Open-shell pair interaction energy decomposition analysis (PIEDA): Formulation and application to the hydrogen abstraction in tripeptides

Mandy C. Green,1 Dmitri G. Fedorov,2 Kazuo Kitaura,3 Joseph S. Francisco,1 and Lyudmila V. Slipchenko1

1Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, USA
2NRL, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Umezono, Tsukuba, Ibaraki 305-8568, Japan
3Graduate School of System Informatics, Kobe University, 1-1 Rokkodai, Nada-ku, Kobe, Hyogo 657-8501, Japan

(Received 13 November 2012; accepted 24 January 2013; published online 21 February 2013)

An open-shell extension of the pair interaction energy decomposition analysis (PIEDA) within the framework of the fragment molecular orbital (FMO) method is developed. The open-shell PIEDA method allows the analysis of inter- and intramolecular interactions in terms of electrostatic, exchange-repulsion, charge-transfer, dispersion, and optional polarization energies for molecular systems with a radical or high-spin fragment. Taking into account the low computational cost and scalability of the FMO and PIEDA methods, the new scheme provides a means to characterize the stabilization of radical and open-shell sites in biologically relevant species. The open-shell PIEDA is applied to the characterization of intramolecular interactions in capped trialanine upon hydrogen abstraction (HA) at various sites on the peptide. Hydrogen abstraction reaction is the first step in the oxidative pathway initiated by reactive oxygen or nitrogen species, associated with oxidative stress. It is found that HA results in significant geometrical reorganization of the trialanine peptide. Depending on the HA site, terminal interactions in the radical fold conformers may become weaker or stronger compared to the parent molecule, and often change the character of the non-covalent bonding from amide stacking to hydrogen bonding. © 2013 American Institute of Physics.

[http://dx.doi.org/10.1063/1.4790616]

I. INTRODUCTION

Non-covalent interactions govern structure and function of biological macromolecules such as DNA and proteins, the state of liquids and colloids, and adsorption processes. For many years, there has been an inexhaustible effort to understand and model non-covalent interactions. For smaller systems, very accurate high-level quantum-mechanical studies of molecular interactions have been conducted.1-10 The basis set superposition error (BSSE) can be eliminated with the counterpoise (CP) correction,11 with self-consistent field for molecular interactions,12-14 or avoided with model potentials such as the effective fragment potential (EFP) method.15-20

Non-covalent interactions are typically thought of consisting of Coulomb, polarization, charge-transfer, and dispersion attractive forces that are counterpoised by a repulsive term arising due to the quantum nature of electronic wave functions. Decomposition of non-covalent interactions into these components is important for creating physically meaningful models for their description, such as new-generation force fields. Decomposition of non-covalent interactions may be achieved by either supermolecular or perturbative energy decomposition schemes. The former typically treats fragment pairs variationally, while the latter uses a perturbation approach based on the fragment electronic states. The Green’s function formalism21 provides some insight into the connection of the two approaches.

Perturbative treatment of fragment interactions has been developed in the symmetry-adapted perturbation theory (SAPT) by Moszynski, Jeziorski, and Szalewicz22,23 in which the interaction energy is expressed in orders of an intermolecular interaction operator V and a many body perturbation theory (MBPT) operator W. Recent developments in this area include the use of SAPT with density functional theory (DFT), which lowers its scaling to O(N⁵) (compared to the O(N⁷) scaling of full SAPT)26,27 and a combination of SAPT ideas with XPol variational method by Herbert.28 Efficient implementation of SAPT algorithms enabled applications to systems containing hundreds of atoms.29-31

In variational Hartree-Fock (HF) energy decomposition analysis (EDA) methods,32-45 pioneered by Morokuma and Kitaura,32 the total energy is typically represented as a sum of a frozen density interaction energy (resulting in Coulomb and exchange-repulsion terms), a polarization energy, and a charge-transfer energy. Dispersion energy may be obtained as a correlated part of the interaction energy. The frozen density term is calculated as the interaction of the unrelaxed electron densities on the interacting fragments (frozen density refers to the electron density of isolated fragments). The polarization term originates from the deformation of electron clouds of the interacting fragments in the fields of each other, while the charge transfer arises from the electron flow between the fragments. Quantum mechanically, polarization and charge transfer terms can be described as lowering of energy due to the intra- and inter-fragment relaxation of the
molecular orbitals, respectively. The main differences in the variational EDA schemes come from the manner in which the intermediate self-consistent energies, corresponding to the variationally optimized antisymmetrized wave functions constructed from molecular orbitals localized on the individual fragments, are determined.

