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The Rate Enhancement for Prolyl Cis-to-Trans Isomerization of Cyclic CPFC Peptide Is Caused by an Increase in the Vibrational Entropy of the Transition State

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The conformational preferences and prolyl cis−trans isomerization of oxidized and reduced Ac-Cys-Pro-Phe-Cys-NH_2 (CPFC peptides) have been carried out using the ab initio HF/6-31+G(d) and hybrid density functional B3LYP/6-311++G(d,p) levels of theory. The most preferred conformations of oxidized and reduced CPFC peptides with the trans prolyl peptide bond have a type-I β-turn for the Pro-Phe sequence in common. In particular, the transition states for both forms are stabilized by the intramolecular hydrogen bonds between the prolyl nitrogen and the N−H group of the Phe3 residue. The rotational barrier $\Delta G_{ct}^{\ddagger}$ to the cis-to-trans isomerization for the oxidized CPFC peptide is calculated to be 19.37 kcal/mol at the B3LYP/6-311++G(d,p)//HF/6-31+G(d) level of theory, which is lower by 0.88 kcal/mol than that of the reduced CPFC peptide. This may indicate that the rate constant $k_{c\rightarrow t}$ of the prolyl cis-to-trans isomerization for the oxidized form is about 4 times larger than that of the reduced form, which is reasonably consistent with the value deduced from NMR experiments. In particular, the increase in vibrational entropy for the transition state of the oxidized form over that of the reduced form contributes to enhance the rate constant for the prolyl cis-to-trans isomerization of the oxidized form.

Introduction

The CXXC sequence is known to be a motif located at active sites of thiol-disulfide oxidoreductases, which include thioredoxins, glutaredoxins, protein disulfide isomerase (PDI), and DsbA.[1] It has been known that oxidoreductases play a role in maintaining the redox status of thiol groups in the cell and in catalyzing or regulating various cellular functions.[1] In particular, pig and poxviral glutaredoxins have the Cys-Pro-Phe-Cys sequence, of which the N-terminal cystine is located in the loop between the first β-strand and the second α-helix, whereas the C-terminal cystine is in the first turn of the second α-helix,[2,3] as found for other oxidoreductases.

Rabenstein and his co-workers studied the cis−trans isomerization of the Cys-Pro peptide bond in oxidized and reduced Ac-CPFC-NH_2 (hereafter, Ac-CPFC-NH_2 will be denoted as the CPFC peptide) using ^{1}H NMR experiments in 90% H_2O/10% D_2O at pH 3.0.[4] They reported that the rate of prolyl cis-to-trans isomerization for the oxidized form is about 10 times faster than that of the corresponding reduced form, which is opposite to the previous experimental findings on other proline-containing peptides with Cys residues.[5] On the basis of NMR solution structures of oxidized and reduced *E. coli* thioredoxin, it has been proposed that functional differences between the two forms are probably related to differences in local conformational flexibility in and near the active site loop.[6] It has been suggested that this might include an increase in the conformational flexibility of the peptide backbone at the active site when it is in the oxidized form.[4a] Recently, they also found that the prolyl cis−trans isomerizations in oxidized Ac-Cys-Pro-Phe-$(Ala)_n$-Cys-NH_2 peptides (n = 1 and 2) are faster than those of their reduced forms.[7]

A few computational studies have been carried out about conformational preferences of the CPFC peptide.[8] From Monte Carlo simulations on oxidized and reduced CPFC peptides,[4a] it has been suggested that the disulfide bond may serve to align the prolyl imide nitrogen with the amide hydrogen of the Phe residue in both the cis and trans conformers, as proposed for the catalysis of prolyl isomerization by the enzyme FKBP,[9] by which the rate enhancement of the oxidized form is caused. However, these previous works did not report any structural or thermodynamic properties of transition states, which would be of consequence in reasonably explaining the above NMR experimental results. Thus, we here studied the conformational preferences and thermodynamic properties of local minima and transition states for oxidized and reduced CPFC peptides using quantum chemical methods in order to figure out the factors that cause the rate enhancement of prolyl cis−trans isomerization in the oxidized form.

