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Molecular Dynamics Study of Onset of Water Gelation around the Collagen Triple Helix

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ABSTRACT **The onset of water gelation around a collagen-like triple helix peptide was studied at ambient temperature and pressure by performing Molecular Dynamics simulations. The radial distribution functions of the oxygen and hydrogen atoms of water are distorted below 4 Å from the peptide. The distortion is accompanied by the breakdown of the tetrahedral coordination of the hydrogen-bonded network of water molecules. The water shell around the peptide consists of alternating regions of higher and lower density. In agreement with experiments we find that the first hydration shell is kinetically labile, with a residence time in the order of picoseconds for a water molecule. From the computed diffusion coefficient, a key measure of the collective dynamics, we estimate the average diffusion speed decreases by a factor of 1.5 close to the peptide compared to the liquid. Our results give new insight in gel formation and structure on a molecular level. Proteins 2006;64:711–718.**
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Key words: gelation; collagen triple helix; liquids; water; molecular simulation

INTRODUCTION

The discovery and design of small organic molecules capable of gelling aqueous solvents, so-called hydrogelators, is a rapidly expanding area of research. This is due to their possible practical applications in tissue engineering, vehicles for controlled drug release, and pollutant capture.[1] Some examples of recently developed potentially strong hydrogelators are *N*-alkyl disaccharides[2] and bis(amino acid)oxalyl amides.[3] The latter also form gels with organic solvents and mixtures of organic solvents and water.

In addition to synthetic gelators also some classes of proteins are able to form gels in aqueous environment. The best known example is the family of fibril-forming collagens.[4] Collagen is the most abundant protein found in mammals, and represents the major structural protein in the extracellular matrix. It meets structural and mechanical requirements for a wide variety of biological systems, among which are hair, horn, nail, and muscle. Together with the α-helical coiled coil, collagen represents a group of proteins with a supercoiled motif.[4] In the 1990s the molecular structure of the collagen protein was determined by X-ray crystallography at a resolution of 1.9 Å.[5,6] It was found that the basic structural motif of collagen is a triple helix consisting of three intertwined chains that are staggered by one residue, allowing for interchain backbone-to-backbone hydrogen bonds. In 1996, Melacini and coworkers showed that the triple helix structure was also stable in aqueous solution by performing NMR experiments on template-assembled collagen peptides with varying chain lengths.[7,8]

In collagen, the most commonly found amino acid sequence is the Gly-Pro-Hyp triplet, where Hyp denotes 4-hydroxyproline resulting from the posttranslational enzymatic modification of proline. In general, a hydrogelator should be amphiphilic in nature, possessing both hydrophobic and hydrophilic pockets on the outer surface. With its cyclic imino group, proline is hydrophobic in nature. The modified cyclic imino group of Hyp bears an additional hydroxyl group that is hydrophilic. Hence, the amphiphilic requirement is satisfied. The amphiphilic nature is also found in "antifreeze" proteins used in the artic regions by certain fish such as the skulpin to overcome to problems associated with the extreme cold climate.[9]

Up until now a wide range of experimental techniques have been used to probe the gelation effect of hydrogelators. For an overview, see Estroff and Hamilton.[1] Recently, a high-resolution NMR study probing the hydration dynamics of a small collagen-like triple-helical peptide in aqueous solution was reported by Melacini et al.[10] They concluded that the first hydration shell is kinetically labile, with upper limits for water molecule residence times in the nanosecond to subnanosecond range. In addition, they argued that the lability of the hydration shell will have important implications for the kinetics of triple-helix self-recognition when the helices arrange to form the gel network.

To our knowledge, the only atomistic computational study concerning the gelating properties of collagen was

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reported by Monti and coworkers et al.[11] Using five short triple helices, each three times 24 amino acids long, arranged in a pentagon, a so-called microfibril, they studied its interaction with different solvents or solvent mixtures. Only in the case of water they found major changes in the conformation of the microfibril. Very recently they extended this work to an assembly of a dimer of microfibrils in solution and found again that only in the case of water major changes are observed in the conformation of the dimer.[12]

Most research efforts until now have been directed towards a detailed understanding of the (molecular) properties of a typical hydrogelator and how these properties determine the gel network. We focus on the solvation shell surrounding the hydrogelator, more specifically, the aqueous hydration shell of the collagen triple helix. As model for collagen we use the small triple helix peptide studied experimentally by Melacini et al., consisting of three intertwined Ac-(Gly-Pro-Hyp)$_6$-NH$_2$ chains (see Fig. 1 for a model). We address the influence of the triple helix peptide on the structure, dynamics, and binding properties of the water molecules in the liquid phase, which should provide an improved understanding of how a hydrogel is formed and which factors govern its stability. The article is organized as follows: Section 2 gives the computational details, in Section 3–5 the results are presented and discussed, and in Section 6 we give the conclusions.

