A Semi-empirical Quantum Mechanical Modeling Study on the Interaction of Collagen-like Peptides with Polyphenolic Molecules: An Attempt to Gain Insights into Vegetable Tanning

by

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Abstract

A semi-empirical quantum mechanical PM3 hamiltonian based molecular modeling has been used to study the interactions of collagen-like peptide with polyphenolics. A collagen-like peptide 9-mer sequence has been built and interacted with the polyphenolic molecules. Most of the complexes of the peptide sequence and polyphenolic molecule exhibited hydrogen bonding. The binding energies of the complexes were in the range of 6.5 - 20 kcal/mol. Complexes of gallic acid exhibited the least binding energies. Epicatechin complexes exhibited higher binding energies with the collagen-like peptide sequence.

Introduction

Studies on collagen - polyphenols interactions are essential to understand structure, properties and stability of collagen upon interaction with vegetable tannins. The stabilization of collagen-ligand interaction has attracted wide attention due to the stability of collagen-ligand complex against the collagen-degrading enzyme, collagenase.[1,2] Collagen is a structural protein, which provides mechanical strength and structural integrity to the connective tissue in the body.[3] Nineteen different types of collagen have been identified.[4] The structure, properties and function of various types of collagen have already been attributed to the triple helical nature of collagen.[5] Collagenase is an enzyme, which is known to cleave collagen thereby altering its stability.[6] Structure, function, mechanical stability and thermal stability of collagen are influenced by the attack of collagenase on collagen. Various molecules provide stability to collagen matrix against the degrading enzyme collagenase through the process called tanning. And those molecules, which bring stability to the collagen matrix, can interact with collagen through covalent, hydrogen bonding, electrostatic and non-bonded interactions. Various Cr(III) complexes have been shown to provide thermal and mechanical stability to collagen[7,8] through co-ordinate covalent interaction.[9] Natural vegetable tannins are also known to provide stability to collagen through the process of vegetable tanning, one of the oldest tanning method employed by mankind.

Tannins are secondary plant metabolites, which have the ability to precipitate protein.[10] Commercial use of tannins, especially in the leather industry, is based on their interaction with the protein - collagen.[11] Therapeutic uses of these tannins have also been investigated.[12] Tannin extract is a complex mixture of polyphenols. Vegetable tannins are classified as hydrolysable and condensed tannins as suggested by Freudenberg.[13] Classification of hydrolysable tannin is usually made on the basis that phenolic acid is liberated on hydrolysis.[14] Those yielding gallic acid are referred as gallotannins and those yielding ellagic acid as ellagitannins. Gallotannins are well known for their involvement in the stabilization of skins.[15] Gallic acid and their derivatives have been found to present in the chromatograms of Chinese gallotannins.[16] Condensed tannins of plants are phenolic polymers comprising catechin and its stereoisomer epicatechin linked by carbon-carbon bonds between C-4 of flavan-3-ol monomer and C-6 or C-8 of the adjacent monomer.[17] Catechin derivatives are also found in tea. Green tea polyphenols are shown to posses anticarcinogenic effects.[18,19] It has been proposed that polyphenolic tannin molecules interact with collagen through H-bonded and non-bonded interactions, the quantification of such interaction is necessary to gain further insight into the stabilization of the collagen matrix. Earlier Hatano et.al. have demonstrated the interaction of catechin derivatives with peptides and small proteins using NMR techniques.[20] In this investigation, molecular modeling and simulation methods have been used to understand the stabilization of collagen-like peptides by various polyphenols. The main objective of this study is to investigate the mechanism and energetics involved in the stabilization of model collagen-like peptides by polyphenolic compounds using the semi-empirical quantum mechanical PM3 method. This study helps to under-

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stand the nature of interaction involved in the stabilization of binary complex, collagen-polyphenolic compound and also provides insight to study the forces involved in the tanning process using tannin molecules. It is possible to predict the effects of vegetable tannins on collagen function by using computational approaches and by identifying binding sites on model collagen peptides. Semi-empirical calculations have been performed to analyse the interaction of various polyphenols with collagen like peptide sequence. It is possible from these modeling studies to understand the effects of hydrogen bonding and non-covalent interactions, which leads to the stability of collagen.

