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Density Functional Theory (DFT) Study of Gas-phase O–C Bond Dissociation Energy of Models for *o*-TEMPO-Bz-C(O)-Peptide: A Model Study for Free Radical Initiated Peptide Sequencing†

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The bond dissociation energy (BDE) of the chemical bond between the carbon and oxygen atoms of a simple TEMPO-derivative is calculated by employing the density functional theory, the 2nd order Møller-Plesset (MP2) perturbation theory, and complete basis set (CBS) methods. We find that BDE of the positive ion of the TEMPO-derivative is larger at least by 7 kcal/mol than that of the negative ion, which implies that the dissociation reaction rate of the positive ion should be slower than that of the negative ion. Such theoretical predictions are contrary to the results of our previous experiments (*Anal. Chem.* **2013**, *85*, 7044), in which the larger energy was required for negative *o*-TEMPO-Bz-C(O)-peptides to undergo the dissociation reactions than for the positive ones. By comparing our theoretical results to those of the experiments, we conclude that the dissociation reaction of *o*-TEMPO-Bz-C(O)-peptide should occur in a complicated fashion with a charge, either positive or negative, probably being located on the amino acid residues of the peptide.

Key Words : Bond dissociation energy (BDE), Free radical initiated peptide sequencing, Density functional theory (DFT), Tandem mass spectrometry, TEMPO

Introduction

The advent of soft ionization techniques such as electrospray ionization (ESI) and matrix assisted laser desorption/ionization (MALDI) have made it possible for mass spectrometry (MS) to place itself as a key technology for biological sciences involving proteins.[1-5] Since their developments, intensive research efforts have been made to understand their mechanisms and expand their uses in more sophisticated applications. In particular, Prof. Kim and coworkers have recently dedicated themselves towards the better understanding of the MALDI mechanism and the expansion of the MALDI analysis as a quantitative tool.[6-9] Another notable contribution made in his group was the coupling of UV light with MALDI technique as a photodissociation (PD) tool,[10-12] which has made an impact on the ensuing use of UV light in peptide sequencing mass spectrometry.[13-16]

On the other hand, radical chemistry of gaseous peptides/proteins has drawn extensive attention from the researchers in the community of biological mass spectrometry since the discovery of electron capture dissociation (ECD) tandem mass spectrometry method.[17-30] In ECD-MS, a hydrogen atom radical (H•) is introduced on the peptide/protein backbone through a combination reaction between a proton (H^+) and a low kinetic-energy electron.[17-24] The unique radical chemistry on the peptide/protein backbone was found to account for peptide/protein backbone dissociations that made the sequencing of peptides/proteins more feasible. Later, a series of ECD variant technologies were developed, which include electron transfer dissociation (ETD), electron detachment dissociation (EDD), and electron induced dissociation (EID).[31-37] These techniques show the unique peptide dissociation behavior readily discernible from the collision-based tandem mass spectrometry methods such as collision activation dissociation (CAD). Specifically, ECD and its variant methods produce ***a***/***x*** or ***c***/***z***-type peptide fragments, while CAD mainly yields ***b***/***y***-type products.[38] In addition, post-translational modifications (PTMs) are known to be well preserved during their applications, in contrast to the CAD that readily lose PTMs.[39]

In ECD and its variant technologies, it has been recognized that the generation of a radical site on the peptide/protein manifold is a key step in achieving the radical-driven peptide/protein dissociations. This recognition has led to a variety of methods that can introduce a radical site on peptides/proteins. For example, collisional activation of the ternary complexes of a metal cation with a peptide and ligand(s) was shown to produce a peptide radical ion.[40-42] UV photodissociation of an aryl-iodine bond in a manifold of non-covalent complexes of a crown ether and peptide or in an iodinated tyrosine-containing peptide was demonstrated to introduce a radical site on the peptide.[43,44] Another innovative approach is the development of the so-called free radical initiated peptide sequencing mass spectrometry (FRIPS-MS).[45-49] In FRIPS, a radical precursor is incorporated as a

†This paper is to commemorate Professor Myung Soo Kim's honourable retirement.