COMPUTATIONAL DETAILS

Molecular Dynamics (MD) simulations were performed using the 1999 release of the AMBER force field,[13,14] which has been successfully exploited for the study of collagen triple helices.[15] Water was described by the TIP3P water model[16] augmented with an Urey-Bradley crossterm. A similar combination of AMBER/TIP3P was recently used in MD simulation studies of collagen-like microfibrils in aqueous solution by Bronco et al.[11,12] A cutoff of 9.0 Å was used for all nonbonded interactions, and electrostatic interactions were computed using a smooth particle mesh Ewald summation. All calculations were performed with the TINKER software package (TINKER—software tools for molecular design, version 4.1, developed by Jay William Ponder, 1990–2003).

For the missing atom types of the Hyp residue we used the corresponding atom types of the sugar fragment of the adenosine nucleotide as available in the AMBER parameter set.[14] Charges were recomputed both for the Hyp residue and the N-terminal Hyp residue, at the Hartree-Fock level of theory. [ESP charges were computed for 4-hydroxyproline, N-terminal 4-hydroxyproline and adenosine at the HF/6-31G* level using the SPARTAN package (SPARTAN '02, Wavefunction Inc., 1991–2001). A charge scaling factor was calculated from the ESP charges of adenosine and the corresponding RESP literature values.[14] Using the scaling factors we calculated the RESP charges of 4-hydroxyproline and the N-terminal analog, which were found to be within 0.03 *e* of available RESP literature values of similar atom types. The final RESP charges we used given by atom type are: 4-hydroxyproline: OH −0.6173, HO 0.4268, H1 0.1135, CB 0.0137, CD 0.0398, CG, 0.0972; N-terminal 4-hydroxyproline: OH −0.5930, HO 0.4588, H1 0.1135, CB −0.0032, CD −0.0081, CG 0.0908.] A list of the missing atom types with the parameters is provided in the Supporting Information.

A hydrated crystal structure of collagen was obtained from the RSCB protein database (PDB ID: 1CGD). The structure was modified with Viewmol (Viewmol, Version 2.4, developed by Jörg-Rüdiger Hill and Andreas Bleiber, 2004) to the triple helix peptide, studied by Melacini et al., having a total of 54 amino acid residues. We checked with a simple energy minimization whether the resulting structure formed a stable triple helix. We then performed a 50-ps NVT (constant number of particles, volume, and temperature) simulation on a periodically repeated rectangular box containing 3450 water molecules at the experimental density of water at 298 K (0.997 g/mL).[17] The temperature was fixed using the Nosé-Hoover thermostat[18] and the Beeman algorithm was used to integrate the equations-of-motion with a time step of 0.5 fs. This algorithm was preferred over the Verlet algorithm because of the more accurate expression for the update of the velocities. No constraints were employed for the water intramolecular bonds. Subsequently, the triple helix peptide was "soaked" into the equilibrated water box. Water molecules that were too close to peptide were removed by hand.

The final system we used for the MD simulations consisted of one [Ac-(Gly-Pro-Hyp)$_6$—NH$_2$]$_3$ triple helix peptide submerged in a periodically repeated rectangular box filled with 3323 water molecules, for a total of 10,356 atoms. Initially we performed a 0.1-ns NPT (i.e., constant number of particles, pressure, and temperature) simulation at ambient pressure using the Berendsen barostat.[19] During the simulation the box lengths decreased by 0.31, 0.15, and 0.14 Å along the x, y, and z direction. We verified that the system was indeed at equilibrium by monitoring the fluctuations in the temperature, pressure, density, and potential energy of the system. For the final 5 ps of the simulation the average temperature was 298.00 ± 0.02 K, the pressure 1.52 ± 3.03 ATM, the density 1.023 g/cm^3 with fluctuations less than 0.1%, and the fluctuations in the potential energy were less than 0.05%.

We used for all further calculations a simulation box of 77.50 × 37.31 × 35.41 Å. We gathered statistical averages from a 0.5-ns NVT simulation. By visual inspection at regular intervals we verified that the triple helix structure of the peptide was stable during the total length of the simulation. In addition, we monitored the RMSD, see Figure 1 of the Supporting Information. The averaged value along the trajectory is 1.86 ± 0.42 Å, which indicates that the peptide structure is equilibrated and does not undergo large structural changes along the trajectory.

