A MMFF94 study of the structure and interactions of ocular solutions containing hyaluronic acid with collagen models

Obinna Chiedozie Ikeora
University of East London
Simi Sabu Joseph
University of East London
Constantinos D. Zeinalipour-Yazdi (c.zeinalipour-yazti@mdx.ac.uk)
University of East London https://orcid.org/0000-0002-8388-1549

Research Article

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Abstract

Molecular modelling techniques have been widely used in drug discovery fields for rational drug design and compound screening. They have however not been used extensively to understand the mechanism of action of drugs within the human body. In this computational study we have used molecular mechanics modelling to describe a complicated biochemical system that exists in the human eye and to describe the mechanism of action of hyaluronic acid in hydrating the collagen from which the cornea of the eyes are made of. Our studies include atomistic level details about what kind of interactions water has with hyaluronic acid and collagen and in systems containing both hyaluronic acid and collagen models. We observe that water has a greater binding energy to collagen than to hyaluronic acid and that hyaluronic acid binds to collagen via H-bonding and maintains its ability to become hydrated with water based on energetic considerations of the binding energy of water with these biomolecules. The study demonstrates the mechanism of action of hyaluronic acid hydration in the human eye which can be used as model system to study the hydrating effect of other ocular solutions based on the quantitative determination of the binding energy of water with these biological molecules.

1 Introduction

Chronic dry eye disease (DED), which primarily affects adults. It results in discomfort, irritability, inflammation, and, in rare instances, significant problems that lower quality of life and vision. Insufficient hyaluronic acid has been linked to a number of eye-related disorders, including dry eye disease. Nowadays, hyaluronic acid eye drops are frequently used to treat DED due to its advantages over eye drops made of polyethylene glycol and hypromellose. Numerous treatments have been tried to treat DED, but prior research has indicated that the most popular form of therapy is the topical administration of pharmaceuticals over an extended period of time. Dry eye disease (DED) is caused by either excessive tear evaporation or insufficient tear production. According to the International Dry Eye Workshop Study Group's estimations, 5–30% of adults over 50 are thought to have DED (The Epidemiology of Dry Eye Disease: Report of the Epidemiology Subcommittee of the International Dry Eye Workshop (2007)). Johnson and colleagues estimated that between 10–20% people have dry eye disease worldwide.[1] Tear evaporation is increased in DED due to less frequent blinking, although tear secretion is decreased due to lacrimal dysfunction.

If DED is not treated, severe symptoms such as dry eye surface, pain, visual impairment, aching and inflammation of the ocular surface, and damage to the cornea and conjunctiva may manifest.[2] Demulcents, a class of polymers used to treat DED, come in many different varieties. These demulcents include polyacrylic acid, carboxymethylcellulose (CMC), dextran, HP-guar, hydroxypropyl methyl cellulose, polyvinyl alcohol, polyvinylpyrrolidone, and polyethylene glycol. Other eye disorders including Sjogren's syndrome and keratoconjunctivitis sicca, which cause inflammation and a drop in eye moisture, have symptoms that can be controlled with the help of medications made from these substances. According to the literature [3], osteoarthritis, eye irritability, and cancer have all been related to a drop in hyaluronic acid levels.

In all vertebrates, HA is present in the extracellular matrix of the skin, all mucous membranes of the body, ocular fluid, vitreous body of the eye, joint cartilage, and synovial fluid.[4, 5] Having the molecular weight of 1000 to 5,000,000 Da, it is a naturally occurring polysaccharide with the chemical formula $(C_{14}H_{21}NO_{11})_n$.[6] Weissman and Meyer discovered the chemical structure of HA in 1954, but Laurent and Fraser revealed that the polysaccharide's basic structure is an unbranched linear chain with the monosaccharides connected by alternating 1,3 and 1,4 glycosidic linkages.[7] HA is made up of two compounds: β-d-glucuronic acid (GCU) and β-d-N-acetylglucosamine (NAG), which are linked by glycosidic bonds at their C1 and C3 and at their C1 and C4 positions, respectively.

Typical hyaluronate molecules can include up to 10,000 disaccharide units. They are made up of N-acetyl-D-glucosamine, which is linearly repeated to create HA, and D-glucuronic acid (GCU). The total negative charge on HA is caused by repeating units with anionic carboxylic sites when conditions are physiological. The interaction of these sites with metal cations is a crucial aspect of the overall supermolecular structure of HA. The degree of hydration, pH, temperature, and counter ion type are a few of the variables that determine HA's properties.

HA is found in practically every eye compartment and due to its biocompatibility, non-toxicity, and viscoelastic qualities, there have been increased applications in cosmetics and medicine.[8] HA is referred to as fake tears since it can be administered topically (as eye drops) to treat DED.[9] The complicated analytics and pharmacokinetics of HA make quantitative and qualitative measurements difficult, according to Šimek and colleagues.[10] To achieve the best medicine delivery effectiveness under varied ocular situations, the molecular weight (MW) and amount of HA should be logically established and used to construct distinct delivery strategies.[11]

According to one study, hyaluronic acid's molecular weight and place of origin directly influence how effective it is as a treatment, leading to a variety of ways that HA is used.[12] Because of its distinct physicochemical and intrinsic characteristics, HA is employed as a medication carrier for ocular formulations.[13] HA as seen in Fig. 1 comes in two different forms: potassium salt and sodium salt, but the sodium salt is mainly produced commercially.

1.1 Sodium Hyaluronate

Sodium hyaluronate (SH) originates from hyaluronic acid. The sodium derivative of HA has many advantages over HA and can be used independently. SH has a lower molecular weight and can easily permeate the top layer of skin. This feature ensures that the skin receives more internal hydration.[14] The sodium hyaluronate in synovial fluid serves as a lubricant and filters large molecules and cells as they move through the joint. Sodium hyaluronate molecules align with one another during blinks, creating an elastic, non-viscous solution that spreads easily across the surface of the cornea. The precorneal tear film is stabilised by the SH knots that form between blinks, and the longer the solution remains on the surface, the better it lubricates and protects the ocular surface.

