



***In silico* Study of Solvent Effects on the Intramolecular Hydrogen Bond of Hydroxy Proline**

JOSÉ COTUÁ^{1*} and SANDRA COTES²

¹Grupo de Investigación Max Planck, Facultad de Química y Farmacia, Universidad del Atlántico, Km 7 Antigua Vía Puerto Colombia, Barranquilla, Colombia.

²Grupo de Investigación en Química y Biología, Departamento de Química y Biología, Universidad del Norte, Km 5 Antigua Vía Puerto Colombia, Barranquilla, Colombia.

*Corresponding author E-mail: jcotuaval@gmail.com

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ABSTRACT

The hydrogen bond strength and stabilization energy of hydroxyproline–water complexes were investigated by performing density-functional theory calculations. In particular, the hydrogen bond formation between carbonyl groups serving as proton acceptors and amino groups as proton donors in the hydroxyproline–water was examined. Hydroxyproline–water exhibit higher energy of their hydrogen bond when the carbonyl groups of their hydroxyproline moieties acts as a proton acceptor. Furthermore, the infrared spectra of isolated water and hydroxyproline molecules were compared with those of the hydroxyproline–water complexes, and the observed frequency shifts were discussed.

Keywords: Hydroxyproline-water, Stabilization energy, Hydrogen bonding, Collagen.

INTRODUCTION

Collagen consists of helix-shaped chains, and its XiYiGly sequence (where XiYi denotes an amino acid and Gly is glycine) is repeated after every three residues. The final collagen structure has been refined and studied in detail by Rich and Crick¹, who suggested that hydrogen bonds are formed between the N–O and O–C groups of different halogen chains with a degree of rotational symmetry equal to 28.6°.

The nature of interactions between macromolecules and water is a topic of many

research studies. It is well known that water cannot be considered a passive solvent. Moreover, the coupling between water and biomolecules plays an important role in various biochemical processes such as binding proteins to ligands,² protein–protein interactions, and conformational changes of proteins. Hence, extensive computational studies have been performed to examine the physicochemical properties of the interactions between amino acid and the surrounding water molecules as well as the importance of polarization using the laws of quantum mechanics. However, the degree of involvement of water species in the solvation of hydrophobic molecules remains



unknown; furthermore, solvation effects are mainly associated with the electronic responses of polar amino acids, while the polarization and charge transfer effects of hydrocarbon chains are still poorly understood.

Proline and hydroxyproline are integral constituents of collagen, which form the main structural proteins in various fibrous tissues of biological systems.³

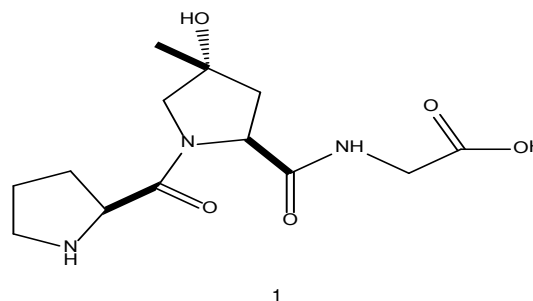
In a previous work, the interactions of proline with water molecules were investigated, and relatively strong effects of proline and hydroxyproline ring sizes on the stability of triple collagen helices were demonstrated⁴. On comparing hydroxyproline to other amino acids, it was found that the embedded pyrrolidine ring was an important part of the collagen structure. Hydroxyproline and proline are exceptional among the amino acids because their N-terminals contain secondary amines with relatively high basicity,^{5,6} thus endowing this special characteristic makes the chemistry of hydroxyproline with unique chemical properties quite interesting. Furthermore, hydroxyproline is expected to be more polar than proline because of the presence of an additional OH hydrophilic group in its structure.

Computational studies of molecular complexes have been extensively performed by various methods. They included calculations of potential energy surfaces, thermodynamic properties, and parameters of hydrogen bonds. Unlike bulk materials, molecular complexes are dominated by surface atoms, and both their structures and properties significantly differ from the bulk ones. Although some parameters of amino acids in the solution phase were examined previously, a large number of their important physicochemical characteristics depended on the nature of solute-solvent molecular interactions.

Proline-hydroxyproline-glycine (labeled ProHypGly) is the most common triplet present in the collagen structure.⁷ The most important part of collagen is its framework, which determines the mechanical strength and thermal stability of the protein.⁸ When hydroxyproline is located at the Yi position of the triple helix (but not at the Xi position), the collagen stability increases.^{9,10} Moreover, the electronegative substituent at the 4-position of the proline ring strongly affects the formation of stable

triple helices because of a stereoelectronic effect¹¹ rather than a simple inductive effect.