Most of the EDA and perturbation schemes have been designed to analyze interactions in non-covalently bound fragments (intramolecular interactions), with a notable exception of a density-based EDA by Wu et al. that is applicable to covalent systems and intramolecular interactions. To analyze intramolecular interactions in biological systems, Fedorov and Kitaura have extended the EDA method to covalently bound fragments by introducing the pair interaction energy decomposition analysis (PIEDA) within the fragment molecular orbital (FMO) framework. Recently, Tanaka et al. have developed an entropic contribution to pair interaction energies, taking into account configuration averaging in a classical model.

FMO is one of a number of fragment-based methods recently reviewed. Most of these methods are developed for closed shell wave functions and only relatively few permit open-shell treatment. In the FMO formalism, one performs fragment calculations in the electrostatic field of other fragments, mutually self-consistent with each other. PIEDA based on the FMO formalism has been applied to studies of protein-DNA binding, protein-ligand binding, investigating intramolecular interactions in synthetic γ-peptides, and in quantitative structure activity relationship (QSAR)-related studies.

Alternatively to PIEDA, for FMO there is the configuration analysis for fragment interactions (CAF) and the fragment interaction analysis based on local second order perturbation theory (MP2) (FILM). BSSE corrections have been attempted in the FMO framework. However, because of the absence of a general way to introduce BSSE corrections in PIEDA with its complicated evaluation of components and in view of a critical assessment of the CP method, we did not use the CP method in the present study.

In this work, we develop an extension of the PIEDA scheme to open-shell fragments. This extension allows one to quantify inter- and intramolecular interactions in systems containing open-shell fragments or molecules, such as radicals or high-spin species. The new technique is applied to a simple tripeptide trialanine upon hydrogen abstraction (HA) from various sites, to characterize and compare the intramolecular non-covalent interactions in the peptide.

Radicals in biological systems play a dual role as both harmful and beneficial species. Radicals specific to radical chemistry are difficult to measure, and as a result they are poorly understood. Radical reactions are not site-specific; many product variants are generated, hindering systematic experimental investigation. New methods are required to investigate radical mechanisms and to understand their effects in large biological systems. Ab initio methods suitable for systematic investigation of open-shell systems are typically limited to small molecules on the order of tens of atoms. Therefore, biological systems that often contain thousands of atoms have previously been beyond the reach of computational methods appropriate for open-shell molecules. Combining FMO with open-shell PIEDA provides a new means to investigate the inter- and intramolecular properties of large open-shell systems.

Oxidative stress is a physiological condition resulting from overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which damage biological structures such as lipids in membranes, DNA, and proteins. Oxidative stress is linked to the pathogenesis of many diseases in biological systems such as cancer, cardiovascular disease, atherosclerosis, hypertension, ischemia/reperfusion injury, diabetes mellitus, multiple neurodegenerative diseases, rheumatoid arthritis, and aging. ROS and RNS are usually open-shell radical molecules such as hydroxyl radical, superoxide, and nitric oxide, although they can be non-radical species such as hydrogen peroxide. Damage to biological molecules, in this case via protein oxidation, occurs by the direct attack of ROS and RNS. HA reactions are the first step in the oxidative pathway initiated by radical ROS and RNS. In this work, we consider products of a prototypical HA reaction in which hydroxyl radical attacks a capped trialanine peptide: OH• + AAAH → HOH + AAA•. Hydrogen abstraction can occur at any atom in the molecule bonded to a hydrogen atom. In this study, we consider the product variants to HA at the α-carbons, the terminal carbons, and nitrogen to characterize and compare the intramolecular non-covalent interaction between the radical sites. The extension of the PIEDA scheme to open-shell fragments allows one to quantification of the molecular interactions in the open-shell fragments trialanine peptide radicals.

The structure of the paper is as follows: Sec. II provides theoretical details on FMO, PIEDA, and open-shell PIEDA methods. Section III summarizes computational details. Section IV describes application of PIEDA analysis to parent and HA-trialanine radicals, while the main conclusions are summarized in Sec. V.