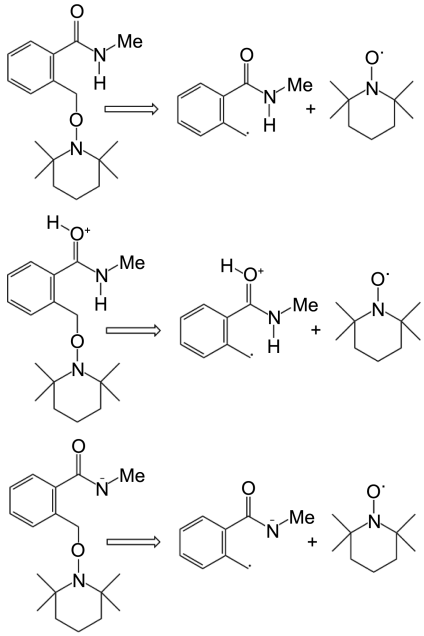
tagging molecule into peptides/proteins at the *N*-terminal amino group or lysine side chain ε-amino groups. Upon collisional activation, the tagged molecule is homolytically cleaved out to produce a radical site at the tagged location. The subsequent collisional activation on the generated radical peptide radical ion species produces extensive peptide backbone dissociations, particularly through radical-driven peptide backbone fragmentation pathways. So far, a variety of free radical initiators have been demonstrated to show the FRIPS effect. For example, peroxycarbamate and Vazo 68 were demonstrated to be good free radial initiators in the early stage of FRIPS development.[45,46] A little later, nitrosopeptides were also shown to introduce a radical site upon thermal activation.[50-52]

Recently, Oh and coworkers utilized the extraordinary stability of a TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) as a thermodynamic boost to facilitate the free radical generation in the framework of *o*-TEMPO-Bz-C(O)-peptide.[47-49] Through two thermal activation steps, that is, the radical generation and radical-driven peptide backbone dissociation steps, sequencing of peptides could be achieved in a universal manner. Furthermore, the TEMPO-based FRIPS approach was shown to lead to the rupture of the disulfide (S-S) or the adjacent C-S bond in an exclusive manner, prior to peptide backbone dissociations.[48] Very recently, it was found that in negative ion mode, the radical-driven peptide backbone dissociations can be achieved in a single step, in contrast to the two-step peptide dissociations in positive ion mode.[49] The relative collision energy requirements for the two thermal activation steps in the TEMPO-based FRIPS were found to be responsible for the different peptide sequencing behaviors in the positive- and negative-ion modes. In negative ion mode, the homolytic cleavage of the bond between the oxygen of TEMPO and the carbon at the benzylic position was shown to require higher thermal energy than in positive ion mode.

The above-mentioned different peptide dissociation behavior in positive and negative ion modes clearly suggests that the bond dissociation energy (BDE) of the C–O bond in *o*-TEMPO-Bz-C(O)-peptides plays a significant role in determining peptides dissociation energetics and dynamics. Based on the recognition of the importance of the BDE of the C–O bond in the TEMPO-based FRIPS, we performed density functional theory (DFT) calculations on a model compound that is thought to reflect the bond dissociation features of *o*-TEMPO-Bz-C(O)-peptides.