STRUCTURE

Figure 2 shows the computed three-dimensional (3D) O–O, O–H, and H–H radial distribution functions (RDFs) of water molecules as a function of the distance to the collagen-like triple helix peptide, in the following denoted as THP. In addition, the figure shows RDFs at four given

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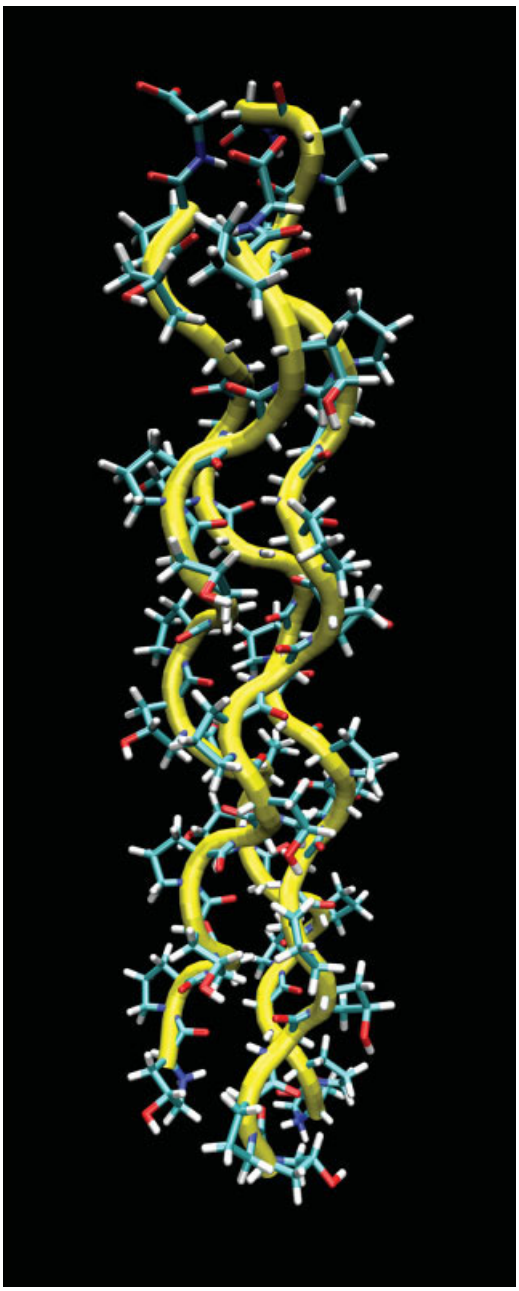

Fig. 1. Molecular structure of the collagen-like [Ac-(Gly-Pro-Hyp)$_6$-NH_2]$_3$ triple helix peptide.[10] The intertwined amino acid chains are indicated schematically by yellow tubes.

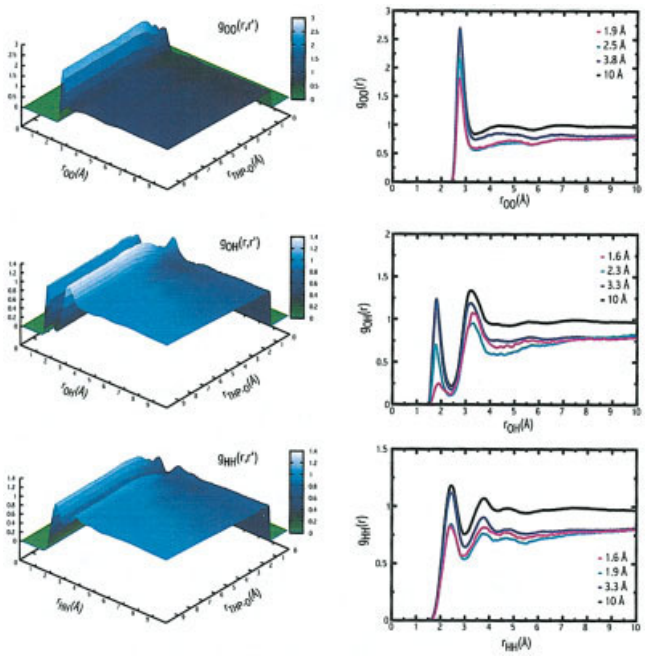

Fig. 2. (Left) Three-dimensional (3D) O–O, O–H, and H–H radial distribution functions of water molecules as a function of the distance from the collagen-like triple helix peptide (THP). (Right) O–O, O–H, and H–H radial distribution functions at four r_{THP-O} distances. Data for $r_{THP-O} <$ 1.45 Å are not shown due to insufficient statistics.