SH, as depicted in Fig. 2, has an extremely compact helix, a high affinity for water, and mucoadhesive properties, which aid in its ability to stay on the surface of the eye for a longer period.[5] It has peculiar viscoelastic properties, which cause it to behave differently during blinks and between blinks. Particularly in the treatment of DED or to ease symptoms following eye procedures, sodium hyaluronate is a crucial component of solutions made for ocular use. Studying the precorneal tear film break-up period by Johnson and his associates provided confirmation of this. They learned that the severity of symptoms prior to and following therapy was minimal when SH is used.[1] In eyes treated with sodium hyaluronate, it was found that the tear film's stability had significantly increased. One can purchase and apply sodium hyaluronate in a variety of ways, including as an injection, eye drops, nasal spray, face wash, serum, lotion, and gel.

1.2 Collagen Model Polypeptide, Prolyl-Prolyl-Glycine

Collagen (CG) is a viable ophthalmic drug-delivery system carrier, according to research, since it helps transfer large concentrations of water-soluble antibiotics to the eye.[15] For instance, the glaucoma medication pilocarpine has a limited bioavailability and a short half-life. To have the desired result, the medicine must be applied repeatedly and continuously. However, pilocarpine has negative effects on the eyes when given in excessive doses. Unwanted side effects including myopia and miosis occur when there is a high concentration of the medicine in the eye.

The shortcoming of pilocarpine and hyaluronate based eyedrops can be overcome by the stabilization of HA-CG linkage with H_2O such that the eyedrops is retained in a significant quantity in the eye for an extended period.

Mammals primarily produce collagen, which makes up around 30% of all proteins in the body.[16] The skin, cartilage, tendons, eyes, bones, and ligaments are the principal locations where it is present.[17] Its rate of synthesis decreases with ageing, and it is quickly deposition during periods of fast growth. Inconsistencies or mutations in the collagen structure are often the cause of connective tissue disorders including infantile cortical hyperostosis and osteoporosis. In order to form an extended triple helix, the amino acids that make up collagen are joined in a certain order. There are more than 20 distinct varieties of collagen. However, the fundamental collagen molecule has a molecular weight of around 300 kDa, is shaped like a rod, and measures about 3000 Å long and 15 Å wide,

Based on their α chains and quaternary structures, collagens are categorised.[18, 19] While Type II and Type IV are unique to the cartilage and some portions of the eye (vitreous) and inner ear, Type I is present in nearly all tissues throughout the human body. In this study, a model interaction of a Type I collagen (Prolyl-Prolyl-Glycine), hyaluronic acid and water in the human eyes were investigated. There are other types of prolyl-prolyl-glycine, but the protein with the chemical formula $[(\text{Pro-Pro-Gly})_{10}]_3$ depicted in Fig. 3 was chosen since it was the first synthesised and commonly used collagen model to examine varied collagen activities.[20] It is known chemically as 2-[[(2S)-1-[(2S)-pyrrolidine-2-carbonyl]pyrrolidine-2-carbonyl]amino]acetic acid and has the formula $C_{12}H_{19}N_3O_4$. It is a polypeptide with three helix strands

and an orthorhombic crystal structure. Figure 4 depicts this helix and coiled structural arrangement. The orderly arrangement of the three strands is maintained through hydrogen bonding as well as intra- and intermolecular van der Waals contact.[21] Prolyl-Prolyl-Glycine has two isomers, D- and L-, but the L isomer, L-Prolyl-L-Prolyl-Glycine, was chosen for the study.

1.3 Interaction of Hyaluronic acid, Pro-Pro-Gly and water

The production of water molecules and their patterns of bonding were examined in the work by Keutsch and his collaborators. According to research, the electrostatic forces and donor-acceptor interactions between the hydrogen and oxygen atoms are what cause the weak bonding between the two elements.[22] The newly created water molecules can interact with other water molecules to form the complex (O-H...O) of intermolecular bonding partners. It was found that the surface functional groups of hyaluronic acid, carboxylic acid (-COOH) or hydroxyl (-OH), can easily bond with hydrophilic substances to produce a persistent thin layer even in the presence of water.[23] The researchers assert that this characteristic of HA results from intra- and intermolecular hydrogen bonding in addition to its molecular weight.

It has been determined that the interactions between hydrogen and oxygen bonds are 5 to 10 times less than those between covalent bonds.[24] This is seen from the hydrogen bonds and O-H covalent bonds' respective binding energies of 21–42 kJ/mol and 456 kJ/mol in water molecules.[25] When an oxygen atom, acting as a hydrogen acceptor next to a water molecule, and a hydrogen donor (electron-deficient H-atom) combine to form an O....H+-O bonding pattern, hydrogen bonds are created. Each mole of material contains between 21 and 42 kcal due to the effects of electrostatic forces and donor-acceptor interactions on water molecules.

Nuclear magnetic resonance spectroscopy (NMR), X-ray diffraction, and molecular modelling have all been used to demonstrate the existence of up to five hydrogen bonds between two neighbouring disaccharide units and the secondary structures. It has been suggested that the structure has secondary hydrophobic faces, which are produced by the axial hydrogen atoms of around 8 CH groups on the opposing sides of the secondary structure. The hydrophobic feature is energetically advantageous for the formation of meshwork-like tertiary structures because of molecular aggregation. The tertiary structure is further stabilised by intermolecular hydrogen bonding.[26] Hydrophobic and hydrogen bonding interactions produce several molecules that reject one another, forming HA molecular networks.

A small number of inner electron shells and a small atomic radius are characteristics of hydrogen. As a result, it can get close to the hydrogen atom of another molecule without experiencing much electrostatic repulsion. According to reports, hydrogen bonds mostly possess electrostatic properties with limited covalent aspects, which are typically represented as 90% and 10%, respectively.[27]

Research on ocular drug delivery is currently focused on enhancing pre-corneal contact time, total resident time, drug permeability, and drug clearance rate. The frequency of eyedrop applications will be decreased, which is a primary benefit of extended medication residence time in the eye. The ocular surface becomes enriched in HA over an extended length of time thanks to a stable complex of CG, HA,

and water. It is still unclear how hydrogen bonding affects the stability of hyaluronic acid and the underlying chemistry that underlies the effective use of sodium hyaluronate eye drops to treat DED. Therefore, the purpose of this study is to use computational methods to explain how hydrogen bonding activity affects the development of the HA-CG-H_2O complex, which serves as a gauge of hyaluronate stability and drug retention duration. The following have been selected as guidelines to achieve the purpose of this study. These are.

I. Analysis of HA's structure and intramolecular H-bond configuration.
II. Examination of HA interactions with CG and computing structural and binding energy parameters.
III. Research on the mechanism of hyaluronate, collagen, and water interaction.
IV. An explanation of how the complex's activities compare to those of its individual components.
V. Research on the position of water molecules, their impact on the strength of intermolecular and intramolecular H-bonds as well as structure-binding energy relationships.