II. THEORY

Development of the restricted open-shell PIEDA (ROPIEDA) scheme is based on a recent spin-restricted extension of FMO to open-shell systems and the original (closed-shell) PIEDA. In this work, the unrestricted extension of FMO is not used. A brief overview of FMO and PIEDA is provided below. Correlation is included using MP2. Alternatively, the coupled cluster (CC) approach can be used instead.

A molecular system in FMO is divided into N fragments, also referred to as monomers. In the first step, each monomer is calculated using the restricted Hartree-Fock (RHF) wave function in the Coulomb field exerted by all remaining monomers. In the open-shell FMO, one of the fragments is open-shell; it is calculated using restricted open-shell Hartree-Fock (ROHF wave function). The monomer calculations are repeated until self-consistency is reached. In the second step, each dimer (pair of monomers) is computed in the Coulomb field exerted by all remaining monomers. This corresponds to the two-body FMO expansion (FMO2). The PIE is then
obtained by subtracting monomer energies from dimer energy

$$\Delta E_{IJ}^{\text{int}} = (E_{IJ}' - E_{IJ}'') + Tr(\Delta D_{IJ}^{\text{mix}} V_{IJ})$$

where $I$ and $J$ are fragments. In FMO, RHF or ROHF calculations are performed in the embedding potential acting upon fragments $I$ ($V^I$) or fragment pairs $IJ$ ($V^{IJ}$), yielding the monomer $E_I$ and dimer $E_{IJ}$. $E_I'$ and $E_{IJ}'$ are the internal energies, calculated as $E_{IJ}' = E_{IJ} - Tr(D_{IJ}^{\text{mix}} V_{IJ})$, i.e., subtracting the contribution of the embedding potential. $\Delta D_{IJ}^{\text{mix}}$ is the difference of the dimer $D_{IJ}$ and two monomer $D_I'$ and $D_J'$ electron densities. $E_{IJ}^{\text{corr}}$ and $E_{IJ}^{\text{corr}}$ are the monomer and dimer correlation energies, respectively.

PIEDA decomposes $\Delta E_{IJ}^{\text{int}}$ into the electrostatic (ES), exchange-repulsion (EX), charge-transfer with higher order mixed terms (CT + mix), and dispersion (DI) contributions as follows:

$$\Delta E_{IJ}^{\text{int}} = \Delta E_{IJ}^{\text{ES}} + \Delta E_{IJ}^{\text{EX}} + \Delta E_{IJ}^{\text{CT+mix}} + \Delta E_{IJ}^{\text{DI}}. \quad (2)$$

For covalently connected fragments, the pair interaction energy and its components are corrected for the corresponding bond detached atom (BDA) energies. This is accomplished by doing an appropriate model calculation, for example, for a detached C–C bond, one calculates CH3–CH3, divided into two fragments. The corresponding interaction energy between the two methyl fragments is taken to be the BDA energy correction.

III. COMPUTATIONAL DETAILS

In order to avoid artifacts due to terminal interactions, the N-terminal of trialanine is capped with an acetyl and the C-terminal is capped with methyamide functional groups. Hydrogen abstraction in the capped trialanine is considered at three $C_\alpha$ sites ($C_\alpha 1$, $C_\alpha 2$, $C_\alpha 3$), $N'$ and $C'$ terminal caps, and at the $N_3$ position (see Figure 1). The $N'$ and $C'$ caps have their methyl functional groups in pseudo $C_\alpha$ positions in the backbone. All considered HA products are doublets.

Geometry optimizations of parent and HA-trialanines in their strand and fold conformations are performed at the restricted and unrestricted MP2(Ref. 87)/6-31G* (Refs. 88 and 89) levels of theory, for closed and open-shell systems, respectively. Cartesian coordinates of the optimized structures are provided in the supplementary material. For radical products, single-point energy calculations using ROHF reference were also performed. FMO2 single point energies are obtained at the same level of theory ((RO)MP2/6-31G*), followed by PIEDA calculations. ROHF wave function was used for a HA radical fragment in the self-consistency cycle of FMO and PIEDA;

FIG. 1. (a) Capped trialanine with considered HA sites (orange circles) and its (b) unfolded (β-strand) and (c) folded (β-turn) conformations.
FIG. 2. Fragmentation schemes used in FMO2 and PIEDA calculations. Scheme D represents a default FMO fragmentation of a polypeptide. Custom schemes A–C are adapted to avoid artifacts due to fragmenting near radical sites.