It is noteworthy that hydroxyproline contains secondary amino groups and forms tertiary amides from protein's peptides. Tertiary amides have considerable populations of both its trans and cis isomers, while all peptide bonds in the collagen structure have trans configurations. The substituent located at the 4-position of proline strongly contributes to the equilibrium constant of the peptide isomerization process. This phenomenon is caused by the stereoelectronic effect¹² involving $n-\pi^*$ interactions between various oxygen atoms of the peptide bond, in which electrons are transferred from the non-bonding pair to the anti-bonding orbital of the carbonyl group in the peptide sequence ProHypGly 1.



Such interactions can only occur if the oxygen-containing peptide bond has a trans configuration. It was reported previously that their contributions stabilized the trans conformation by $\Delta G = -0.71$ kcal/mol.¹¹ Moreover, it has been demonstrated that proline residue located at the Xi position with a $C\gamma$ -endo pucker was able to stabilize the triple helix, whereas the one with a $C\gamma$ -exo pucker destabilized it.¹³

In this study, hydrogen bonding interactions between hydroxyproline and water were investigated theoretically by conducting quantum chemical calculations. In particular, the physicochemical properties of the (2*S*,4*R*)-4-hydroxyproline-water were studied here.

This article describes the predicted physicochemical properties *In silico*. For this purpose, four different conformations of (2*S*, 4*R*)-4-hydroxyproline, in which hydroxyproline served either as a proton donor or proton acceptor, were considered (Fig. 1). All calculations were performed

using the Gaussian 98W and GaussView software packages. The most stable structures with the lowest energies were identified by verifying that all their vibrational frequencies were real. The B3LYP/6-311G density-functional theory method was employed in most computations.

RESULTS AND DISCUSSION

The conformer structures with the lowest energy are presented in Table 1 and Fig. 1. They showed that the energy difference between conformers Hp1 and Hp2 is approximately 0.55 kcal/mol, while the energy differences between them, and the other two conformers vary between 0.74 and 0.88 kcal/mol.

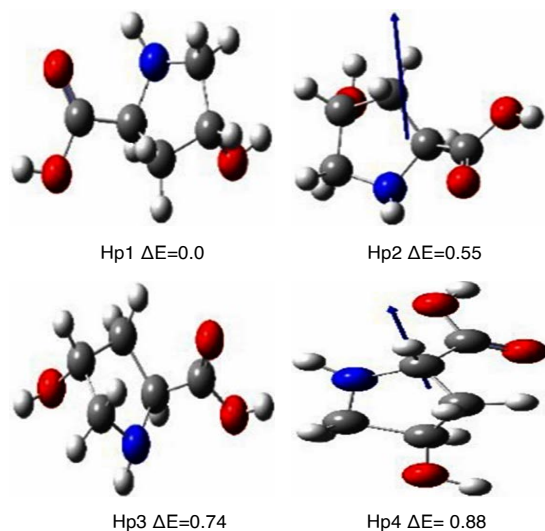


Fig. 1. Calculated equilibrium geometries

Table 1: Calculated electronic energies E , zero-point energies ZPE, relative energies ΔE , and dipole moment μ for four lowest-energy conformers of hydroxyproline

Conformer	E (Hartree)	ΔE (kcal/mol)	ZPE (kcal/mol)	μ (Debye)
Hp-1	-476.0495	0	92.93	1.8
Hp-2	-476.0486	0.55	93.02	3.41
Hp-3	-476.0484	0.74	93.29	1.97
Hp-4	-476.0481	0.88	93.08	1.08

Table 1 also lists the dipole moments of the hydroxyproline conformers, which strongly influence the formation of hydroxyproline–water conformers. Conformer Hp2 exhibits the highest dipole moment of 3.41 D, while Hp4 possesses the lowest dipole

moment because its vector is oriented opposite to the hydroxyl bond of the hydroxyproline group. In contrast, the dipole moment of the Hp2 conformer is oriented in the parallel direction.

The equilibrium structure and thermodynamic properties of the hydroxyproline–water complexes with the lowest energy, in which the hydroxyproline moiety served as a proton acceptor, were also calculated by the B3LYP/6-311++G method.

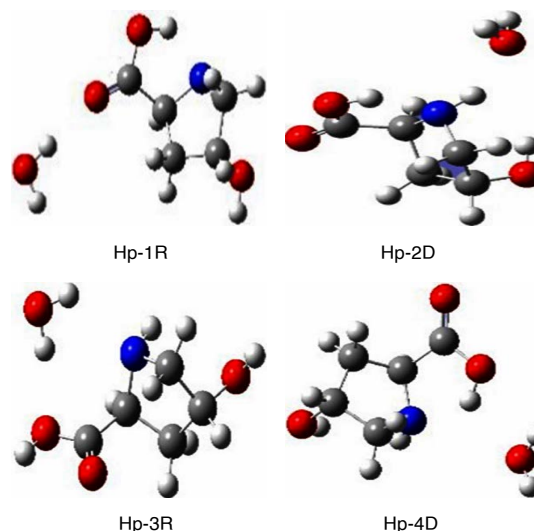


Fig. 2. Structure of hydroxyproline-water complexes

Table 2: Calculated electronic E , relative energy ΔE , zero-point energies ZPE and dipole moment of low hydroxyproline- H_2O complexes