2. Methods

2.1 Literature Search

Science direct, Google Scholar, PubMed, ResearchGate, and Web of Science were searched for current problems, related studies and acquisition of knowledge required for the study. Some the search terms were (“dry eye” or “dry eye disease” or “Sjögren's syndrome”), (“molecular modelling of hyaluronate” or “molecular dynamics of collagen interaction” or “molecular simulations of hyaluronate eyedrops” or “computational modelling of hydrogen bonding” or “Computer-aided drug design”), (“collagen interaction with hyaluronate” or “collagen interaction with hyaluronic acid” or “reaction of Pro-Pro-Gly and hyaluronic acid”), (“hydrogen bond stability” or “hydrogen bond contribution to peptide stability” or “calculation of hydrogen bonding binding energy”) and (“hyaluronic acid” “hyaluronate” or “hyaluronan”). Only sources in English were taken into consideration.

2.2 Computational methods

This study made use of Avogadro and Visual Molecular Dynamics (VMD) molecular modelling programmes. Avogadro Software was used to visualise and construct the molecular architectures. The preferred force field, MMFF94, was used to optimise the constructions. The length and bond angles of the hydrogen bond and the covalent link that it is adjacent to, were measured and recorded.

2.2.1 Computational Modelling of Hyaluronic acid and Pro-Pro-Gly

Avogadro was used to simulate molecules, examine H-bond characteristics, and optimise structures while crystal structure imaging was done using VMD.

2.2.2 Literature search for Hyaluronic Acid and Collagen (Pro-Pro-Gly)

Hyaluronic acid and collagen (Pro-Pro-Gly) with reference IDs 2BVK and 1K6F, respectively, were chosen from the Protein Data Bank (PDB) search results.

2.2.3 Upload of model compounds and Optimization

The following steps are a summary of how to add hyaluronate, collagen, and water to the Avogadro software to evaluate hydrogen bonds.

i. **Hyaluronate addition**: Using Avogadro's File → import → Fetch from PDB option, hyaluronic acid was added to the main display. Two hyaluronic acid units make up the hyaluronic acid extracted from PDB (2BVK). One of the units was taken out to create the monomer that was needed for this study.

ii. **Collagen (Pro-Pro-Gly) Addition**: The peptide was added to Avogadro in the following order: Build Tab → insert sub-Tab → Peptides. These procedures offer steps used to upload the peptide to the main display. Pro-Pro-Gly was created by clicking the Pro, Pro and Gly Tabs from the different proteins provided. No other settings were altered because the L isomer was the default option. The display tab's "align to axes" option was chosen to preserve the axes alignment, which is crucial for model reproducibility.

iii. **Water addition**: The "Build" Tab was selected, followed by the insert option where water was added as a fragment. The Simplified Molecular Input Line Entry System (SMILES) in the insert Tab or the insert "fragment water" can also be used to add water to the main display. Water is represented by the SMILES symbol "O".

iv. **Optimization**: Based on the chosen orientation of the molecule and computer calculations, optimization produces a conformation derived from the structural arrangement and binding energy (E) in kJ/mol. The water molecule was shifted about and tuned to produce global binding energies when HA, CG and H_2O interacts. Avogadro optimization is carried out by selecting the "**E**" tab and then selecting the MMFF94 force field.

2.4 Hydrogen Bond Energy Contribution

To comprehend the strength of the contact, the lengths of the hydrogen bonds in the models of biological molecules were examined. In general, short hydrogen bonds with bond angles near to 180^o are stronger than longer ones with bond angles farther away. In this study, an important parameter, the hydrogen bond binding energy contribution to the complexes form, was examined, and calculated using this formula:

ΔE (binding) = E (A-B-C) complex − E (A) − E (B) − E (C) (1)

2.5 Flow Chart of Experimental Methods

The flow chart outlines the step-by-step procedures formulated in this study. HA and CG interact with water individually while HA and CG bind in the absence of water. Additionally, a combined reaction of all three components, HA, CG, and H_2O was investigated as illustrated in Fig. 5.

3. Results

3.1 Binding of water to Hyaluronate (HA)

7 lowest energy conformations were found when HA dimer interacts with water as shown in Fig. 6. These structures differ in the positions and number of atoms involved in hydrogen bonding. Water molecule with atom labels, H19-O13-O20, was rotated around the HA molecule and optimized to yield the various structures in which the water molecule was interacting with various functional groups of HA. In some structures the water molecule would form 2 H-bonds and in some structures only a single H-bond. In most structures water did form two H-bonds to the hyaluronic acid due to simultaneously interacting with two adjacent functional groups.

In Table 1 we present the binding energy and the structural parameters of the H-bond formed between the water molecule and HA dimer. We have investigated if a correlation could be found between the binding energy and the H-bond length/angle but such a correlation could not be established. This indicates that the binding energy is not entirely based on H-bonding interactions and that other interactions such as dipole-dipole and dipole-induced dipole may be important in the interaction of water with HA.

The average binding energy of hyaluronan to water was found to be -36.6 ± 7.6 kJ/mol. This binding energy is smaller than the binding energy water has to collagen as we will show in the following sections. The binding energy was calculated based on the following formula,

$\Delta E(\text{binding energy}) = E(\text{X-Water}) - E(\text{X}) - E(\text{Water}),$ (2)

where X is HA or Collagen, using the MMFF94s force field and a convergence criterion of 10^{-7} kJ/mol. We have basically optimised the HA and collagen fragment first and then added a water molecule in the periphery of HA and the collagen fragment. The energy was optimised for the complex of HA-water and collagen-water, respectively, and the E(X-water) was recorded. Subsequently, the water molecule was removed, and the energy of the HA or collagen given by E(X) was recorded. We then used Eq. 2 to calculate the binding energy, ΔE (binding energy).

Table 1
Binding energy of water to HA, H-bond angle, O-H bond distance and O…H bond length. When two values are shown, that means that two H-bonds are formed.