ROMP2 was employed for subsequent monomer and dimer calculations including the HA fragment.

By default, fragmentation for proteins in FMO is performed at the bonds between an \( \alpha \)-carbon and a carbonyl. This fragmentation scheme, containing four fragments, is employed for the parent trialanine (scheme D, see Figure 2). Three other fragmentation schemes A, B, and C shown in Figure 2 contain three fragments each. These schemes are employed for describing radical HA products to avoid fragmenting the bond near a radical center. In particular, scheme A is used for HA on \( C_{\alpha 1} \), scheme B for HA on \( C_{\alpha 2} \), and scheme C for HA on \( C_{\alpha 3} \). The \( N_3^* \) product was fragmented with schemes B and D; while the \( N'\text{cap}^* \) product was fragmented with schemes A and D; and the \( C'\text{cap}^* \) product was fragmented with schemes C and D. In the three latter cases, the fragmentation schemes with a larger radical-containing fragment (A, B, or C) provided consistently more accurate results than scheme D. Thus, presentation of the \( N_3^* \), \( N'\text{cap}^* \), and \( C'\text{cap}^* \) results will be limited to fragmentation schemes B, A, and C, respectively. For consistency, FMO and PIEDA computations with the same fragmentation schemes are performed for the parent trialanine as well.

All calculations were performed in the GAMESS electronic structure package.84,85

IV. RESULTS AND DISCUSSION

A. Intramolecular interactions in parent trialanine

The goal of this section is to characterize intramolecular non-covalent interactions in the parent closed-shell trialanine and to provide a reference for comparing the interactions in radical HA products. The same fragmentation schemes are used for parent trialanines and HA products to ensure that there are no artifacts due to differences in fragmentation.

Interactions between terminal amide planes are decomposed by PIEDA and shown in Figure 3. We also call the terminal interactions as “1-4 interactions” based on the numbering in the D scheme even though they are formally 1-3 interactions in schemes A–C. The 1-4 interactions are non-covalent in all fragmentation schemes. Not surprisingly, the terminal interactions between amide planes 1 and 4 are much stronger in the turn conformation than in the strand isomer. Terminal interactions in the turn are attractive by \(-7\) to \(-9\) kcal/mol; they are much weaker (\(-0.4\) to \(+2.0\) kcal/mol), depending on the fragmentation, in the strand isomer. The leading contribution to the interaction energy in both the turn and strand is the electrostatic term that determines a sign and magnitude of the total interaction energy. However, the other contributions in the turn (dispersion, charge-transfer, and exchange) are also non-negligible. Strong electrostatic interaction between terminal planes in the turn can be explained by dipole-dipole interactions between the peptide bonds typical for \( \beta \)-structures.

For 1-4 terminal interactions, fragmentation schemes B and D produce almost identical results (see Figure 3). This is because these schemes have identical terminal fragments. The total interaction energies and their components are somewhat different between schemes A, C, and B/D, because one of the interacting fragments in schemes A and C is larger than the terminal fragments in schemes B and D. Interestingly, electrostatic interactions in schemes A and C become less attractive (or even repulsive for the strand), suggesting some electrostatic repulsion between central and terminal parts of the peptide. The decrease in electrostatics is partly compensated by the increase in charge-transfer and dispersion terms, which is understandable as one of the fragments is larger.
TABLE I. Electronic energy differences $\Delta E$ (kcal/mol) for hydrogen abstraction reaction for selected sites in capped-trialanine with full $ab\ initio$ and FMO methods.

