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Huge Disulfide-Linkage'S Electron Capture Variation Induced by α-Helix Orientation

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Abstract: An active site containing a Cys-X-X-Cys motif (CXXC), where X denotes any amino acid, is always found in the thiol-disulfide oxidoreductase superfamily. Because of its very high propensity for N-termini of α-helices, we examine the effect of this secondary structure on the disulfide-linked CXXC electron affinity. A Cys-Gly-Pro-Cys motif (CGPC) is chosen as an example, as it is the canonical motif found in thioredoxins. QM/MM calculations (MP2/6−31+G**:CHARMM) establish that the electron capture is strongly favored by an N-terminal α-helix, due to the positive electrostatic potential in the vicinity of the active site. The enhancement of adiabatic electron affinity accounts for ca. 0.9 eV for a 12-residues helix and rapidly converges as the number of alanine residues increases. A close agreement between a reference thioredoxin (Trx h) and the corresponding model peptide is found (respectively +2.20 and +2.12 eV), in parallel with experimental redox potentials [Iqbalsyah et al. *Protein Sci.* **2006**, *15*, 2026−2030]. This suggests a simple additive rule for geometrical and electrostatic effects. The electron affinity of the CXXC active site is first considered in an isolated way. Then, the strong modulation of the electrostatic field created by the α-helix can be added up. This simple partition scheme allows a proper quantification of the ease of attachment of a low-energy electron.

Introduction

Rationalizing the outcome of biomolecules under ionizing radiations is of the utmost importance for the development of radiation therapy.[1] These radiations massively lead to the *in situ* formation of low-energy secondary electrons. Strong experimental evidence for their highly specific attachment on disulfide linkages of protein has recently been reported.[2] In this Letter, hybrid calculations establish that electron capture is strongly favored by an N-terminal α-helix (ca. 1 eV), due to the positive electrostatic potential in the vicinity of the active site. Conversely, a C-terminal helix lowers the disulfide-linkage electron affinity. We find a close agreement between a reference thioredoxin (Trx h, PDB ID code 1TOF) and the corresponding model peptide (respectively +2.20 and +2.12 eV). This provides a simple way to predict the ease of attachment of a low-energy electron.

Disulfide bridges are essential for protein structure and reactivity. However, they are prone to damage, due to the relative weakness of covalent sulfur−sulfur bonds. Many questions arise concerning the following: the ease with which a disulfide linkage can capture an electron, the factors which govern the site of attachment, and the outcome of so-formed disulfide radical anion intermediates. Previous studies have brought some important partial answers: pulse radiolysis,[3] electron capture dissociation,[4] Coulomb-stabilization cleavage,[5] etc. Yet, we are still far from a sound understanding of the one-electron addition on disulfide-linkages. In order to gain more insights into this reaction in macromolecular systems, both geometrical and electrostatic contributions have to be considered and properly evaluated. Disulfide linkages are most often cyclic in proteins, and we recently demonstrated that disulfide electron affinity is governed by ring strain (1,2-dithiacycloalkanes)[6] or topological frustration (peptides).[7] The scope of this Letter is to assess the contribution of electrostatic effects occurring in larger biomolecules.

Enzymes of the thioredoxin superfamily are extensively studied due to their importance in many biological redox processes.[8] Their common active site is a CXXC motif forming a hairpin-like loop (β-turn), where X denotes any amino acid. Isolated CXXC peptides exhibit different redox potentials, and the whole tertiary structure (thioredoxin fold) induces an overall constant shift toward more reducing values.[9] More specifically, the key role of the N-terminal α-helix,[10] although well established: lowering of pK_a,[11] enhancement of redox potential,[12] etc., needs a more quantitative assessment for an in-depth understanding of proteins biochemistry. Recent experimental studies have used intermediate size designed peptides. Neat agreement between the many-complicated protein and a small peptide containing *solely* the α-helix has been reported. For instance, Doig and co-workers recently established that the redox potential for the model peptide NH_2−CAACAAAAKA-

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AAAKGY-NH_2 is very close to the characteristic value of thioredoxins.[12] This has been *a posteriori* related to the very high propensity of the CXXC motif to be located at the N-terminus end of α-helices. Theoretical calculations have also proved their usefulness for providing new insights into the role and action of terminal helices in a wide range of situations.[13]

Computational Details

QM/MM calculations have been performed (MP2/6−31+G**: CHARMM, see the Supporting Information) to study the effect of the α-helix on the disulfide bond's electron affinity.