Computational Methods

The conformational Gibbs free energies were computed at the B3LYP/6-311++G(d,p)//HF/6-31+G(d) level of theory using the Gaussian 03 package.[10,11] The starting conformations for optimization of oxidized and reduced CPFC peptides at the HF/3-21G(d) level of theory were generated with the build-up procedure using the ECEPP/3 force field.[12] The selected local minima at the HF/3-21G(d) level of theory were reoptimized at the HF/6-31G(d) level of theory, and this was followed by the further optimization at the HF/6-31+G(d) level of theory.

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TABLE 1: Backbone Torsion Angles and Thermodynamic Properties of Preferred Conformations and Transition States for Oxidized and Reduced Ac-CPFC-NH_2 at the B3LYP/6-311++G(d,p)//HF/6-31+G(d) Level of Theory[a]

	Cys1			Pro2			Phe3			Cys4			thermodynamic properties			
conformer[b]	ϕ_1	ψ_1	ω_1	ϕ_2	ψ_2	ω_2	ϕ_3	ψ_3	ω_3	ϕ_4	ψ_4	ω_4	ΔE_e[c]	ΔH[d]	ΔS[e]	ΔG[f]
							Oxidized									
DtBdBC	−118	105	−170	−82	−7	177	−116	12	−179	−87	70	−174	0.00	0.00	0.00	0.00
FcBdCB	−58	139	20	−87	1	177	−87	59	−170	−135	15	175	1.35	1.17	−3.49	2.21
ts_{ox}	−66	145	133	−107	−25	−173	−97	45	178	−87	72	−175	21.80	20.79	−2.66	21.58
							Reduced									
CtAuBB	−106	110	−171	−63	−26	−176	−73	−7	175	−89	−7	177	0.00	0.00	0.00	0.00
FcBdAB	−92	146	3	−91	−6	−166	−66	−28	−177	−106	12	172	1.70	1.59	0.59	1.41
ts_{red}	−107	70	123	−110	−6	−168	−79	−5	173	−86	−10	177	21.62	20.47	−3.97	21.66

[a] Torsion angles are defined in Figure S1 of the Supporting Information; units in degrees. [b] See the Supporting Information for definition. [c] Relative electronic energies in kcal/mol. [d] Relative enthalpy changes in kcal/mol at 25 °C. [e] Relative entropy changes in cal/K·mol at 25 °C. [f] Relative Gibbs free-energy changes in kcal/mol at 25 °C.

The transition states for reduced and oxidized CPFC peptides were located from the lowest-energy conformations CtAuBB and DtBdBC, respectively, at the HF/6-31+G(d) level of theory, as done for Ac-Pro-NHMe.[13] Vibrational frequencies were calculated for all feasible stationary points at the HF/6-31+G(d) level of theory, which were used to estimate relative enthalpies, Gibbs free energies, and entropies at 25 °C and 1.0 atm. Single-point energies were calculated at the B3LYP/6-311++G(d,p) level of theory for all feasible stationary points of oxidized and reduced CPFC peptides located at the HF/6-31+G(d) level of theory. Because of practical problems encountered,[11] solvation free energies were not considered in this work.

Results and Discussion

The torsion angles and thermodynamic properties for the most preferred conformations of oxidized and reduced CPFC peptides with the trans and cis prolyl peptide bonds and their transition states at the B3LYP/6-311++G(d,p)//HF/6-31+G(d) level of theory are listed in Table 1. The most preferred trans conformation DtBdBC of the oxidized CPFC peptide is more stabilized by 2.21 kcal/mol in Gibbs free energy (ΔG) than the most preferred cis conformation FcBdCB. The hydrogen bond between the C═O of the Phe3 residue and the N−H of the C-terminal group, with r(C═O···H) = 2.17 Å, appears to play a role in stabilizing the trans conformation DtBdBC. The cis conformation FcBdCB has two hydrogen bonds between the C═O of the Pro2 residue and the N−H of the Cys4 residue, with r(C═O···H) = 2.14 Å, and between the C═O of the N-terminal group and the N−H of the Phe3 residue, with r(C═O···H) = 2.12 Å. The transition state ts_{ox} for the oxidized CPFC peptide, which has quite similar backbone conformations for Cys1 and Phe3 residues to those of the cis conformation FcBdCB, has two hydrogen bonds between the C═O of the Phe3 residue and the N−H of the C-terminal group, with r(C═O···H) = 2.21 Å, and between the C═O of the Pro2 residue and the N−H of the Cys4 residue, with r(C═O···H) = 2.50 Å.

