. Indeed, it has been shown previously [74,75] that the description of the ground state electronic structure of hydrogen-rich peptide radicals is often inaccurate with B3LYP, a method that was extensively applied to tackle this problem in the past [31–56]. Indeed, B3LYP, and other "conventional" exchange-correlation functionals tend to yield an excessively delocalized spin density, and it has been demonstrated that the more severe the self-interaction error, leading to incorrect long-range behavior, the less reliable the results [75]. Reciprocally, the relative performances of range-separated hybrid (RSH) functionals, which provide more accurate electronic structure, for yielding accurate optimized geometries, relative energies and activation energies have not been assessed in the present context. It is therefore of primary importance to check that the conclusions obtained from an in silico mechanistic study are not influenced by the selected level of calculation.

2. Methods

All DFT and post-HF calculations have been performed with the Gaussian 09 program [76]. All structures were fully optimized without any constraints at the DFT levels and, for selected cases, at the CCSD level. The 6-311++G(2d,p) atomic basis set was applied for all atoms throughout the study. For each stationary point obtained at the DFT level, we carried out a vibrational frequency calculation at the same level of theory to characterize its nature as true minimum or transition state as well as to obtain Gibbs free energies at 298 K. To obtain accurate geometries and energies, the SCF convergence criterion was systematically tightened to 10^{-8} au, and the force minimizations were carried out until the rms force became smaller that (at least) 1×10^{-5} au ("tight" optimization in Gaussian 09). For selected cases, final single-point energies have been obtained at both the MP2 or CCSD(T) levels with the 6-311++G(2d,p) basis set using the DFT-optimized geometries.

Five different exchange-correlation functionals, which cover different categories from "conventional" to range-separated hybrid (RSH) functionals, have been applied for these calculations: (i) the hybrid GGA functional B3LYP [77,78], which includes 20% of Hartree-Fock exchange (X^{HF}); (ii) the hybrid meta-GGA functional M06-2X [79], which includes 54% of X^{HF}; (iii) the RSHs ωB97X-D [80], ωB97 [81], and LC-BLYP [82], which present a growing fraction of X^{HF} with increasing interelectronic separation in the 22.2–100, 0–100 and 0–100 range, respectively. The speed of the transition from Kohn-Sham exchange to X^{HF} is governed by the attenuation parameter ω whose value is 0.20, 0.40 and 0.47 au for these three functionals, respectively. Previous studies using peptide models and highly correlated wavefunction reference values [74,75] have shown that among these functionals, only ωB97 and LC-BLYP are able to give accurate electronic structure of electron-rich peptide radicals obtained by vertical reduction, whereas the other functionals may give unphysical results.

Calculations on all open-shell species used the spin-unrestricted formalism, and the resulting spin operator $\langle S^2 \rangle$ values are between 0.750 and 0.763. In order to ascertain that the wavefunctions of open-shell species correspond to the minimum, their stability has been tested with the "stable" approach implemented in Gaussian 09. The MP2 energies reported in the following correspond to the spin-projected ones (PMP2). The spin densities were visualized applying a contour threshold of 0.001 au.

The conformational landscapes of the diprotonated dipeptide $[GlyLys + 2H]^{2+}$ were explored using molecular dynamic simulations (MD) with the Tinker molecular modeling package [83] and the polarizable AMOEBA force field [84,85]. AMOEBA was shown to be reliable for charged systems due to an efficient treatment of electrostatic and polarization effects [86–88]. The parameters for the neutral COOH termination were adjusted based on those of the carboxylic group of the aspartic acid [89]. For a given starting structure, MD calculations have been conducted at 500 K for a total simulation time of 0.16 ns during which structures were sampled every 0.16 ps. All minima obtained at the AMOEBA level have been re-optimized at the DFT level as described above. No extensive conformational search was performed on our studied models **3⁺**, **6⁺–9⁺**, **3•** and **6•–9•** (vide infra), but several minima have been obtained for some compounds. In that case, only the lowest energy minimum is discussed in the text.

3. Results and discussion

The first part of this work is dedicated to the assessment of relative performances of the B3LYP, M06-2X, ωB97X-D, ωB97 and LC-BLYP exchange-correlation functionals. This includes studies of the relative energies of the doubly protonated dipeptide