The second-order Møller-Plesser perturbation theory (MP2) method has been used to ensure a proper description of a two center three electron bond. MP2 calculations, alongside with large basis sets including diffuse functions, have proved to give accurate results for treating electron capture by simple disulfides.[6,7] The choice for the Gaussian basis set (6−31+G**) is based on previous benchmark calculations on diethyl disulfide.[6]

The MM surrounding is described with the CHARMM force field using the CHARMM27 parameters for proteins.[14] The QM/MM calculations are performed with a modified version of the Gaussian 03 package[15] linked to the Tinker software[16] for the MM calculations. In the present case, the QM/MM frontiers are set at the C_α-C_β bonds of the two disulfide-linked cysteine residues. This frontier has been discussed in a previous article:[7] the key idea is to take advantage of the highly localized character of the disulfide bond density.

Results and Discussion

We consider a CGPC tetrapeptide, commonly found in thioredoxins, grafted onto an alanine homopolymers chain in an α-helix conformation. We test both possible orientations (N- and C- termini) for the helix, and we let its length n vary from 0 to 24 residues.

Geometrical parameters for both neutral and radical anionic forms are given in the Supporting Information. As observed for other cyclic disulfides,[6,7] geometries of radical anion compounds are highly malleable. The initial tetrapeptide Ace-CGPC-NHMe ($n = 0$) has a positive adiabatic electron affinity (EA_{ad} = 1.20 eV), which simply reflects the topological frustration of the neutral tetrapeptide.[7] This value is strongly enhanced by the presence of an N-terminal α-helix (ca. 1 eV). The variation of EA_{ad} as a function of n is displayed in Figure 1a.

A short alanine chain ($n = 1-4$) induces weak and irregular variations of electron affinity. They come from the subtle interplay of electronic and geometrical contributions, as structures are highly flexible. Disulfide linkage propensity to capture an electron increases monotonously with the number of Ala residues, ranging from +2.07 ($n = 8$) to +2.43 eV ($n = 24$). This enhancement simply corresponds to the usual representation of the α-helix, which creates an intense dipole (see the Supporting Information). The N-terminal orientation imposes a positively charged extremity of the associated electric dipole in the vicinity of the CXXC motif. The radical anion is preferentially stabilized, hence the higher electron affinities. Our calculations provide a quantitative assessment of electrostatic contributions, which account for ca. 1 eV. This proves that disulfide electron affinity is considerably modulated by the environment (tertiary structure); by comparison, mutation of intraloop residues X accounts for less than 0.3 eV.[7] Interestingly enough, a similar result has been recently obtained for redox potential of a CAAC motif placed at the N-terminal of a 12-residue-long α-helix.[12]

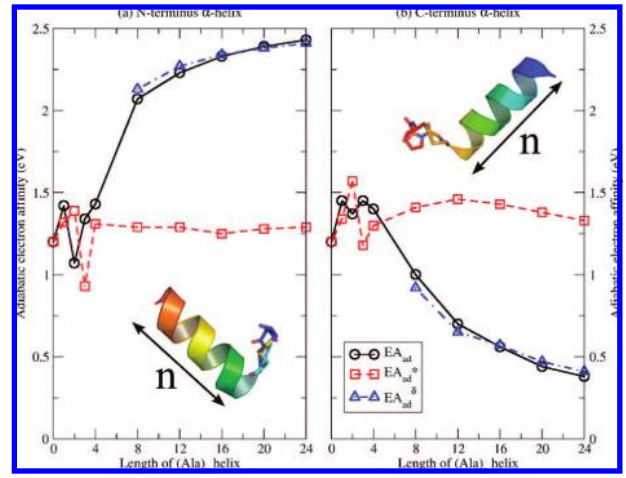

Figure 1. Electron affinity (in eV) of a disulfide-linked CGPC tetrapeptide grafted on a (a) N-terminus and (b) C-terminus α-helix, as a function of the number of Ala residues (n). The EA_{ad}, EA_{ad}*, and EA_{ad}^{δ} values are represented by black solid (circles), red dashed (square dots), and blue dashed lines (triangle dots), respectively. EA_{ad} and EA_{ad}* correspond to the QM/MM calculations with and without the electrostatic embedding respectively. EA_{ad}^{δ} corresponds to simplified calculations where the helix dipole is modeled with two point charges.