EXPERIMENTAL

Computation details

Semi empirical methods are the only alternative to molecular mechanics for most molecules of biological interest, especially for the study of hydrogen bonding systems. A 9-mer sequence representing collagen-like peptide sequences containing residues Ace-Gly-Pro-Hyp-Gly-Ala-Ser-Gly-Glu-Arg-Nm has been chosen for the study. Using standard model building tools in the biopolymer package, collagen-like peptides 9-mer has been generated. The dihedral angles of the backbone of the model peptide sequence have been chosen to represent dihedral angles corresponding to collagen-like peptide region in Ramachandran (ϕ,ψ) plot.[21] The polyphenolic molecules have also been built using the geometry building tools in the Cerius2 package (Molecular Simulations Incorporation[MSI], USA). The geometry of the polyphenolic molecules and the peptide sequence has been minimized using steepest decent method and followed by conjugate gradient method employing INSIGHT II package (MSI, USA). Interaction of the polyphenolic compounds has been studied at different sites of the peptide sequence. Molecular mechanics calculations have been made using Consistent Valence Force Field (CVFF). Dielectric constant of 80.0 has been used to mimic the interactions in the water medium.[22,23] The geometry of peptide-polyphenolic complex has been minimized using both steepest decent and conjugate gradient algorithms. The geometry and energy of the peptides and the peptide-ligand complex have further been minimized using PM3 Hamiltonian using Gaussian 98W suite of program.[24] The semi-empirical quantum chemical methods have been used for biological systems[25,26] and are known to provide realistic results.

The binding energy (E_{bind}) of the model complexes can be calculated using the following equations.

$$E_{int} = E_{complex} - (E_{peptide} + E_{ligand})$$

$$E_{bind} = - E_{int}$$

Where E_{int} is the interaction energy, $E_{complex}$ is total energy of the complex and $E_{peptide}$ and E_{ligand} are the total energies of the peptide and ligand (polyphenolics) respectively.

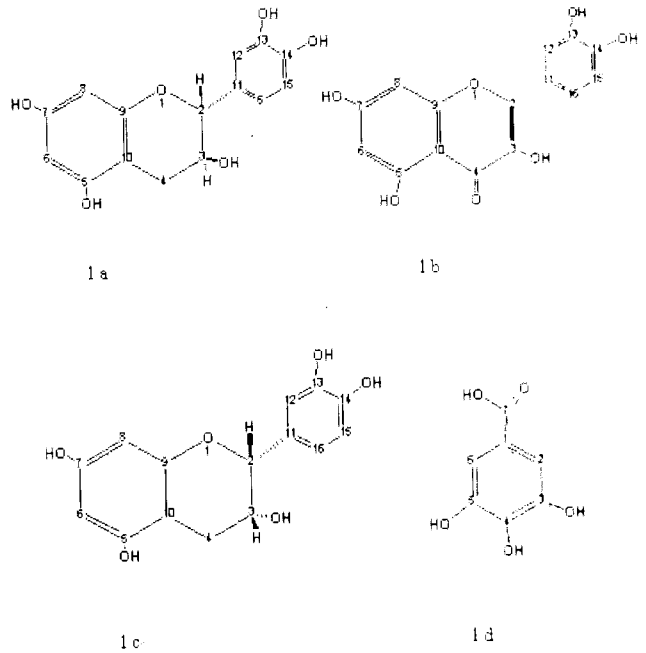
In order to validate the PM3 semi-empirical calculations, binding energy for simple systems, gallic acid-water and gallic acid-formaldehyde has been calculated using PM3,[27] AM1[28] (semi-empirical) and Hartree-Fock - HF[29] (*ab initio*) method of basis sets 6-31G*.

RESULTS AND DISCUSSION

AM1 (Austin Model 1) and PM3 (Parameter Model 3) is the two major computer program used for obtaining semiempirical solutions to the electronic Schrodinger wave equation. PM3 hamiltonian has been used for the present study and validation of PM3 is carried out based on the binding energies of simple complexes of gallic acid-water and gallic acid-formaldehyde. The results on the binding energy of the gallic acid-water and gallic acid-formaldehyde complexes calculated using PM3, AM1 and HF/6-31G* are depicted in Table I. The value of binding energies exhibited by PM3 appeared to be less compared to HF/6-31G* but the trend has been observed to be similar. In the case of binding energy calculations using AM1 method, two complexes exhibit-

TABLE I
Comparison of Binding Energies Obtained Using PM3, AM1 and *ab initio* Hortee Fock (HF) Methods