Label	Binding energy	H-bond angle	O…H distance	O-H distance
	(kJ/mol)	(°)	(Å)	(Å)
A	-47.3	142.9	1.956	0.980
		161.5	1.842	1.025
B	-33.0	168.8	1.784	0.987
		150.8	1.786	0.982
C	-35.0	166.4	1.794	0.987
		152.7	1.766	0.984
D	-30.9	176.5	1.93	0.979
		152.3	1.898	0.986
E	-27.4	167.7	1.766	0.981
F	-36.0	173.0	1.874	0.985
		142.1	1.881	0.981
G	-46.4	162.5	1.802	1.025
average	**-36.6**	st.dev.	**7.6**	

Bond formation between the O8 of the Hyaluronate (HA) with the H19 of the water molecule was found to have a binding energy of -47.3 kJ/mol which was the highest among the different conformations investigated. This bond is relatively strong, and it is mainly due to the formation of two H-bonds with water and an inter-molecular H-bond between N-H and O7. The average bond length of the two H-bonds (H…O) is 1.899Å and the bond angle between O7 H19 O13 is 152.2°. Because of this strong interaction the eye drops containing HA hold a good amount of water in the eye and prevent dryness. This can also find use for the treatment of dryness in hair.

3.2 Binding of water to collagen (CG)

4 low energy conformations were found when CG interacts with water as shown in Fig. 7. These structures differ in the positions of atoms involved in hydrogen bonding. The atoms of water, H20-O5-O21 were rotated around the structure and optimized. Both H20 and H21 took part in the formation of hydrogen bonding that occurs when CG binds with water. Half the structures form a single H-bond with the CG fragments that consist of six amino-acids Pro-Pro-Gly-Pro-Pro-Gly and have three strands. Half of the structures had two H-bonds to the water molecule due to the bonding primarily to the C = O and N-H functional groups. It is noted that this model of collagen was not completely folded to the structure that

is found by X-Ray crystallography, so it is possible that the binding energies of water to this model are slightly overestimated from those that water would have in a folded structure of collagen without any defects. This model of collagen does represent better the edges of the real collagen in the human eye cornea or collagen that is slightly misfolded. This is advantageous to our modelling studies which are concerned with the hydration of collagen, which has a small degree of defects. This collagen would also be the one that benefits the most from the hydration offered via the hyaluronic acid polymer binding to the collagen.

In Table 2 we present the binding energy and the structural parameters of the H-bond formed between the water molecule and CG. We have investigated many more starting structures that would optimise to the four structures given in Table 2. From the structural parameters of the H-bond it is obvious that all CG-water complexes form moderate to strong H-bonds with the water molecule.

Table 2
Binding energy of water to CG, H-bond angle, O-H bond distance and O…H bond length. When two atom labels are shown, that means that two H-bonds are formed.

Label	Binding energy	H-bond angle	O…H distance	O-H distance
	(kJ/mol)	(°)	(Å)	(Å)
L	-47.7	146.6	1.775	0.985
M	-48.4	152.8	1.832	0.980
		154.1	1.829	0.979
N	-47.9	146.8	1.775	0.985
		171.3	1.833	1.024
O	-36.3	171.3	1.853	0.985
average	**-45.1**	st.dev.	**5.9**	

In structures optimised we found that the average binding energy is -45.1 ± 5.9 kJ/mol which is slightly more exothermic than the binding energy found between HA and water. This indicates that water can easily transfer from HA to CG due to the similar strength of the binding energy of water with these biomolecules. In structure **L** the hydrogen bond between water and HA is due to the interaction with a C = O group. Structure **M** forms two H-bonds both of which are with C = O groups. This is the strongest interaction of water with this collagen model and has a binding energy of -48.4 kJ/mol. Structure **N** forms also two H-bonds one with a C = O group and another with an N-H group. The weakest interaction of water with the collagen model is when it H-bonds to the N-H group of a 5-membered ring of the prolyne aminoacid which has a binding energy of -36.3 kJ/mol. These results suggest that water can transfer from its adsorbed position on the HA to the CG via a spillover effect or just due to more exothermicity of the hydration enthalpy. What remains to be established is that HA can bind to CG while maintaining its

tendency to adsorb water. This will be studied next by first modelling the interactions between CG and HA and then modelling the interactions between CG, HA and water in the following sections.

3.3 Binding of HA and CG and water

The binding of HA to CG was simulated with a three stranded Pro-Pro-Gly-Pro-Pro-Gly model that was placed adjacent to the HA dimer. The HA dimer showed that it prefers to bind to the edge to the CG model, interacting via a H-bond of the O-H group of HA and the N-H group of the Proline aminoacid. This binding of HA to CG is shown in Fig. 8a and in Fig. 8b we show the binding of water to HA that is bound to CG. The binding energy of HA to CG is moderate and was calculated to be -24.0 kJ/mol. The binding of the water molecule to the HA in this configuration was found to be slightly more exothermic with a binding energy of -26.7 kJ/mol. This clearly shows that water can effectively be adsorbed to the HA while the HA is bound to the CG. By comparing the last binding energy with that of water adsorbed to the side of CG which was found earlier to be -45.1 ± 5.9 kJ/mol it is obvious that the water would like to realease energy by adsorbing to a more exothermic position which places it on the CG side. This spillover effect can cause the water originating on the HA to transfer to the CG hydrating therefore the collagen of the cornea in the human eye.

These relative strong binding energies indicate that HA strongly binds to the collagen of the cornea once its is dispensed via ocular solutions containing HA or SH. The polymeric structure of HA/SH will bind its backbone with the collagen fibers and therefore offer an extended high surface area substrate for water hydration to happen and to maintain the CG hydrated by a spill-over effect explained earlier. The strong binding energy of HA to CG indicates that the complexation of HA to CG will occur even if HA is diluted significantly in water, as the strong exothermicity of its binding to CG will dictate the enhanced adsorption of the polymeric strands of HA to the CG fibers. What remains to be shown is that once the HA has bound to CG that, it maintains its inherent property to adsorb significant amounts of water molecules, acting therefore a water storage for the CG fibrils. Therefore, we have modelled the adsorption of multiple water molecules to HA that is bound to CG and found that HA dimer can adsorb as many as 8 water molecules that are strongly coordinated to HA via H-bonding. This water storage capacity is reduced in polymeric HA due to the unavailability of two O-H groups present in the dimer. Additionally, the polymeric form of HA may prefer to align its strands to the CG fibers, which may reduce its water storage capacity even further.