This evolution of adiabatic electron affinity EA_{ad} (Figure 1), once the CXXC motif is grafted onto the $(Ala)_n$ chain, arises from two contributions: (i) the outer electric field and (ii) the geometrical interplay between the α-helix and the β-turn fragments. The rapid and regular increase suggests the predominance of the dipole field effect: the positive charge remains almost fixed with respect to the disulfide-linkage barycenter, while the negative charge (C-terminus) moves away from it as the size of the helix increases. In order to ascertain this hypothesis and to gain further insights into the nature of the electrostatic modulation, we performed single point calculations where the partial charges of alanine residues were turned off. In this way, the electrostatic embedding of the α-helix is switched off: the corresponding electron affinities are denoted EA_{ad}*. Their values, given in the Supporting Information, are very close to the reference for isolated tetrapeptide (Figure 1a). This clearly establishes that the α-helix modulation almost exclusively comes from electrostatic contributions. The small deviations observed for $n = 1-4$ are caused by the limited structural reorganization. A simple yet efficient model for the α-helix dipole[13] is given by placing two point charges δ, respectively positive and negative, on the nitrogen and carbon termini atoms of the α-helix. Electron affinities EA_{ad}^{δ} were computed following this scheme, with all other helix point charges set to zero. A value of ±0.4 e accurately reproduces the helix effect (Figure 1a).

Conversely, a C-terminal helix tends to disfavor electron capture by the disulfide-linkage (Figure 1b). The previous interpretation is directly transferable and is further confirmed by the electrostatic potential (see Figure 2) . Quantitatively, a

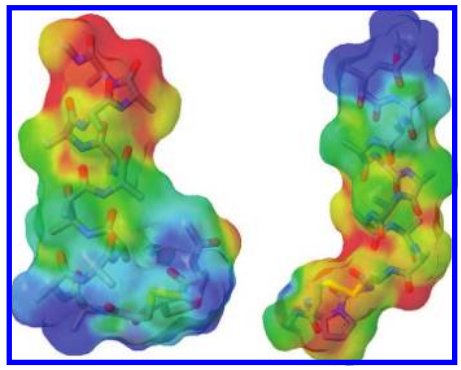

Figure 2. Electrostatic potential mapped on the van der Waals surface of a disulfide-linked CGPC tetrapeptide grafted on a 12-residues δ-helix (neutral form), at the N-terminus (left) or at the C-terminus (right). Red corresponds to negative values of the electrostatic potential, blue to positive ones.

noticeable weakening of the effect is observed: for the same number of alanine residues, the shifting toward lower electron affinities is roughly halved in comparison with the enhancement produced by an N-helix. Variations of the C-terminal domain is used in proteins of the thioredoxin-like family to reach a modulation of redox potential.[17]

If the previous results provide a reliable measure of α-helix electrostatic contributions, the comparison with a full protein structure is legitimate. We chose thioredoxin h, a protein from the eukaryotic green alga *C. reinhardtii*, as an example. It features a CGPC motif linked to the N-terminal group of the α_2 motif and the C-terminal group of the β_2 motif (C-terminal strand, see Figure 2). The difference between the isolated (capped) tetrapeptide Ace-CGPC-NHMe and the full protein structure comes from the specific environment, the highly characteristic thioredoxin fold. Our QM/MM calculations predict a value of +2.12 eV for the adiabatic electron affinity EA_{ad}, which is significantly higher than the +1.20 eV value obtained for the Ace-CGPC-NHMe tetrapeptide. It is noteworthy that this value (2.12 eV) is very close to the value obtained for the model peptide Ace-A_{12}-CGPC-NHMe (EA_{ad} = 2.23 eV). This agreement could seem rather surprising given the difference between the real full protein and the model peptide. However, a similar result was recently reported by Doig and co-workers[12] The modeled CAAC peptide, also grafted on a 12-residue-long N-terminus helix, was found to possess a redox potential of −220 mV, which is very close to the experimental value for the corresponding protein (−231 mV). This parallel ascertains the value of these designed peptides as model systems.

Conclusion

In the present study, the modulation created by an N- or C-terminal α-helix on disulfide electron affinity of a common disulfide-linked CGPC motif has been investigated. The electrostatic effects are of the utmost importance in proteins and lead to a dramatic modulation of disulfide-linkage electron affinity. A simple partition scheme based on a decomposition of geometrical and electrostatic effects provides a robust and simple way to investigate the propensity of thioredoxins to fix an electron on their unique disulfide bridge. This work sheds new light on the factors which govern the site of attachment of low-energy electrons. The analogy between studies on redox potentials and electron affinities is insightful and may contribute to the general understanding on disulfide reactivity.

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Supporting Information Available: Representation of the thioredoxin h structure, additional details of the QM/MM scheme, frontier orbitals and electrostatic potentials, scheme of the dipole created by an α-helix, and table of geometrical parameters and adiabatic electron affinities. This material is available free of charge via the Internet at http://pubs.acs.org.

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