distances from THP. The pronounced structure of all three RDFs indicate the formation of a tetrahedral network of hydrogen bonds, a well-known feature of liquid water. The positions of the first peak at a $r_{THP—O}$ distance that corresponds to bulk water match, within statistical accuracy, experimental data[20,21] and an *ab initio* MD study of liquid water.[22] In addition, both the position and height of the first peak of the O–O RDF match, within statistical accuracy, the TIP3P results of Jorgensen and Tirado-Rives.[23] The position of the peak of the O–H and H–H RDF match, within statistical accuracy, the TIP3P data of Jorgensen et al.,[24] whereas the peak heights are 4 and 8% lower. This discrepancy could be due to fact that we used a cutoff of 9.0 Å for the Coulomb interactions, whereas in the original paper 7.75 Å was used. Overall, although currently better water models exist in the literature, we conclude that the computed structural properties using the TIP3P model are rather accurate. A similar result was recently reported by Rascke and Levitt, who concluded that the hydration structure of the hydrophobic solutes benzene and cyclohexane is remarkably insensitive to the the water model used.[25]

At $r_{THP—O}$ distances below ~4 Å all three RDFs show an unusual behavior. The O–O RDFs has two shallow minima at 1.8 and 2.4 Å, and it reaches its highest value at ~3.7 Å. For the O–H RDF, which most directly probes the hydrogen bonding, the structure is completely distorted at distances below ~1.8 Å, where the first peak has almost disappeared and the second peak at ~3.2 Å has a maximum. Apparently water molecules of the first solvation shell have migrated to the second solvation shell. The same picture emerges from the distorted H–H RDF, although less evident, showing the same minima as the O–O RDF.

By integrating the 3D O–O RDF we estimated the number of nearest neighbors, in essence, the number of hydrogen bonds (H-bonds) per water molecule (see Fig. 3). At large $r_{THP—O}$ the number of H-bonds is slightly less than 4, as one would expect from a tetrahedrally coordinated network. However, at a distance smaller than 4 Å, on average, a water molecule has significantly less H-bonds, with a lowest value of 2.7 H-bonds at $r_{THP—O}$ = 2.1

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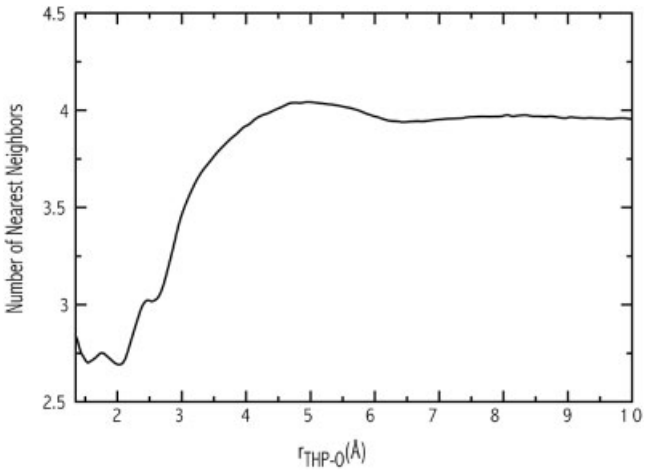

Fig. 3. Number of nearest neighbors per water molecule as function of the distance from the collagen-like triple helix peptide, estimated by integrating the 3D oxygen–oxygen radial distribution function up to the first minimum at 3.45 Å.

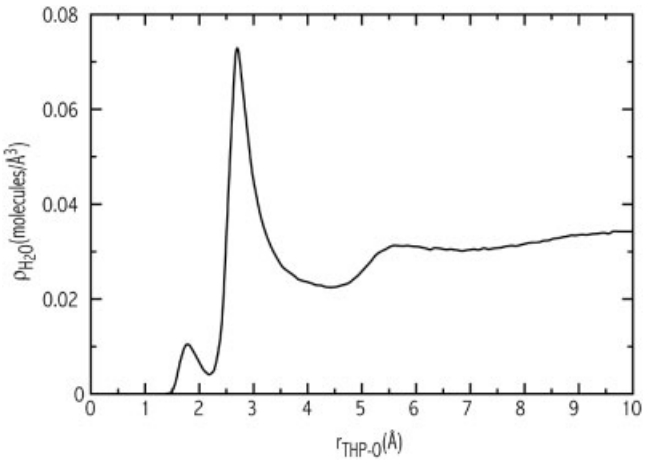

Fig. 4. Averaged water molecular number density as function of the distance from the collagen-like triple helix peptide.

Å. As was already clear from the RDFs, the tetrahedral network of water breaks down in the vicinity of the triple-helix peptide. Moreover, not until a distance of 6 Å from the peptide the number of H-bonds reaches a constant value, indicating that the effect of the peptide reaches at least up to a distance of 6 Å in solution. Hence, a relatively small collagen peptide already interacts with at least 1000 water molecules. This order of magnitude relates well to the work of Bhattacharya and Acharya,[2] who find that very good hydrogelators, such as *N*-alkyl disaccharides, have water gelating capacities of more than 6,000 molecules.