Computational Details

Electronic structure calculations were carried out for three model reactions in order to predict the BDEs of the C–O bond in *o*-TEMPO-Bz-C(O)-peptide (see Scheme 1). In Scheme 1, the reactant of the first reaction is of the neutral form, in which the peptide part of an *o*-TEMPO-Bz-C(O)-peptide is simplified as a methyl (CH_3) group. The reactants of the second and third reactions are its positive and negative ion counterparts, respectively. In the second reactant, a proton is placed on the carbonyl oxygen, and in the third reactant the deprotonation is made at the position of the amide nitrogen. All three reactions under study are the homolytic cleavage of C–O bond, *i.e.*, between the oxygen of TEMPO and the carbon at the benzylic position, producing a stable TEMPO radical. In the present study, we employ a relatively simple model in which a charge is allocated to either a carbonyl oxygen (protonated, the 2nd reaction) or an amide nitrogen (deprotonated, the 3rd reaction). Our study is limited to the cases where a charge is topologically close to the TEMPO moiety of the reactants. Therefore, careful caution should be given because it is generally thought that a charge, either positive or negative, can be located at some different places of peptides depending on the acidity of the constituting amino acids. One caveat is, therefore, that such possibility is excluded in our model reactions.



Scheme 1. Three model chemical reactions employed in this study to predict the BDEs of C–O bond. Three different reactants are investigated in this study: neutral (top), positive (middle) and negative (bottom) reactants. They all produce the same stable TEMPO radical.

In this study, the following three theoretical methods are employed; the density functional theory (B3LYP), the 2nd order Møller-Plesset (MP2) perturbation theory, and complete basis set (CBS) methods. For B3LYP calculations, both 6-31G(d) and 6-31+G(d,p) basis sets are used, and the 6-311+G(2d,p) basis set is combined with MP2 calculations. The optimized geometries and frequencies of all reactants and products are obtained using each theoretical method. The zero-point energies are also estimated by taking the proper scaling factors into account.

Results and Discussion

Figure 1 shows the structures of the neutral reactants

Table 1. Calculated bond dissociation energies (BDEs). The BDE values are given in the unit of kcal/mol.

BDE (kcal/mol)	Neutral	Positive	Negative
B3LYP/6-31G(d)	29.1	36.1	29.3
B3LYP/6-31+G(d,p)	25.1	32.4	24.9
MP2/6-311+G(2d,p)	2.4	10.1	2.9
CBS-4	104.3	106.8	55.2

optimized by B3LYP/6-31+G(d,p). The structures of all three neutral, positive, and negative reactants do not differ significantly from each another even though there are some differences in bond length and angles. For example, the distances of the C–O bond are 1.45, 1.47, and 1.44 Å, respectively, for neutral, positive, and negative reactants.

However, in terms of the BDE for the C–O bond between the TEMPO and the benzylic carbon, B3LYP, MP2 perturbation theory, and CBS method provide quite different results. In the B3LYP DFT calculations with two different basis sets, the BDE values range from 25 to 36 kcal/mol for three different reactions (see Table 1). On the other hand, MP2 perturbation theory underestimates BDEs quite significantly, which ranges from 2 to 10 kcal/mol. In contrast, CBS method overestimates BDEs compared to those of B3LYP calculations. Even though the thermodynamic properties (for example, the bond dissociation energy, the proton affinity, etc.) from the CBS method are expected to be generally more accurate than the density functional theories and the perturbation theories, it is difficult to decide which theoretical methods would provide the most accurate BDE values for the particular reactions examined in this study.

A recent theoretical study by Hodgson et al. showed that predicting the thermodynamic properties of the homolysis of alkoxyamines should be a demanding task.[53] They performed extensive and systematic calculations to obtain the enthalpy of the homolysis of the C–O bond of various alkoxyamines. They found that the homolysis of the C–O bond should be dependent on the alkyl fragment and that B3LYP DFT methods would be appropriate for gas-phase reactions. In addition, according to pervious experiments BDEs of the C–O bond in various neutral TEMPO derivatives range from 18 to 48 kcal/mol.[54] In particular, when there exists a benzylic ring in the TEMPO derivative, BDEs range from 26 to 32 kcal/mol. This also suggests that the BDE values of B3LYP are more likely to agree with the experimental results.

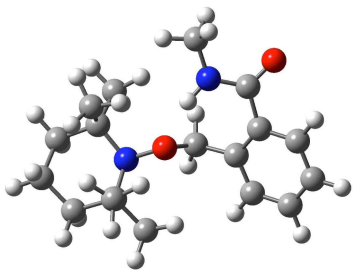


Figure 1. The optimized structure of the neutral reactant obtained *via* B3LYP/6-31+G(d,p).