Conclusions

The vital element of the vitreous body of the human eye, 200 g/ml of HA, is present in just a small quantity in the phakic eye vitreous. [28] The viscoelasticity and inherent water characteristic of hyaluronan aid in the healing of the corneal and conjunctival epithelium.[29] It is believed that the HA's antioxidant capabilities have a high molecular weight and can protect against reactive oxygen species. Various experts advise patients with persistent dry eyes caused by oxidative stress to utilize eye drops. The rabbit's sclera, conjunctiva, cornea, and contact lenses were each subjected to the experiment independently (HAB pep-polymer system). The polymer was shown to be effective in preserving HA and reducing contact lens evaporation, it was determined.[30] Here we have used computational chemistry to

describe the action of HA in hydrating the cornea of the human eye. We study systematically what the binding energy of water is to HA, CG and HA-CG. Our study suggests a mechanism of action for the binding of HA to CG and the subsequent hydration of the HA, which via a spillover effect, transfers water from the HA to the CG. This mechanism of action is supported by detailed atomistic simulations of the binding energy of water using the MMFF94 force field.

Declarations

Conflicts of Interest

There are no conflicts of interest to declare

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Code availability: For all calculations in this manuscript the Avogadro software was used.

Authors' contributions: This computational study was conceived and designed by Dr. Constantinos Zeinalipour-Yazdi. CDZ also collected the data and wrote parts of the paper. SSJ and OCI did the computational analysis and wrote the paper.

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Figures

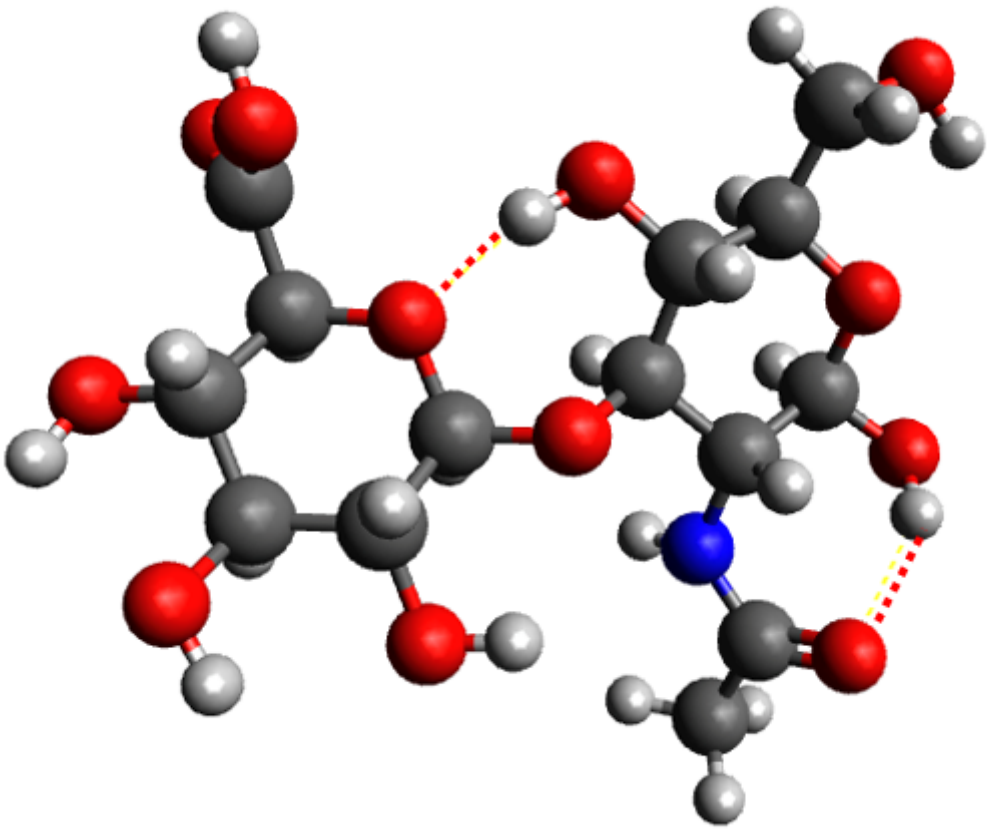

Figure 1

Monomer of hyaluronic acid showing intra-molecular H-bonds. White is hydrogen, red is oxygen, blue is nitrogen and grey is carbon.

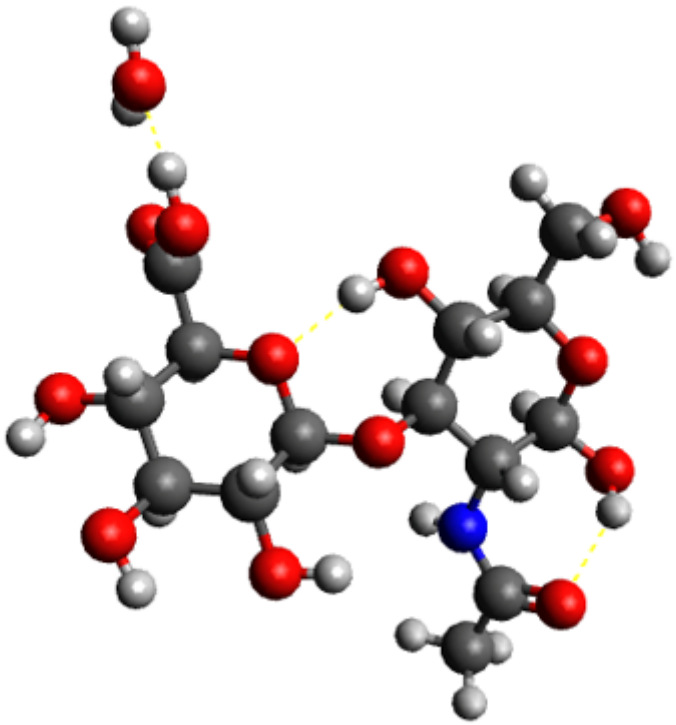

Figure 2

Illustration of hyaluronic acid and water (H_2O) binding. White is hydrogen, red is oxygen, blue is nitrogen and grey is carbon.