The overall picture of a distorted hydration shell is also found when we plot the (averaged) molecular number density of water (see Fig. 4). Two distinct maxima are seen at $r_{\mathrm{THP—O}} = 1.8$ and 2.7 Å, and a more shallow maximum at ~5.6 Å. The molecular number density of liquid water at 298 K and ambient pressure is 0.033 molecule/Å^3;[17] hence, at the maximum at 2.8 Å the number density is roughly two times higher, whereas at 4.5 Å from the peptide the density is only two-thirds of the value at ambient conditions. This seems to indicate that the water molecules around the collagen-like triple helix are arranged in alternating hydration shells of lower and higher density. A similar oscillating behavior was found when a vacuum is introduced in liquid water. It was shown by Lum et al.[26] that the water density oscillates below 10 Å from a hard sphere (i.e., the vacuum) caused by the microscopic granularity of liquid water. In contrast to our results only the second peak at 2.7 Å is found, while no peak is found at 1.8 Å from the hard sphere. The shallow minimum in the density at approximateley 5 Å corresponds to the broad, shallow maximum at 5 Å in Figure 3 where we report the number of nearest neighbors. At the maximum there are slightly more than four neighbors, which indicates an apparent higher packing of the water molecules. However, in Figure 4, where the number density is plotted, the density decreases in the same region. This phenomenon is

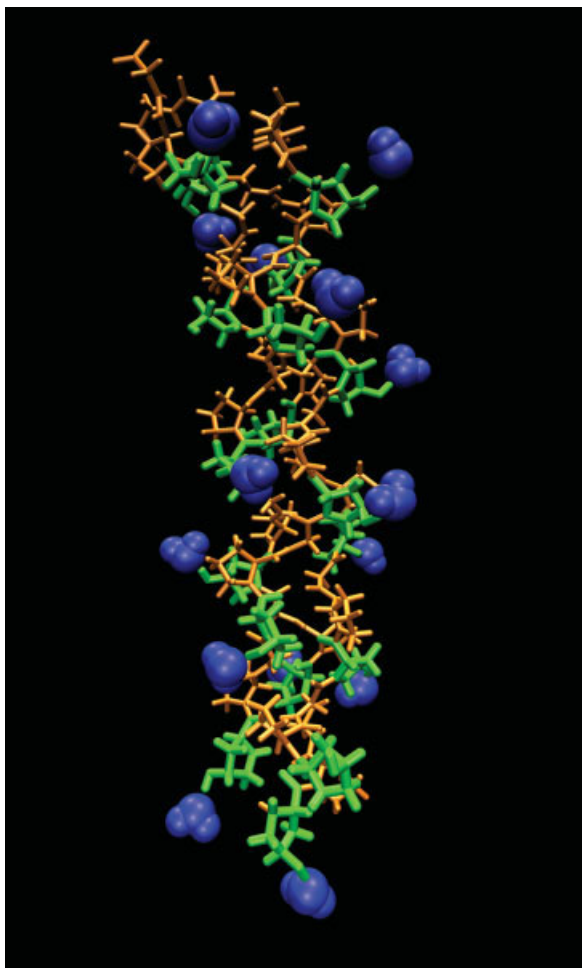

Fig. 5. Graphical representation of first solvation shell of the collagen-like triple helix peptide for an instantaneous configuration of the MD simulation. Hyp residues (18) are shown in green, other residues (36) in orange. Water molecules (15) in the interval of 1.5 to 2.0 Å from the peptide are indicated in blue.

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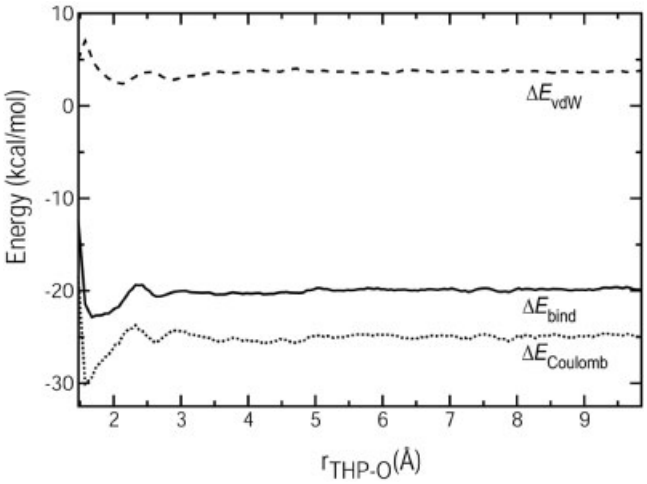

Fig. 6. Water molecular binding energy (full line) as function of the distance from the collagen-like triple helix peptide. Van der Waals and electrostatic (Coulomb) contribution are indicated by the dashed and dotted line. Besides the latter two contributions the water molecular binding energy has additional small intramolecular contributions (not shown). Results are based on 30 statistically independent MD configurations. See the main text for details of the computation of the energy terms.

similar to the decrease in density in water when it starts freezing where better packing is accompanied by a lower density.