The most preferred trans conformation CtAuBB of the reduced CPFC peptide was more energetically favorable than the most preferred cis conformation FcBdAB by 1.41 kcal/mol in ΔG, which is 0.80 kcal/mol lower than that of the oxidized CPFC peptide. The trans conformation CtAuBB has two hydrogen bonds between the C═O of the Cys1 residue and the N−H of the Cys4, with r(C═O···H) = 2.25 Å, and between the C═O of the Pro2 residue and the N−H of the C-terminal group, with r(C═O···H) = 2.26 Å. The cis conformation FcBdAB has hydrogen bonds between the C═O of the N-terminal group and the N−H of the Cys4 residue, with r(C═O···H) = 2.16 Å, and between the C═O of the Pro2 residue and the N−H of the C-terminal group, with r(C═O···H) = 2.28 Å. The transition state ts_{red} of the reduced CPFC peptide has a hydrogen bond between the C═O of the Pro2 residue and the N−H of the C-terminal group, with r(C═O···H) = 2.27 Å.

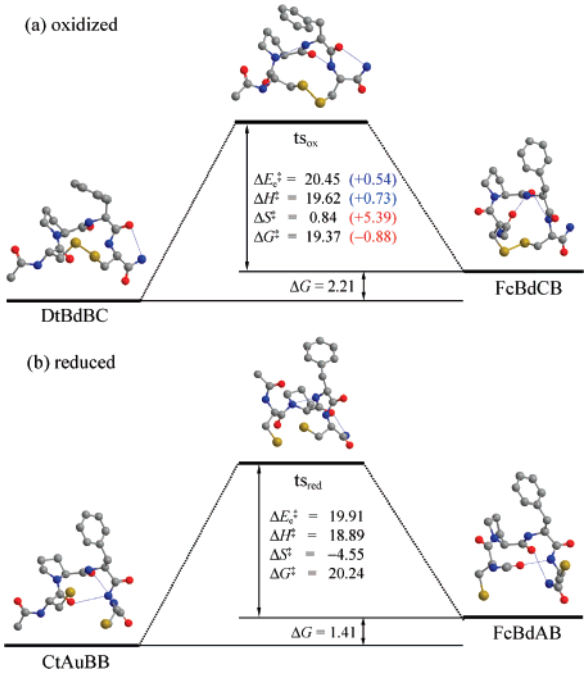

Figure 1. Free-energy profiles for prolyl cis−trans isomerization and preferred trans and cis conformations and transition states for (a) oxidized and (b) reduced CPFC peptides at the B3LYP/6-311++G(d,p)//HF/6-31+G(d) level of theory. The relative changes in energies and entropy of the transition state for the oxidized form to those of the reduced form are shown in parentheses. Energies are in kcal/mol, and entropies are in cal/K·mol.

The statistically weighted cis populations of oxidized and reduced CPFC peptides are computed to be 1.6 and 5.7%, respectively, by relative free energies of all feasible local minima described in the Computational Methods of the Supporting Information, whereas the corresponding values were determined to be 9.3 and 12.0%, respectively, from NMR experiments in 90% H_2O/10% D_2O at pH 3.0.[4a]

The two most preferred trans conformations of oxidized and reduced CPFC peptides have type-I β-turns for the Pro-Phe sequence in common, as found experimentally[8a] and theoretically[8b] for oxidized CPXC peptides, although they have different puckerings. In particular, the transition states ts_{ox} and ts_{red} have another hydrogen bond between the prolyl nitrogen and the N−H group of the Phe3 residue in common, with r(N···H−N)