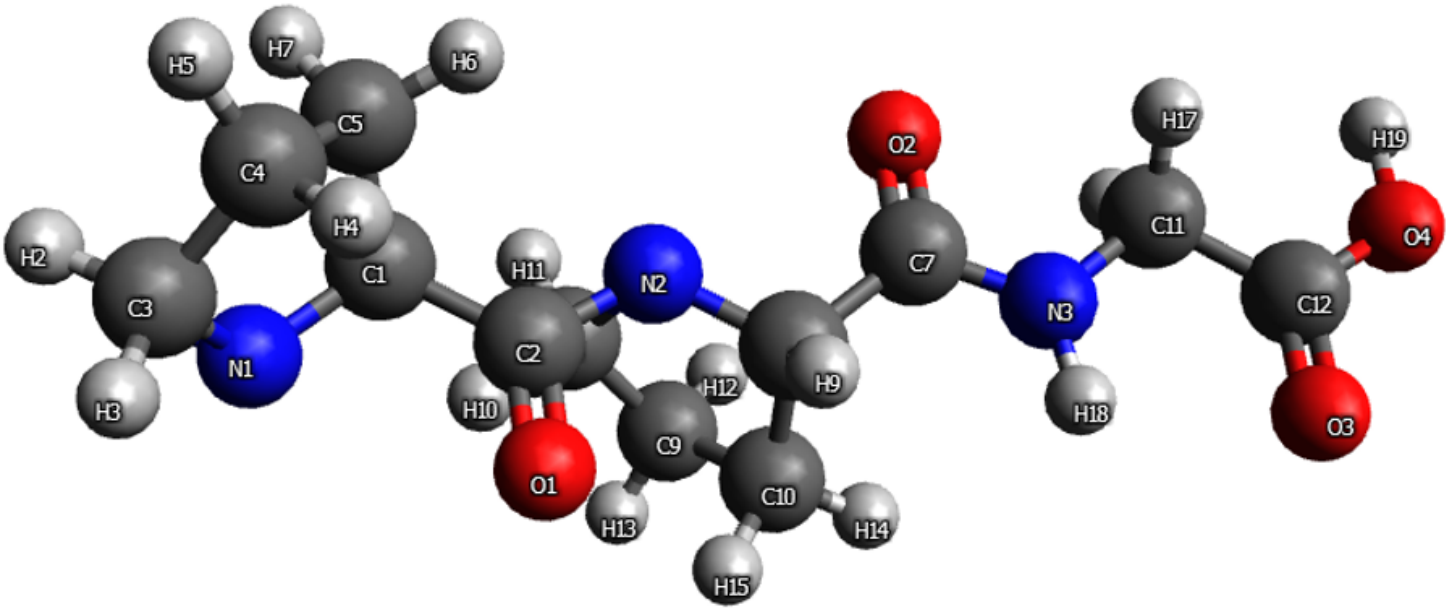

Figure 3

Illustration of Pro-Pro-Gly Collagen

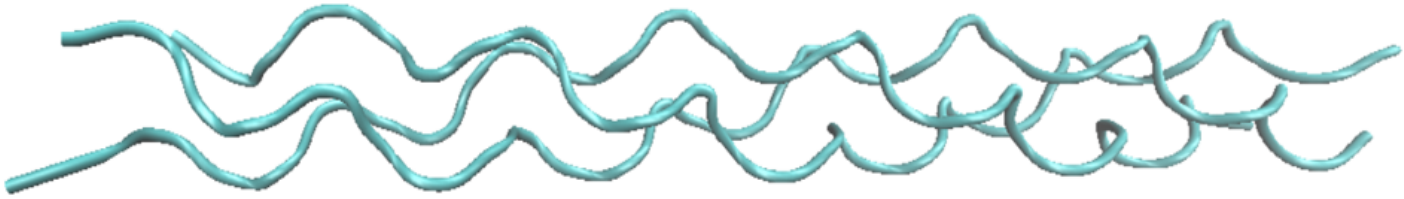

Figure 4

Triple helical structure of Pro-Pro-Gly Collagen.

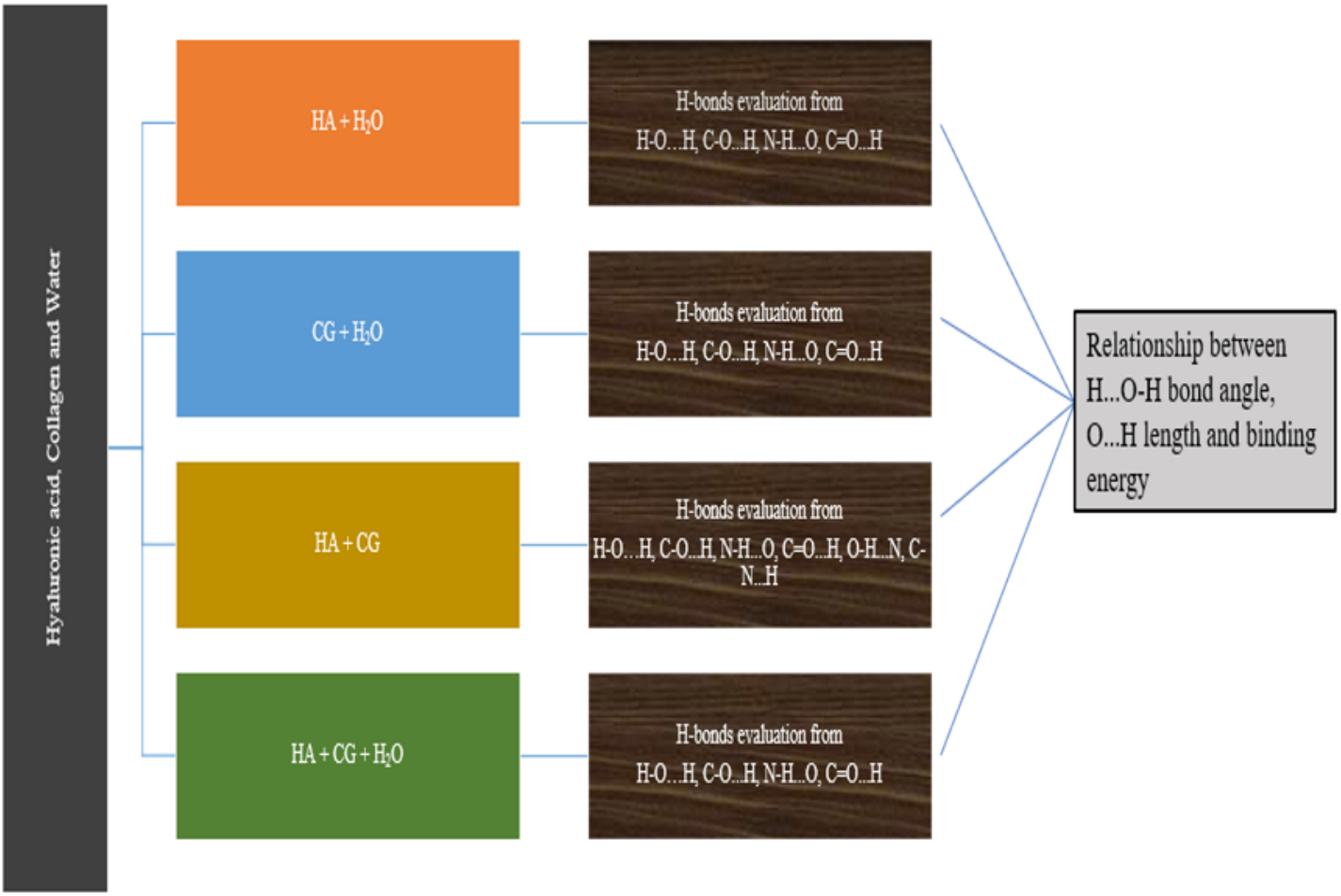

Figure 5

Flow chart of the experimental methods showing interactions of HA, CG with H_2O and HA-CG without water.