Figure 5 shows a graphical representation for a typical configuration of the first hydration shell, that is, the first peak in the molecular number density profile (Fig. 4). Water molecules are located exclusively close to one of the Hyp residues. This is due to the formation of a hydrogen bond between the OH group of a Hyp residue and a water molecule. Using the hydrogen bond formation criteria of ref. 31 we computed a hydrogen bond length of 1.8 ± 0.2 Å, equal to the hydrogen bond in liquid water.[22] The distribution of values (not shown) ranges from 1.4 to 2.6 Å and is asymmetric. The significant variations in the hydrogen lengths are due to thermal motion, and indicate that the first hydration shell has a dynamic character. On average, 15 of the 18 available Hyp residues have formed a hydrogen bond with a water molecule. This means that 83% of the Hyp sites of the peptide are occupied. Related to this is the lifetime of the hydrogen bonds that will be discussed in Section 5. The second, large peak at 2.7 Å in the water density profile, proved much more difficult to assign. However, by examining different MD configurations we believe that the formation of clusters of water molecules around hydrophobic residues emerges as the driving force for the appearance of the second peak.

BINDING PROPERTIES

In Figure 6, we plotted the (averaged) water molecular binding energy defined as

$$\Delta E_{\text{bind}} = E_{\text{tot}} - E_{N-1} \quad (1)$$

where E_{tot} is the total energy of the system and E_{N-1} is the energy of a system where one water molecule has artificially been removed. The final values were obtained by taking the average over all water molecules of 30 statistically independent MD configurations. Close to the triple-helix peptide ΔE_{bind} shows an oscillating behavior with two minima at approximately 1.8 and 2.7 Å. The corresponding binding energies are 23.9 and 20.8 kcal/mol. Compared to value of 20.0 kcal/mol at $r_{\text{THP—O}} = 10$ Å, there is a significant energy stabilization of 3.9 and 0.8 kcal/mol, respectively. The two energy minima have a one-to-one correspondence with the two maxima in the molecular number density plot (Fig. 4). If we look at the dominant contributions to ΔE_{bind}, that is, the electrostatic ($\Delta E_{\text{coulomb}}$) and van der Waals ($\Delta E_{\text{vdW}}$) interactions, we find, as expected, that $\Delta E_{\text{coulomb}}$ dominates ΔE_{bind} and is binding, whereas ΔE_{vdW} is antibinding and about six to seven times smaller. Again, both contributions show an oscillating behavior in the vicinity of the peptide, however, remarkably, they are not "in phase" effectively canceling each other out. For example, $\Delta E_{\text{coulomb}}$ has a minimum at a distance of 2.5 Å, while ΔE_{vdW} has a broad maximum at this distance, and reaches a minimum only at 2.8 Å. This shows that, although the electrostatic interactions are the dominating contribution to the water molecular binding energy, the van der Waals interaction cannot be neglected and contributes substantially to the overall structure of the hydration shell around the triple-helix peptide.

The finding that the van der Waals interaction contributes significantly to the change of the water structure concurs with earlier work that claim that the hydrophobic interaction is the driving force in many (self)-assembly processes in aqueous solution. Hummer et al.[28] developed a theoretical model that quantifies hydrophobic hydration on a molecular scale. They argue that the presence of a hydrophobic core, such as a protein, results in "energetic frustration: of water molecules in the liquid phase. In other words, the balance between the attractive and directional hydrogen bonding forces and the repulsive hydrophobic packing forces is disturbed. In this respect it is interesting to note that the van der Waals contribution to the water molecular binding energy is antibinding and increases by a factor of two in the vicinity of the peptide.

We are aware that the energetic frustration has an entropic component. This component can be deduced in qualitative manner from the number of H-bonds a water molecule forms.[29] A tetrahedrally coordinated water molecule has six possible arrangements of H-bonds that it can form. When it has only three neighbors, the number of possible arrangements drops to 3. Using the data of Figure 3 we estimate that the entropy of a water molecule decreases by 30% at distances below 3 Å from the peptide compared to the entropy of a water molecule in the bulk solution.

To quantify which energetic factors precisely govern the perturbed water structure we computed the nonbonding energy interactions of the different amino acid residues of the collagen-like triple helix peptide with the hydration shell (see Table I). For all residues the dominating interaction with the hydration shell is the electrostatic interaction. Both for the C-terminal as the N-terminal residues the electrostatic contribution is large, due to the charged

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TABLE I. Van der Waals and Electrostatic (Coulomb) Interaction Energies of the Amino Acid Residues of the Collagen-Like Triple Helix Peptide with Its Aqueous Hydration Shell

Residue	ΔE_{vdW}	$\Delta E_{Coulomb}$
Gly (C-term.)	+6.3	−157.7
Gly	−0.6	−8.2
Pro	−4.4	−9.5
Hyp	−3.3	−19.8
Hyp (N-term.)	−3.0	−82.9
H_2O (min. val.)	+2.4	−32.1
H_2O (max. val.)	+9.0	−23.3

Data were obtained by averaging over 40 statistically independent MD configurations. For comparison the minimum and maximum value of a water molecule are also given (see Fig. 6).