<table>
<thead>
<tr>
<th>Conformer</th>
<th>Radical site</th>
<th>FMO $\Delta E$</th>
<th>Full $\Delta E$</th>
<th>FMO $\Delta E$ error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strand</td>
<td>C$_{\alpha}1$.A</td>
<td>$-24.91$</td>
<td>$-25.23$</td>
<td>$0.31$</td>
</tr>
<tr>
<td>Strand</td>
<td>C$_{\alpha}2$.B</td>
<td>$-24.15$</td>
<td>$-24.48$</td>
<td>$0.33$</td>
</tr>
<tr>
<td>Strand</td>
<td>C$_{\alpha}3$.C</td>
<td>$-23.66$</td>
<td>$-23.99$</td>
<td>$0.33$</td>
</tr>
<tr>
<td>Strand</td>
<td>N$_{\text{cap}}$.A</td>
<td>$-12.14$</td>
<td>$-12.47$</td>
<td>$0.32$</td>
</tr>
<tr>
<td>Strand</td>
<td>C$_{\text{cap}}$.C</td>
<td>$-15.23$</td>
<td>$-15.62$</td>
<td>$0.38$</td>
</tr>
<tr>
<td>Strand</td>
<td>N$_{3}$.B</td>
<td>$0.59$</td>
<td>$0.19$</td>
<td>$0.78$</td>
</tr>
<tr>
<td>Turn</td>
<td>C$_{\alpha}1$.A</td>
<td>$-14.20$</td>
<td>$-14.30$</td>
<td>$0.11$</td>
</tr>
<tr>
<td>Turn</td>
<td>C$_{\alpha}2$.B</td>
<td>$-17.25$</td>
<td>$-17.58$</td>
<td>$0.33$</td>
</tr>
<tr>
<td>Turn</td>
<td>C$_{\alpha}3$.C</td>
<td>$-16.17$</td>
<td>$-16.56$</td>
<td>$0.39$</td>
</tr>
<tr>
<td>Turn</td>
<td>N$_{\text{cap}}$.A</td>
<td>$-11.99$</td>
<td>$-12.47$</td>
<td>$0.48$</td>
</tr>
<tr>
<td>Turn</td>
<td>C$_{\text{cap}}$.C</td>
<td>$-15.88$</td>
<td>$-16.27$</td>
<td>$0.38$</td>
</tr>
<tr>
<td>Turn</td>
<td>N$_{3}$.B</td>
<td>$-0.23$</td>
<td>$-0.76$</td>
<td>$0.53$</td>
</tr>
</tbody>
</table>

B. Hydrogen-abstrated tripeptides

1. Validation of open-shell FMO for HA-trialanine

We consider radical products resulting from hydrogen abstraction reaction from tripeptide

$$\text{OH}^* + \text{AAAH} \rightarrow \text{HOH} + \text{AAA}^*$$  \hspace{1cm} (3)

where AAAH is the parent capped tripeptide; AAA$^*$ is a HA-radical product. Hydrogen abstraction on the backbone of the tripeptide can occur at three C$_{\alpha}$ sites (C$_{\alpha}1$, C$_{\alpha}2$, C$_{\alpha}3$). Additionally, HA sites at the N' and C' terminal caps (N$_{\text{cap}}$ and C$_{\text{cap}}$, respectively), which have their methyl functional groups in pseudo C$_{\alpha}$ positions, and the site at the N$_3$ position are considered (see Figure 1). The terminal and N$_3$ sites are selected due to their enhanced 1-4 intramolecular interactions, as analyzed in detail below.

Electronic energy differences, $\Delta E$, for the HA reaction (Eq. (3)) at different sites are reported in Table I. The HA reactions are considered separately for strand and turn conformations of tripeptide; geometries of the reactants and products are optimized within each conformation. The accuracy of FMO2 is compared against full $ab\ initio$ calculations, at the same level of theory (MP2/6-31G*). Negative $\Delta E$ values mean that the HA reaction is exothermic. As seen from data in Table I, the C$_{\alpha}$ sites are the most thermodynamically favorable for HA out of all the sites on the protein backbone.

For the C$_{\alpha}$ sites in both the strand and turn conformations, the relative difference in HA energies between the full $ab\ initio$ and FMO methods is less than 0.4 kcal/mol. FMO-$ab\ initio$ discrepancies for the terminal sites on the caps are also very small (<0.5 kcal/mol).

Different from hydrogen abstraction reactions from carbon sites, HA from the nitrogen site N$_3$ is almost energetically neutral, with the full $ab\ initio$ method producing a HA energy change of $-0.2$ kcal/mol in the strand and $-0.8$ kcal/mol in the turn. A radical site on nitrogen is more challenging for the description by FMO, which produces relative errors of 0.5–0.8 kcal/mol, probably due to the larger amount of charge-transfer between fragments. Generally, compared to full $ab\ initio$, FMO slightly under-stabilizes HA radical products compared to the parent tripeptide; however, the discrepancies do not exceed 1 kcal/mol.