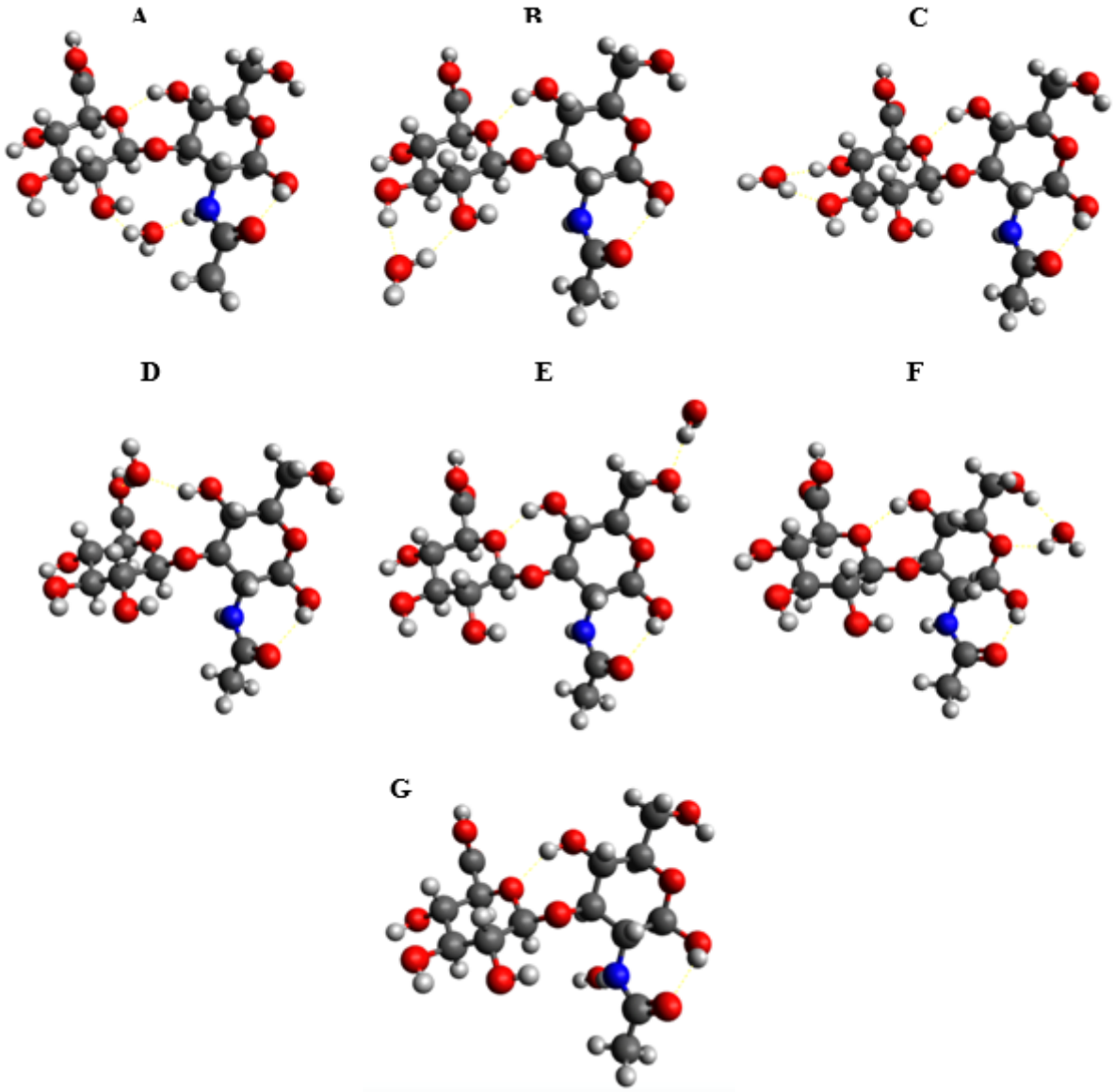

Figure 6

lowest energy conformations of HA-H_2O complex formed after optimization.