Complex		Binding Energies (Kcal/mole)		
		AM1	PM3	HF/6-31G*
Gallic acid : Water	$COOH:OH_2$	0.729	3.42	7.4
	m-$OH:OH_2$	- 4.97	1.93	3.67
	p-$OH:OH_2$	2.99	1.56	2.16
Gallic acid : Formaldehyde	$COOH:OCH_2$	- 0.578	3.89	6.78
	m-$OH:OCH_2$	1.26	2.28	1.18
	p-$OH:OCH_2$	0.998	4.77	5.62



1a 1b

1c 1d

Figure 1. - Structure of the polyphenolic molecules a) Catechin, b) Epicatechin, c) Quercetin, and d) Gallic acid

ed negative binding, and trend is also deviating when compared to the other two methods. PM3 has predicted better binding energies than AM1 for the hydrogen bonded complexes of gallic acid with water and formaldehyde. Hence PM3 method will be plausibly an appropriate choice of semi-empirical calculations for predicting the energies of complexes involving collagen-like peptide sequence with polyphenols.

The polyphenolic molecules that have been chosen for the study viz. catechin, epicatechin, quercetin and gallic acid are shown in Figure 1. These molecules are known to be present in the vegetable tannin extracts, which stabilizes the three-dimensional collagen matrix. These molecules are expected to interact electrostatically with protein, forming hydrogen bonding with relevant donor/acceptor groups in the collagen.[30-33] The model peptide selected in this study has some potential binding sites for the interaction of these polyphenolic molecules with collagen. Catechin, epicatechin and quercetin belong to the category of condensed tannins. The compound gallic acid is found in hydrolyzable tannins like chinese gallotannins, sumac, etc. The interaction of the four polyphenolic molecule with the collagen-like peptide sequence has been studied by minimizing the complex and their energetics were calculated. Interaction of each molecule has been investigated at three positions in the sequence. Based on the energies of the energy-minimized complex and individual energy of the compounds and the sequence, binding energy of the complexes is calculated. Several conformations for each complex have been scanned and the conformation, which has exhibited better interaction energy using CVFF, has been taken for geometry optimization and energy minimization calculation using PM3.

The binding energies of three complexes of each polyphenolic compound are shown in Table II. Complex 1 of epicatechin exhibited the maximum binding energy of 20.4 kcal/mole when it has been positioned near the serine and glutamic acid residue of the peptide sequence. This complex has exhibited multiple hydrogen bonding between epicate-

TABLE II

Binding Energies of Collagen-like Peptide Sequence and the Polyphenolic Compounds

Polyphenolic Compounds	Binding Energy (kcal/mol) Complex 1[a]	Complex 2[b]	Complex 3[c]
Catechin	15.79	16.34	14.24
Epicatechin	20.40	13.98	19.98
Quercetin	14.78	11.02	13.60
Gallic Acid	7.91	10.15	6.45

[a]- Polyphenolic molecule interacted with the serine and glutamic acid residue of the collagen-like peptide sequence.
[b]- Polyphenolic molecule interacted with the arginine residue of the model collagen-like peptide sequence.
[c] - Polyphenolic molecule interacted with the hydroxyproline residue of the model collagen-like peptide sequence.

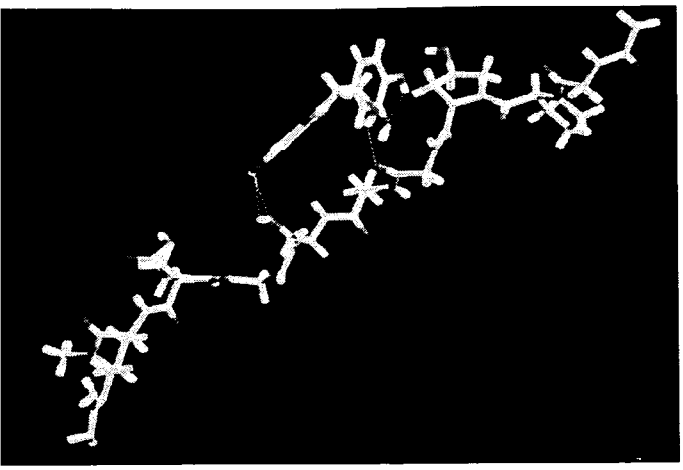


Figure 2. - Complex of Epicatechin and collagen-like peptide sequence.