Cluster	E (Hartree)	ΔE (Kcal/mol)	ZPE (kcal/mol)	μ (Debye)
Hp1-R	-552.9676	0.00	10.16	7.62
Hp2-D	-552.9618	3.65	11.10	5.14
Hp3-R	-552.9589	5.43	11.28	3.59
Hp4-D	-552.9574	6.39	10.71	4.22

Figure 2 shows the structures of the four hydroxyproline-water conformers studied in this work. In two of them, hydroxyproline (R) serves as a proton acceptor, and in the other two (D) as a proton donor. According to Table 2, the dipole moment of the Hp1-R is 7.62 D which exceeds those of the other structure. In this, the hydrogen bond formed with water allows the oxygen atom of the hydroxyproline carbonyl group to act as a proton acceptor. Although the hydrogen atom attached to the oxygen atom of the hydroxyproline OH group in the Hp4-D functions as a proton donor, the

resonance effect of the carbonyl group considerably decreases its dipole moment. In addition, atomic charges in the studied systems were computed to investigate the related charge transfer processes. The electrostatic interaction energy of the hydrogen bond formed between water and the carbonyl group is 25.88 kcal/mol, indicating relatively high bond strength. Meanwhile, a much lower hydrogen bonding energy of 19.53 kcal/mol is obtained for the hydroxyproline–water Hp2-D with the nitrogen atom serving as a proton donor.

It is noteworthy that the Hp1-R has the lowest energy of -552.96759 *au.*, while the Hp4-D has the energy of -552.95740 *au.*, suggesting the absence of a correlation between dipole moment and stability.

Table 3 lists the frequencies of the stretching modes of the carbonyl groups (C=O), carboxyl groups (–COOH), and amino groups (–NH) of hydroxyproline and the hydroxyproline–water as well as their corresponding frequency differences. The Hp1-R exhibits a red shift of approximately 548.96 cm^{-1} . A smaller shift of 161.34 cm^{-1} related to the –NH group is observed for the Hp2-D. Although the Hp4-D produces a red shift of 391.11 cm^{-1} , it is not noticeable because of the resonance effect of the carbonyl group. Therefore, it can be concluded that carbonyl groups are the most active moieties of these conformers, which can explain the observed difference in their chemical properties.

Table 3: Calculated stretching frequencies (cm^{-1}) of low energies conformers of hydroxyproline–water complexes

Complexes	Carbonyl C=O	-NH	$\Delta\nu$
HP-1	1737.36	3599.61	
HP-2	1740.47	3541.95	
HP-3	1745.33	3561.37	
HP-4	1748.48	3644.82	
Hp-1R	1188.40		548.96
Hp2-D	1324.79	3430.11	161.34
Hp4-D	1357.37		391.11

The investigation of the interaction potentials of small molecular systems is very important for predicting their properties. In theory, the interaction potential of two different molecules located at distance R from each other can be computed exactly.¹⁴ However, the quantitative predictions of

the interaction potentials of H₂ molecules are not accurate to adequately determine the properties of the resulting hydrogen bonds. Meanwhile, the stabilization energy SE of a molecular cluster¹⁴ can be computed via the following equation:

$$SE = E_{cluster} - \sum_{i=1}^n E_i$$

Where E cluster is the total energy of the complex, E_i is the energy of the molecule monomer, and n is the number of monomers. The stabilization energy of the HP1-R is 3.07 kcal/mol lower than that of the Hp2-D, which is in good agreement with the calculated energy of their hydrogen bonds (as was found earlier in this work, the bonding energy of Hp1-R exceeded the energy value obtained for Hp2-D).

CONCLUSION

Hydroxyproline–water complexes exhibit higher energy of their hydrogen bonds and lower stabilization energy when the carbonyl groups of their respective hydroxyproline moieties act as proton acceptors. As the presence of water molecules strongly affects the collagen conformation, hydroxyproline species play a key role in its stabilization through the formation of hydrogen bonds. The red shift observed for the calculated infrared peak of the carbonyl group of the molecule in this work strongly supports the importance of hydrogen bonding in the stabilization of Hydroxyproline–water clusters. Even though there are reports of proline–water interactions, the importance of this work lies in the prediction of the interactions of hydroxyproline with water that support the structure of collagen for the first time.

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Conflict of interest

The author declares no conflict of interest.

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