= 2.28 and 2.29 Å, respectively, which seems to play a role in stabilizing the transition-state structure, as seen for Ac-Pro-NHMe.[13] In water, the backbone of small peptides would interact with nearby water molecules, and the hydrogen-bonded conformations such as a type-I β-turn for the Pro-Phe sequence of the reduced form with the trans prolyl peptide bond could not be favored. However, the solution NMR structures of the active site sequence Cys11-Pro12-Tyr13-Cys14 of *E. coli* glutaredoxin in the reduced[14a] and oxidized[14b] forms have shown that two sequences are located at a loop exposed to water and have a type-I β-turn conformation for the Pro-Phe sequence in common, although the active site is somewhat better defined in the oxidized form. In addition, it has been reported that the small peptides with the Pro-X sequence can form β-turn structures in nonpolar (chloroform) and polar (DMSO and water) solvents, although the populations of β-turn structures are somewhat decreased as the solvent polarity increases.[15]

X-ray diffraction structures of CPFC sequences in the active sites of pig glutaredoxin in the oxidized form and poxviral glutaredoxin in the oxidized and reduced forms have the conformations DtAuAA (pdb id = 1kte),[2] DtAuAA (pdb id = 2hzf),[3] and FtAuAA (pdb id = 2hze),[3] respectively. After optimization at the HF/6-31+G(d) level of theory, the first two X-ray structures in the oxidized form were converged to the same local minimum DtAuBB, with the relative electronic energy of 1.12 kcal/mol at the B3LYP/6-311++G(d,p)//HF/6-31+G(d) level of theory. The third X-ray structure in the reduced form was converged to the most preferred conformation CtAuBB.

The free-energy profiles for prolyl cis−trans isomerization of the most preferred trans and cis conformations and the transition states for reduced and oxidized CPFC peptides are shown in Figure 1. The rotational barriers $\Delta G_{tc}^{\ddagger}$ and $\Delta G_{ct}^{\ddagger}$ to the trans-to-cis and cis-to-trans isomerization for the reduced CPFC peptide are estimated to be 21.66 and 20.24 kcal/mol at the B3LYP/6-311++G(d,p)//HF/6-31+G(d) level of theory, respectively. The corresponding values of the oxidized CPFC peptide are 21.58 and 19.37 kcal/mol, which are lower by 0.08 and 0.88 kcal/mol, respectively, than those of the reduced CPFC peptide. This may indicate that the rate constant $k_{c\rightarrow t}$ of the cis-to-trans isomerization is about 4 times larger for the oxidized form than that for the reduced form. From NMR experiments in 90% H_2O/10% D_2O at pH 3.0, the rotational barriers $\Delta G_{ct}^{\ddagger}$ have been determined to be 19.5 and 18.0 kcal/mol for reduced and oxidized CPFC peptides, respectively, which may imply that the $k_{c\rightarrow t}$ of the oxidized form is about 10 times larger than that of the reduced form.[4] In particular, the relative entropy $\Delta S^{\ddagger}$ of the transition state ts_{ox} to the cis conformation for the oxidized form is increased by 0.84 cal/K·mol, but that of ts_{red} for the reduced CPFC peptide is decreased by 4.55 cal/K·mol (Figure 1). The relative increase of 5.39 cal/K·mol in $\Delta S^{\ddagger}$ for the transition state ts_{ox} is mainly caused by vibrational contributions (Table 1 and Table S4 of the Supporting Information).

In conclusion, the rate enhancement for the cis-to-trans isomerization of the Cys-Pro peptide bond of the CPFC peptide in the oxidized form can be ascribed to the increase of the conformational entropy (i.e., flexibility) of the transition state for the oxidized peptide, although two transition states of oxidized and reduced forms have a hydrogen bond between the prolyl nitrogen and the N−H group of the Phe3 residue in common.

Supporting Information Available: Complete ref 10, details of computational methods, torsion angles for backbone and side chains, entropies, and optimized Cartesian coordinates of preferred conformations. This material is available free of charge via the Internet at http://pubs.acs.org.

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