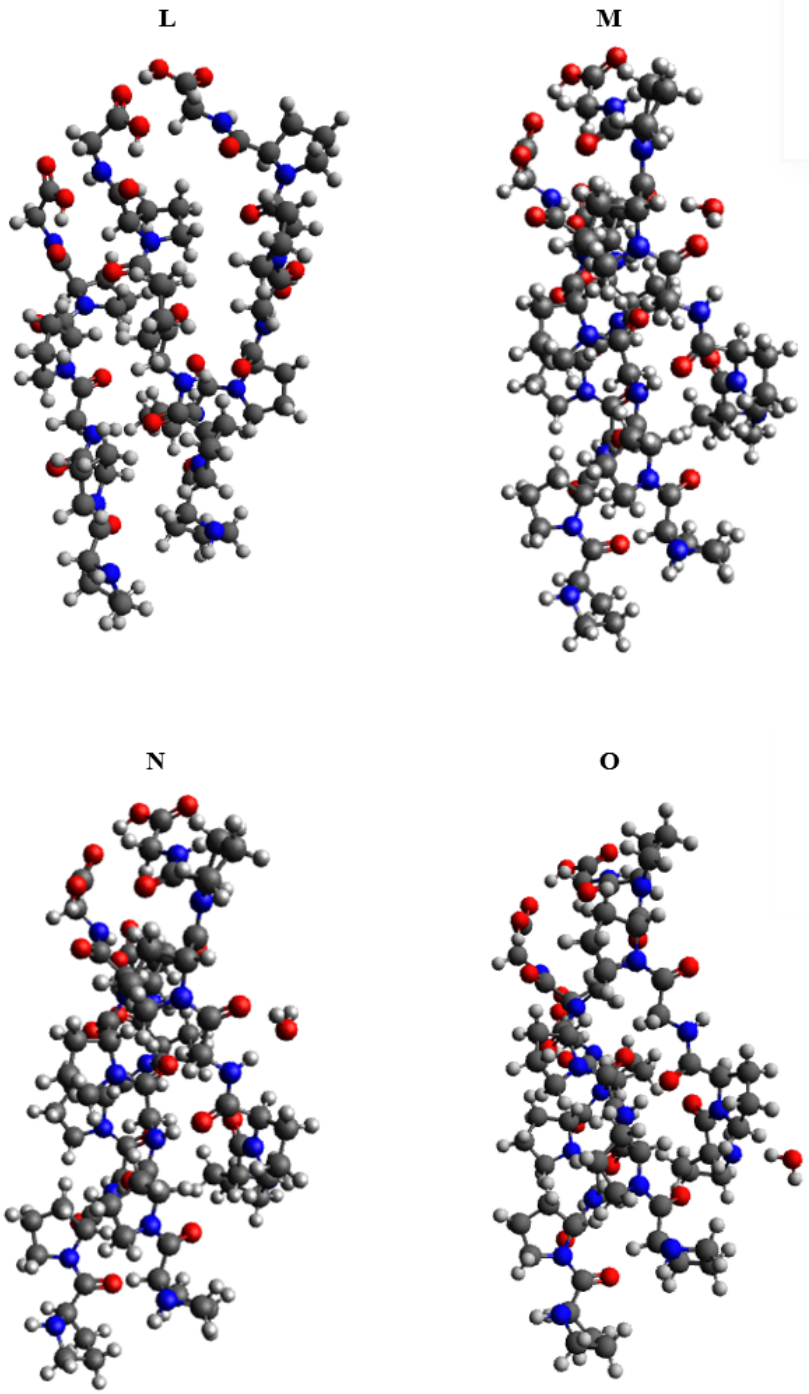

Figure 7

4 low energy conformations of CG-H_2O complex formed after optimization. The CG model was a three stranded model of Pro-Pro-Gly-Pro-Pro-Gly to which a water molecule was added at various positions along the fiber axis. The binding energies are given in Table 2.

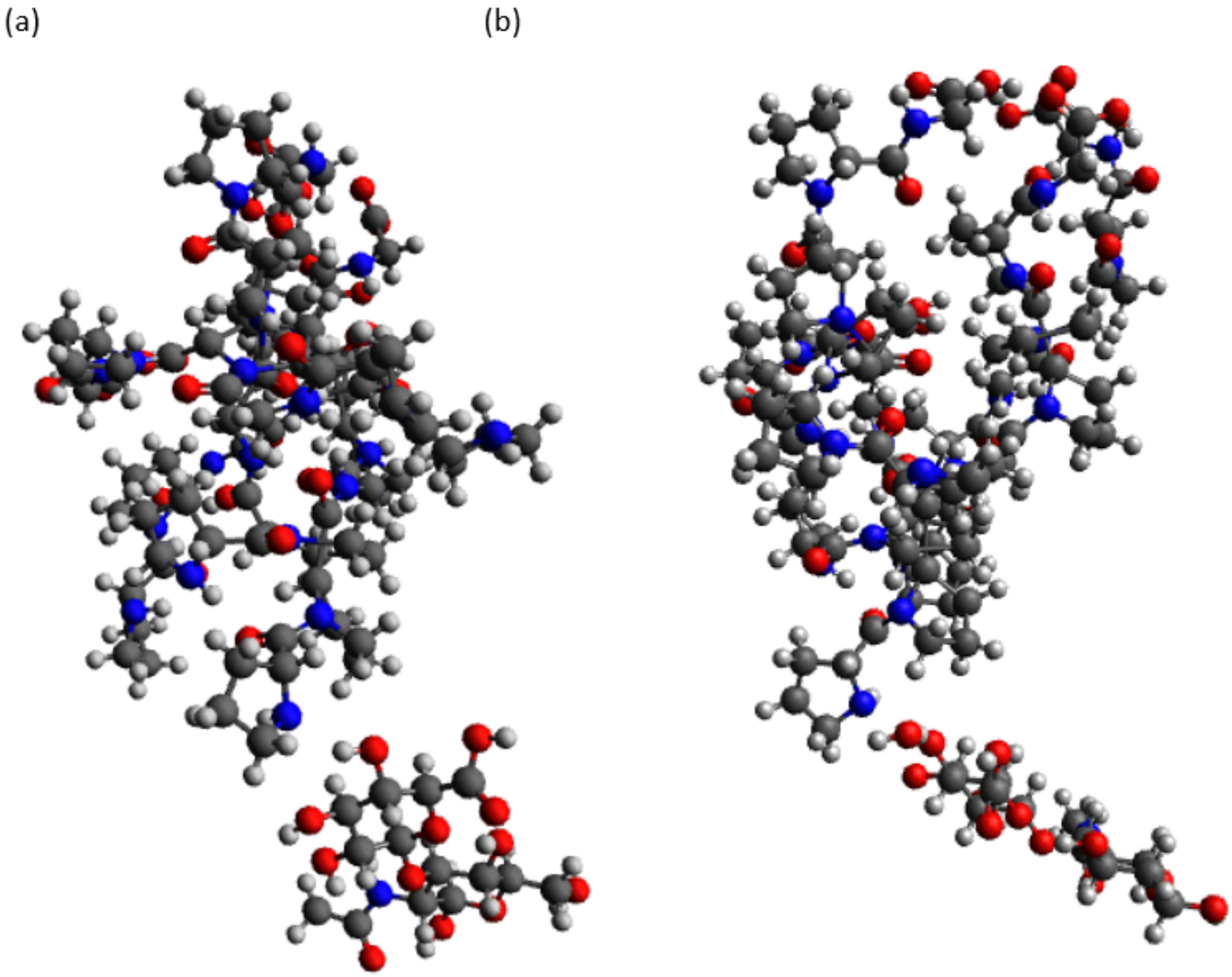

Figure 8

(a) Optimised structure of HA-CG and (b) HA-CG-Water models. The structure of CG is a three stranded Pro-Pro-Gly-Pro-Pro-Gly model and the HA is a dimer.