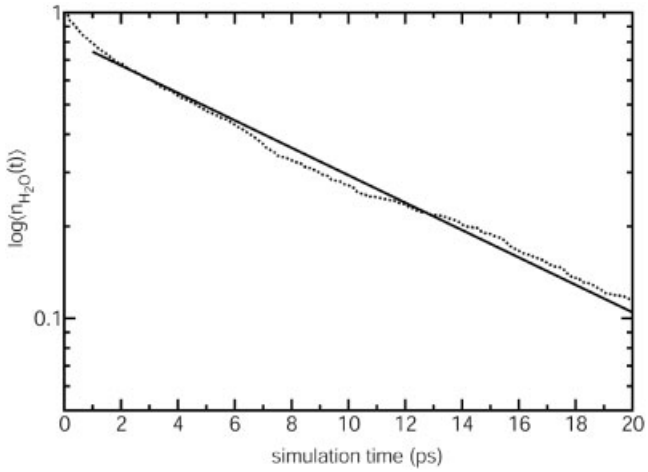

Fig. 7. Logarithmic plot of the time averaged residence time correlation function as defined by Lee and Rasaiah (ref. 32) (dotted line) and the mono exponential fit (full line) of this function with a characteristic time constant of 10 ps.

CO_2^- and NH_2^+ groups. The additional OH group of Hyp leads to an electrostatic interaction twice as large compared to Pro, whereas Gly also has a substantial electrostatic interaction with the hydration shell. More important, except for the C-terminal residues, the van der Waals interactions are negative, contributing to the binding energy, and not canceling, as was found for water molecules in the hydration shell. This raises the question whether a water molecule would rather stay in the bulk solution or in contact with one of the amino acid residues. Certainly, the Hyp residues are competing, for which a water molecule will have roughly the same binding energy either in the bulk solution or in the first hydration shell of the peptide. Our finding that the van der Waals interactions between amino acid residues of the peptide and the water molecules are binding is in contrast with the work of Devarakonda et al.,[30] who studied the interactions of a range of amino acids with the ice {001} surface, to understand which residues contribute the most to the ice inhibiting effect of certain type of antifreeze proteins where they find that for all residues the van der Waals interactions are antibinding.

DYNAMICS

To characterize the kinetics of the first solvation shell around the collagen-like triple helix peptide we follow the work of Impey et al.[31] and compute the average time a water molecule spends in the first solvation shell defined by Lee and Rasaiah[32] as

$$n_{H_2O}(t) = \frac{1}{N_t} \sum_{n=1}^{N_t} [\theta(r,t) \times \theta(r,0)], \qquad (2)$$

where $\theta(r,t)$ is the Heaviside unit step function, which is 1 when a water molecule is within the first solvation shell and 0 otherwise. N_t is the average number of water molecules in this shell. Similar to Impey et al., we used a tolerance time of 2 ps to allow for the rattling motion of the water molecules. A plot of the time average of this function $[n_{H_2O}(t)]$ is shown in Figure 7. The residence time is defined as the mono-exponential fit of this function, for which we find a value of 10 ps. Our computed value in agreement with the NMR experiments of Melacini et al., who find that the residence time has an upper limit in the subnanosecond range. We note that, at odds with the time-average structure of the first shell of hydration, the residence time is rather sensitive to the water model chosen, especially the inclusion of polarization effects in one way or the other is extremely important.[33,34] As the water model we employed does not take into account polarization our computed value gives only a rough estimate, and if we look into the literature, is most probably too short. This is not detrimental to the fact that the residence time of water molecules in the first solvation shell of the peptide will be of the order of picoseconds rather than nanoseconds. The decrease of water dynamics is similar to the work reported by Marchi et al., where the dynamics of the water molecules in the vicinity of lysozyme, a nonhydrogelating peptide, is calculated to be three to seven times slower than that in the bulk.[35] Hence, one should by very cautious in predicting the gelating properties of peptides based solely on the residence time of water molecules in the first solvation shell. Other longer range properties should also be considered.