It is notable that the DFT predictions for the BDEs of *neutral* and *negative* (*deprotonated*) reactants are close to each other within about 0.2 kcal/mol, which is much smaller than the thermal energy at room temperature. However, when the reactant is a *positive* (*protonated*) ion, the DFT prediction for the BDE is larger by about 7 kcal/mol than those of neutral and negative reactants, regardless of the basis-sets employed in the present study. Such a large BDE in the positive ion compared to the neutral and negative counterparts is also observed with the MP2 perturbation theory. To summarize, our electronic structure calculations indicate that the C–O bond dissociation reaction of the positive ion should require more energy than that of the negative ion. Assuming the Hammond's postulate, the transition state structure of the homolytic cleavage of C–O bond would be similar to that of the product and the activation energy should be close to the BDE.[55,56] Therefore, the dissociation reactions of negative ions can be expected to occur kinetically fast.

However, the theoretical predictions of such a larger BDE and a slower reaction rate for positive ions are inconsistent with our previous experiments for *o*-TEMPO-Bz-C(O)-peptide, wherein larger collisional energies were required for the negative ions of *o*-TEMPO-Bz-C(O)-peptides to undergo dissociation reactions than for the positive ions. A discrepancy between theoretical predictions and experiments may be attributed to the relatively simple reaction models employed in this study for the electronic structure calculations. In our model reactions, positive (protonated) and negative (deprotonated) charges are assigned to the oxygen and nitrogen atoms that are close to the benzylic ring. But for *o*-TEMPO-Bz-C(O)-peptide in previous experiments, charges are likely to be located at amino-acid residues of a peptide other than the oxygen and nitrogen atoms that are next to the benzylic ring. In addition, the flexibility of the peptide backbone could affect the dissociation reaction in a more complicated way by expanding the phase space of reactants significantly. Therefore, in order to elucidate the dissociation reaction mechanism of *o*-TEMPO-Bz-C(O)-peptide, a more systematic theoretical approach should be planned in our future study by carefully increasing the number of amino acids in the model reactant.

Conclusions

We perform extensive electronic structure calculations to estimate the BDEs of simple model reactions and further shed light on the dissociation mechanism of *o*-TEMPO-Bz-C(O)-peptides. In the model reactions, we consider the simplest case of *o*-TEMPO-Bz-C(O)-peptides, wherein all peptides are replaced by a methyl group. The reactions occur *via* the homolytic cleavage of C–O bond between the TEMPO and the benzylic carbon, thus forming stable TEMPO radicals. The BDEs are calculated using B3LYP DFT theory,

MP2 perturbation theory and CBS-4 method. The BDE values obtained with B3LYP DFT theory fit in the range of experimental BDE values. We find that the BDE values of neutral and negative reactants are smaller than those of the positive reactant, which does not agree with the trend found in the experimental BDEs of *o*-TEMPO-Bz-C(O)-peptides. For *o*-TEMPO-Bz-C(O)-peptides, a larger energy is required for the negative reactants to undergo the homolytic cleavage of the C–O bond than for the positive reactants. The discrepancy between our theoretical BDE of simple reactions and the experimental BDE of *o*-TEMPO-Bz-C(O)-peptides could be attributed to the fact that the charges (either negative or positive) of reactants used in our theoretical calculations were adjacent to the benzylic ring while the charges of *o*-TEMPO-Bz-C(O)-peptides might be placed at amino acid residues away from the terminal *o*-TEMPO-Bz group. This possibly enables *o*-TEMPO-Bz-C(O)-peptides to undergo the reaction *via* a different mechanism and calls for additional extensive computational investigations. In our future study, we will undergo similar electronic structure calculations but with additional amino acid residues for the model reactants to better understand the dissociation mechanism of TEMPO-based FRIPS.

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