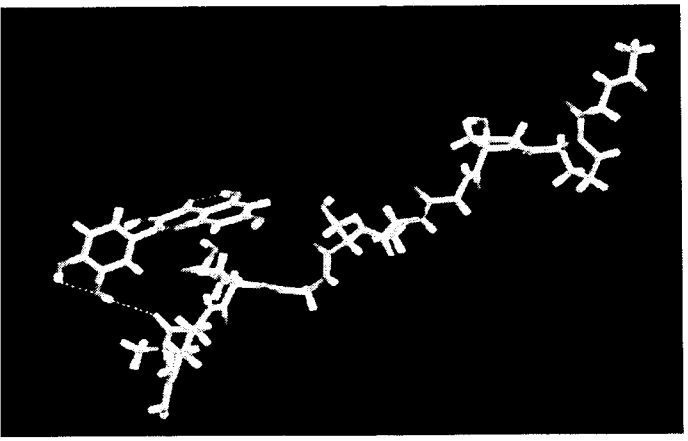


Figure 4. - Complex of Quercetin and collagen-like peptide sequence.

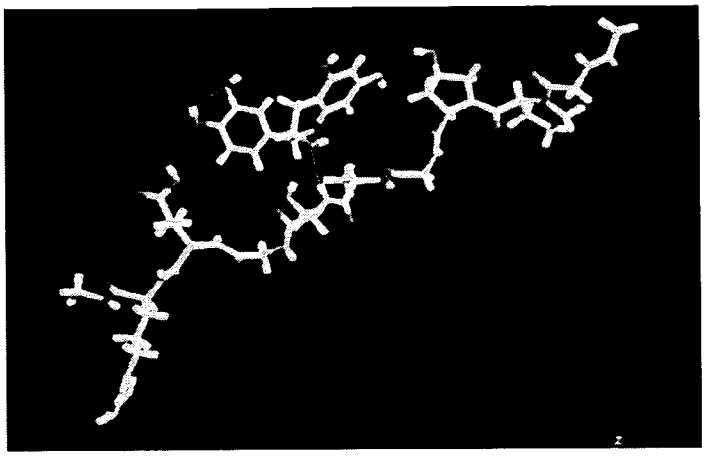


Figure 3. - Complex of Catechin and collagen-like peptide sequence.

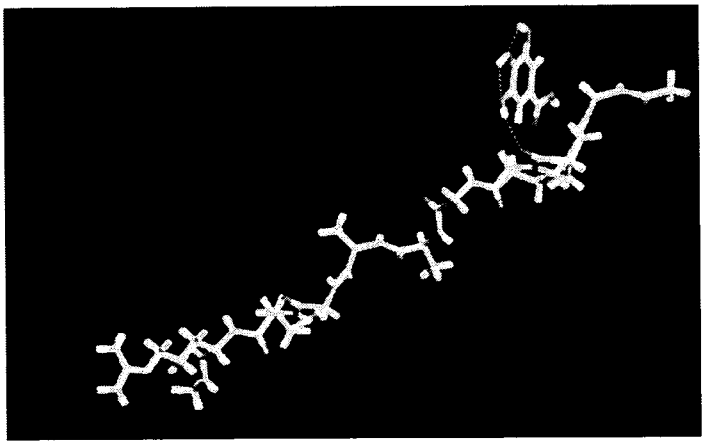


Figure 5. - Complex of Gallic acid and collagen-like peptide sequence.

chin and the peptide sequence, which is shown in Figure 2. Catechin, an isomer of epicatechin had exhibited lesser binding energies when compared to the complex of epicatechin. Complex 1 of catechin, which has been interacted with serine, is shown in Figure 3. In this complex, the oxygen of the hydroxyl group acts as a hydrogen bond acceptor with the hydrogen atom of the amide N-H of the serine residue. The complex exhibited a binding energy of 15.79 Kcal/mol. All the polyphenolic molecules exhibited better binding when interacted with serine and glutamic acid residue of the collagen-like peptide sequence. Gallic acid exhibited lesser binding energies compared to other polyphenolic molecules. This possibly could be because of the lesser number of hydroxyl groups present in it and also because of smaller size of gallic acid than other polyphenols, which can span to a larger portion of the peptide. Majority of the complexes were found to exhibit hydrogen bonds. The respective hydrogen-bonded distance and angle for the complexes that have formed hydrogen bond are mentioned in Table III. Carbonyl group of the backbone amide bond is found to be a hydrogen bond acceptor with the polyphenolic molecules in four of the hydrogen bonded complexes between the polyphenolics and the collagen-like peptide. Only the side chain group (hydroxyl) of serine amino acid residue was involved in the hydrogen bonding with the polyphenolics, where the hydroxyl group acts both as a hydrogen bond donor as well as acceptor.