TABLE II. Relative stability of turn versus strand conformers ($E_{\text{stab}} = E_{\text{turn}} - E_{\text{strand}}$) for parent tripeptide and HA products and stabilization energy ($E_{\text{stab}} = (E_{\text{turn}} - E_{\text{parent}})_{HA} = (E_{\text{turn}} - E_{\text{parent}})_{HA}$) of the turn conformer due to hydrogen abstraction (kcal/mol).

<table>
<thead>
<tr>
<th>Radical site</th>
<th>$E_{\text{turn}} - E_{\text{strand}}$</th>
<th>$E_{\text{stab}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
<td>$-6.40^{\text{a}}$</td>
<td>0.00</td>
</tr>
<tr>
<td>C$_{\alpha}1$.A</td>
<td>4.13</td>
<td>10.72</td>
</tr>
<tr>
<td>C$_{\alpha}2$.B</td>
<td>0.05</td>
<td>6.90</td>
</tr>
<tr>
<td>C$_{\alpha}3$.C</td>
<td>0.78</td>
<td>7.48</td>
</tr>
<tr>
<td>N$_{\text{cap}}$.A</td>
<td>$-6.43$</td>
<td>0.16</td>
</tr>
<tr>
<td>C$_{\text{cap}}$.C</td>
<td>$-7.35$</td>
<td>$-0.65$</td>
</tr>
<tr>
<td>N$_3$.B</td>
<td>$-7.67$</td>
<td>$-0.82$</td>
</tr>
</tbody>
</table>

$^{\text{a}}$FMO relative stabilization energy of the turn using fragmentation scheme D. The corresponding energies using other fragmentation schemes are: $-6.59$ kcal/mol (scheme A), $-6.85$ kcal/mol (scheme B), $-6.70$ kcal/mol (scheme C).

In the parent trialanine, the turn conformer, with favorable dipole-dipole intramolecular interactions, is more stable than the strand by $\sim 6$ kcal/mol (see Table II). As follows from Table II, HAS at alpha-carbon sites destabilize the turn by 7–11 kcal/mol and make the strand formers energetically more preferable. However, HAs at other sites produce much smaller changes (typically within 1 kcal/mol) in stability of radical products. This is the key observation of this study. Destabilization (and potential unfolding) of the turn structures by HA at alpha-carbons may be significant for understanding the influence of oxidative stress on secondary and tertiary structures of proteins. The reasons of destabilization of the turn conformers upon HA are analyzed below using the developed open-shell PIEDA.

2. Intramolecular interactions upon hydrogen abstraction

Section IV B 2 describes changes in the intramolecular interactions upon hydrogen abstraction in the turn conformers. In strand conformers, where 1-4 interactions are already very weak (see Figure 3), almost no changes occur upon hydrogen abstraction.

Decomposition of 1-4 intramolecular interactions in the turn conformers of radical C$_{\alpha}$ tripeptide products by open-shell PIEDA analysis is shown in Figure 4. The absolute energies are shown on the left, followed by the relative energies on the right. The relative energies are determined as a difference between the 1-4 intramolecular energy in a C$_{\alpha}$ product and the corresponding energy in a parent trialanine with the same type of fragmentation. Note that the geometries of HA-products are optimized; thus, Figure 4 shows adiabatic relative energy differences. The presence of radical centers on C$_{\alpha}1$ and C$_{\alpha}3$, i.e., the centers that are involved in 1-4 terminal interactions, dramatically decreases total 1-4 interaction energies. Additionally, the 1-4 interactions change their nature from being predominantly electrostatic in the parent tripeptide to dispersion-dominated in C$_{\alpha}1$ and C$_{\alpha}3$ radicals.
On the contrary, there is a slight increase in the 1-4 interaction energy in the C\(\alpha\)2 product (where the radical center is not explicitly involved in terminal interactions), mainly occurring due to enhanced electrostatic attraction and diminished exchange repulsion.