In a similar fashion to Equation (2), we computed the hydrogen-bond lifetime between hydroxyl hydrogens of the Hyp residues and the oxygen atoms of water molecules in the first solvation shell. Using the hydrogen-bond criteria of Haughney et al.,[27] we compute a value of 1 ps for the hydrogen bond lifetime. This value is similar to the accepted average hydrogen bond lifetime in bulk water,[36] and confirms the average hydrogen bond length we discussed earlier in Section 3. In conclusion, our results indicate that kinetics of the first hydration shell does not show an unusually slow behavior, in agreement with the experiments of Melacini and coworkers, who termed the first hydration shell kinetically labile with upper limits for the water residence time in the subnano- to picosecond range.

The diffusion constant D is key measure of the collective dynamics of the water molecules. Table II gives D, com-

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TABLE II. Water Diffusion Constants as a Function of the Distance from the Collagen-Like Triple Helix Peptide

Δr_{THP-O} (Å)	D (10^{-5} cm^2/s)
< 2.0	2.6 ± 0.7
2.0–4.0	3.0 ± 0.2
4.0–6.0	3.7 ± 0.1
6.0–8.0	4.0 ± 0.1
8.0–10.0	4.0 ± 0.1

Δr_{THP-O} denotes the distance interval from the peptide. Diffusion constants were computed by monitoring the mean square displacement of the water molecules along 10 statistically independent 50 ps MD trajectories.

puted from the mean square displacement of the water molecules, as a function of distance intervals from the collagen-like triple helix peptide. The experimental value of liquid water at 298 K and ambient pressure is 2.3×10^{-5} cm^2/s. The values we computed overestimate this by a factor of roughly 2, which is a known deficiency of the TIP3P water model.[37] Mahoney and Jorgensen al.[37] obtained for liquid water at 298 K and ambient pressure using the TIP3P model a value of $5.19 \pm 0.08 \times 10^{-5}$ cm^2/s. (The value reported in Table II at a $r_{THP—O}$ distance that corresponds to bulk water does not agree with the literature value of Mahoney and Jorgensen.[37] The reason for this discrepancy is because we did not restrain the intramolecular bonds of the water molecules during our simulations. To check this we performed a NVT simulation on a system containing 1000 water molecules at ambient pressure and temperature (1 ATM and 298 K) using the same density as Mahoney and Jorgensen. The intramolecular O—H bonds were restrained to their equilibrium value using the RATTLE algorithm.[38] From this simulation we obtain a value of 5.10×10^{-5} cm^2/s, in good agreement with the NVT literature value (5.06×10^{-5} cm^2/s). From the data in Table II we find that close to the peptide D has decreased by 35%, an important indication of the arrest of water molecules, and hence, of the onset of gel formation. The decrease of D gradually becomes smaller further away from the peptide and reaches bulk values at approximately 6 Å from the peptide.

CONCLUSIONS

Using Molecular Dynamics computational techniques we have modeled how the collagen-like triple helix hydrogelator changes the bulk liquid water state, which might be termed as the onset of gelation. As model for the hydrogelator we used the 54 amino acid residue peptide, experimentally studied by Melacini and coworkers.[10] At a distance of 4 Å and smaller from the hydrogelator the water atom–atom radial distribution functions were partly distorted, especially the oxygen–hydrogen radial distribution function that directly probes the intermolecular hydrogen bonding. The structural distortion is accompanied by a breakdown of the tetrahedrally coordinated hydrogen bonded network. The water structure shows a pattern of alternating regions of high and low molecular density. The 4-hydroxyproline residues play a crucial role due to the formation of hydrogen bonds with water molecules of the first hydration shell. In accord with the structural distortion we found that water molecular binding energy close to the peptide shows a nonregular, oscillating pattern. Although binding energies are dominated by electrostatic interactions the van der Waals interactions cannot be neglected, and seem to substantially contribute to the formation of the gel structure. For the different residues of the peptide we find that both the electrostatic and van der Waals interactions are negative and contribute to the binding energy. This is in contrast with the work of Devarakonda et al.,[30] who found that the van der Waals interactions are positive in a series of calculations on the interaction of amino acids with an the ice {001} surface. In agreement with the experiments of Melacini and coworkers, we find that hydration shell is kinetically labile, with an average residence time of water molecules in the first solvation shell in the order of picoseconds. The computed diffusion coefficient is 35% smaller than the liquid state value, indicative of the onset of gel formation.

Overall, we conclude that our analysis of the water structure and dynamics around the triple helix of a hydrogelating peptide shows that there are a number of phenomena that occur around it. The calculated residence time of water molecules close to the macromolecular surface is similar to that determined experimentally, and is also similar to that found for other protein systems. The first solvation shell therefore is not entirely diagnostic for gelation. However, other longer range changes are computed in the water structure, which are related both to the hydrophobic effect and to the presence of lower/higher packing. Despite the relatively short time of the present simulations, the phenomena are consistent with a signal that could be termed the onset of gelation.

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