Though arginine residue present in the model sequence is expected to act as a hydrogen donor or acceptor, our calculations did not yield any hydrogen-bonded complexes between the arginine moiety in the peptide and polyphnolic molecules. This may be due to the fact that the complexes that have been modeled may not have all their geometrical parameters favorable for hydrogen bonding. Docking of Catechin near the arginine residue has exhibited better interaction energy of 16.34 kcal/mol compared to other polyphenolics at the same region of the collagen-like peptide. The calculated hydrogen bond distance for this binary complex is 2.01 Å and the angle between O....HO is 158°.

Complexes 2 and 3 of quercetin and peptide sequence have exhibited hydrogen bonding. Complex 2 of quercetin has exhibited a co-operative hydrogen bonding with the OH(3') and OH(4') groups of the quercetin molecule and between the OH(4') of quercetin and amide C=O of the arginine

TABLE III
Complexes that are Hydrogen Bonded, Hydrogen Bonding Distance and Angle

Polyphenolic Compounds	Sequence	H - bond (Sequence .... Compound)	H-bonded Distance (Å)	H-bonded Angle
	Complex 1	Ser-NH...O of OH at 3	2.499	160
Catechin	Complex 2	8th Gly C=O...H of OH at 5'	2.01	158
	Complex 3	No H - bond		
		Ser OH...O of OH at 7	2.89	116.1
	Complex 1	Ser HO...H of OH at 7	2.85	118.3
		5th Gly.C=O...H of OH at 3	2.32	138.3
Epicatechin	Complex 2	No H-bond		
	Complex 3	Ala N-H...O of OH at 7	2.71	150
	Complex 1	No H-bond		
Quercetin	Complex 2	Arg C=O...H of OH at 4'	2.48	135
	Complex 3	Hyp C=O...H of OH at 3	2.47	140
	Complex 1	Ser-O...H of OH at 5	2.45	151
Gallic Acid	Complex 2	No H-bond		
	Complex 3	Pro C=O...H of OH at 3	2.48	152.22

residue which is shown in Figure 4. Similar co-operative hydrogen bonding was also observed in the complex 3 of gallic acid, which is shown in Figure 5. Gallic acid also had exhibited hydrogen bond with the serine residue of the collagen-like peptide sequence. It exhibited a binding of 7.91 kcal/mol. We have earlier reported that gallic acid forms hydrogen bonding with the collagen-like peptide sequence using CVFF force field and molecular dynamics of one of the collagen-like peptide gallic acid H-bonded complex has confirmed its existence.[34]

All the complexes exhibited positive binding of polyphenolic molecules with collagen-like peptide sequence and their binding energies were in the range of 6.5 - 20 kcal/mole. It is necessary to point out here that due to complexity of the molecules (size) chosen in this study, it is difficult to perform calculations beyond the semi-empirical level. In addition to this, in strongly hydrogen bonded complexes, the contribution from the intermolecular dispersion (electron correlation) towards binding energy is less significant. On the other hand in the case of weakly bonded systems, in addition to the electrostatic contributions, dispersion energy would also play a decisive role in the calculation of binding energy. Since electrostatic interaction is known to stabilize the collagen-polyphenol complex, it is sufficient to use semi-empirical calculation to derive a perspective view on the interactions. It is a well-known fact that all these interaction takes place in solvent environment. Presence of solvent environment will definitely influence the strength of hydrogen bond energy when compared to gas phase calculations because of the polarization of charges in the presence of bulk solvent. Computationally it is an unfeasible task to carry out solvation for a huge system using semi-empirical calculations. However, the same system using a molecular mechanics (force field) based calculations can be done using explicit solvent medium.

CONCLUSIONS

In summary, through the molecular modeling calculations of various polyphenolic compound with model collagen-like peptide sequence, it is possible to understand the mechanism of interactions and relative roles played by various amino acid residues present in the collagen. The calculated interaction energy for the binary complex rages from 6.5 - 20.0 kcal/mol. There is also strong evidence that hydrogen bonded, electrostatic and non-bonded interactions are involved in the stabilization of collagen by polyphenolic compounds. The interaction energy of the polyphenolic compounds with the collagen follow the order that E(collagen-gallic acid) < E(collagen-quercetin) < E(collagen-catechin) ≅ E(collagen-epicatechin). Though many indirect experimental evidences for the role of hydrogen bonding and electrostatic interaction involved in the stabilization of collagen by polyphenolic compounds, such interactions are qualitatively described in this study by appropriate choice of model peptide and poly phenolic compounds.

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