The intriguing changes in the terminal interaction energies upon HA can be rationalized by geometrical changes in radical products (see Figure 5 and Table III). Compared to the parent trialanine, C\(\alpha\)1\(^*\) and C\(\alpha\)3\(^*\) radicals have more open structure, with larger separation between terminal planes. This happens because of structural reorganizations in the proximity of the radical centers. In particular, HA at the C\(\alpha\) and the cap sites causes a change in hybridization of the carbon atom from \(sp^3\) to \(sp^2\) resulting in a trigonal planar local geometry instead of a tetrahedral one. The changes in a local molecular geometry near the radical sites alter the backbone dihedral angles and the overall backbone shape. As a result, HA at C\(\alpha\)1 and C\(\alpha\)3 sites causes more separation between the first and fourth amide planes and a loss of favorable dipole-dipole interactions. On the contrary, HA at the C\(\alpha\)2 site leads to less separation and increased terminal interactions through the formation of a hydrogen bond (see Table III).

In order to distinguish effects of geometry changes and changes in the electronic structure upon HA, we compared the terminal interaction energies in the parent and radical molecules at the fixed geometry of the parent trialanine. These vertical energy differences are shown in Figure 6. Indeed, a radical center at C\(\alpha\)2 does not affect the terminal interaction energies, while the changes due to radicals in C\(\alpha\)1 and C\(\alpha\)3 are very minor (of the order of 0.2–0.3 kcal/mol) and originate from electrostatics.

Thus, we conclude that the main changes in the terminal interactions upon HA at \(\alpha\)-carbons originate from geometrical rearrangements induced by local changes of bonding and hybridization at the radical centers. The geometrical rearrangements initiated by change of the electronic structure at radical center destabilize the turn with respect to the strand. However, this destabilization may be counteracted by restructuring of neighboring groups and formation of H-bond. Evolution of energetic balance in turn and strand structures upon HA in larger peptides will be a topic of future work.

Hydrogen abstraction at the terminal and N\(_3\) sites increases attraction between the terminal planes (see Figure 7).
The strongest effect is observed in case of the N3 radical site. HA at the N3 site causes the molecular geometry near nitrogen to change from trigonal planar to bent. The backbone shape alternation due to HA at N3 results in a more compact structure of the turn conformer and formation of hydrogen bond between terminal planes, with an O...H separation of 1.997 Å (see Table III). PIEDA analysis is consistent with this conclusion: the increase in the terminal interaction energy occurs due to more attractive electrostatics in HA products. However, similar to the case of HA at \( \alpha \)-carbons, the main effect originates from the structural changes produced by HA. Analysis of relative vertical interaction energies, i.e., when the radical is considered at the geometry of the parent trialanine, reveals only a minor (<0.3 kcal/mol) destabilization of the terminal interactions in the HA product due to less favorable electrostatics (see Figure 8).

The cap sites are unique in that HA there does not alter the backbone shape. However, a decrease in sterical repulsion between the terminal groups when one of them becomes CH2 (instead of CH3) results in a reduction in the separation between the first and fourth amide planes and formation of H-bond (see Table III). This is again consistent with stronger electrostatic interactions in the N'cap* radical compared to the parent trialanine.

Different from other HA sites, HA at terminal caps has a non-negligible effect on terminal interaction energies even when geometrical rearrangements are excluded. As follows from Figure 8, both N'cap* and C'cap* radicals have weaker terminal interactions compared to the parent molecule. However, the effect is more significant for N'cap* (1.7 kcal/mol) than for C'cap* (0.2 kcal/mol). Interestingly, while changes in charge-transfer, dispersion, and exchange-repulsion contributions are very similar between N'cap* and C'cap*, changes in electrostatic energies are of opposite signs. Terminal electrostatic interactions stabilize C'cap* and destabilize N'cap* with respect to the parent trialanine. Changes in electrostatic interactions can be rationalized by radical-induced changes in the dipole moments associated with the terminal groups. The \( \pi \) electrons of \( sp^2 \)-hybridized carbons in C'cap* and N'cap* radicals participate in \( \pi \)-conjugation of adjacent amide groups. As shown in Figure 9, the resonance structure stabilizes uncharged form of the amide group in N'cap*, while the resonance structure in C'cap* possesses charges on nitrogen and oxygen. Thus, the radical center in N'cap* effectively decreases the dipole moment of the amide group, but the radical at the C'cap* increases the corresponding dipole moment. This is consistent with the observed decrease in 1-4 electrostatic (dipole-dipole) interactions in N'cap* product and a slight increase in case of the C'cap*.

**FIG. 6.** Relative terminal (1-4) intramolecular interactions in the C\( \alpha \) HA products in the turn conformation calculated at the geometry of the parent turn trialanine.

**FIG. 7.** Absolute and relative (with respect to parent trialanines) 1-4 terminal interactions in N3 and terminal caps HA products in the turn conformation.

**FIG. 8.** Relative terminal (1-4) intramolecular interactions in the N\( \alpha \) and terminal caps HA products in the turn conformation calculated at the geometry of the parent turn trialanine.
FIG. 9. Representative resonance structures in the parent trialanine and N’cap* and C’cap* radicals. Zwitterionic resonance structures affect the dipole moment of the peptide groups. Radical site in N’cap* stabilizes neutral form and effectively decreases the dipole moment of the terminal peptide group; radical site in C’cap* stabilizes zwitterionic form and enhances the dipole moment.

V. CONCLUSIONS

The PIEDA scheme for analysis of inter- and intramolecular interactions has been extended to open-shell systems. This development allows one to characterize non-covalent interactions (in terms of electrostatic, exchange-repulsion, charge-transfer, dispersion, and optional polarization terms) in systems containing radicals or radical sites or high-spin species, as well as to compare interaction energies in open-shell and related closed-shell molecules. The open-shell PIEDA was applied to investigate non-covalent interactions in capped trialanine upon hydrogen abstraction at various sites.

Hydrogen abstraction in capped trialanine was characterized with respect to geometrical changes, energetic stabilities, and intramolecular non-covalent interactions as a function of a hydrogen abstraction site. HA at α-carbons is the most energetically favorable in strand conformers. However, geometrical changes due to HA at α-carbons destabilize the turn conformers (with respect to the strand conformers). HA at other sites (middle nitrogen N3 and terminal caps) has a negligible effect on the stability of the turn with respect to the strand conformers. As a result, in the turn conformers HA at the caps is energetically comparable to HA at α-carbons.

The strand conformer of the parent trialanine has very weak terminal interactions, while the terminal planes in the turn conformer exhibit non-covalent attractions dominated by dipole-dipole forces. If considered at a rigid geometry of the parent trialanine, HA products experience only minor changes in terminal interactions, with exclusion of HA at the terminal caps. However, strong structural rearrangements associated with HA at any site result in significant changes in terminal interaction energies. In particular, HA at Cα1 and Cα3 sites weakens terminal interactions, but HA at the other considered sites strengthens them. Hydrogen abstraction at Cα2, N3, and terminal cap sites results in formation of hydrogen bonds between terminal planes.

The 1-4 interactions are a model for inter-strand interactions in a beta sheet as they are critical for turn formation and protein folding.91, 92 Protein misfolding and aggregation are the most likely cause of various neurological and systematic diseases, classified together as amyloids because the aggregates all have the same underlying beta structure. Oxidative stress initiates accumulation of amyloid protein aggregates in several diseases93–95 but the mechanism and an understanding for why the aggregates form beta structures remain elusive.96 PIEDA provides specifics for the altered intramolecular interactions and as such is instrumental in examining for how beta structures respond to radical damage. As we found in this study, radical damage local to the 1-4 interactions, where the unpaired electron resonates with the 1st and 4th amide planes, results in formation of a hydrogen bond instead of amide stacking. Thus, amyloid aggregates may adopt the beta structures to compensate for radical damage by delocalizing the unpaired electron into the local amide planes and the hydrogen bonds.
Being highly scalable, FMO and PIEDA can be used to investigate the properties of much larger biological systems than considered here. In our future work, we will employ open-shell PIEDA to gain insight into the origin of radical stabilization in proteins and examine electro/nucleophilic substituents in changing the preferred radical site. Another important effect to be considered in future studies is how solvent alters interaction patterns and stabilization of radical centers. This can be done by using a combination of open-shell FMO with either explicit (for example, treated with EFP97–99) or implicit (polarizable continuum model, PCM100–103) solvation.

ACKNOWLEDGMENTS

L.V.S. acknowledges support from National Science Foundation (NSF) (Grant No. CHE-0955419) and Purdue University. D.G.F. and K.K. have been supported in part by the Next Generation Super Computing Project, Nanoscience Project (MEXT, Japan) and Strategic Programs for Innovative Research (SPIRE, Japan).


