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Collagen stability, hydration and native state

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Abstract

Molecular dynamics simulations of a collagen-like peptide (Pro-Hyp-Gly)$_4$-Pro-Hyp-Ala-(Pro-Hyp-Gly)$_5$ have been done in order to study the contribution of the hydration structure on keeping the native structure of collagen. The simulation shows that the absence of water produces a distortion on the molecular conformation and an increase in the number of intra-molecular hydrogen bonds. This is in agreement with previous experimental results showing the stiffness of collagen under severe drying and its increase in the thermal stability. This dehydrated material does not keep, however, the native structure.

Keywords: Hydration; Collagen; Molecular dynamics simulations

1. Introduction

Collagen, the most abundant protein in mammals, has a particular structural motif, the triple helix, shared only with some host defense and membrane proteins. Because of its function as the major structural protein in the extra cellular matrix and the particularity of the triple helix, it has been extensively studied [1,2]. Also, the model polypeptides chains that contain the repeating sequence X-Y-Gly, with X and Y, often proline or hydroxyproline, have been used to investigate on collagen characteristics. Great tensile strength and thermal stability are characteristics of collagen fibers, properties that are grounded on its molecular structure. The collagen triple helix is formed by three left-handed helices supercoiled right-handed around a common axis. The collagen protein family has around 20 members; among them the types I, II, III, V, and XI, found in bones, tendons, skin, which form periodic fibrils and are well characterized [3].

The function of water as a stabilizing agent of collagen have been often considered and extensive studies on the hydration properties and its possible influence on the collagen structure have been done. The existence of water bridges with potential stabilizing properties has been demonstrated both for the native collagen [4–6] and for different collagen-like peptides [7–9]. However, the concept that water is essential to keep the structure has been challenged [10].

Thermal stability is easily checked, either in fibers or in solution [1,2]. However, this is not a true test for the properties of the native state. It is also known that collagen fibers lose their flexibility upon drying [12]. If the dehydration does not proceed below some threshold value, the flexibility can be recovered by rewetting. This changes can be correlated with the sorption isotherm, in which the threshold value can be seen as the point at which the resorption curve follows the same pattern as the desorption one [11]. This irreversible change after drastic drying is shared with other, if not all, proteins [13,14]. Reaching the irreversible state we are faced with a rigid material for which the properties are no longer those of the native state. Thermal stability, as measured for instance by the shrinkage temperature (T_s), is higher than that for the hydrated (native) state [15]. The existence of a collagen-like substance having thermal stability in anhydrous environments or having other substitute with more efficient inductive effect [10] cannot be used as a proof that the water is not essential to maintain the *native* state.

On the other hand, the static picture of the crystallographic data may induce to believe that the relatively large amount of water involved in bridges are rigidly bound to the protein, having long residence times and low mobility. Under this assumptions, the entropic cost would be indeed huge. However, it has been experimentally proved [4,6] that water residence times in the specific sites are in the nanosecond to sub-nanosecond range. Under the circumstance, it is clear that the relative high exchange rate of most of the hydration water must be considered to compute the entropic change. Moreover, the estimated difference between the chemical

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potential of the specific hydration water and that of the bulk is slightly favorable to the bound state [4], ruling out the entropic argument.

In order to check the effect of drying on collagen at a molecular level, we have performed molecular dynamics simulation of a collagen-like peptide (Pro-Hyp-Gly)$_4$-Pro-Hyp-Ala-(Pro-Hyp-Gly)$_5$, that have been widely used as a model for collagen [7–10]. We have simulated the polypeptide in complete absence of water and fully hydrated. The results from the simulation support the idea that dehydration does produce denaturation, although it increases the thermal stability.

2. Methods

2.1. Computational method

Simulations have been carried out using the GROMOS package (Biomos n.v.Groningen) [16]. The equations of motion are solved with the leap-frog algorithm, the system was weakly coupled to a thermal and a hydrostatic bath to work in the isothermal–isobaric ensemble [17] at $T = 300\,\mathrm{K}$ and $P = 1.013 \times 10^5\,\mathrm{Pa}$.

The time step of integration was held on 0.5 fs. All the simulation runs were made in Pentium based personal computers running under GNU/Linux. Plots were done either under MS Windows or in a Silicon Graphics O2 workstation.

The force field of GROMOS was used for collagen in conjunction with the SPC/E water model [18]. In the GROMOS force field, the interactions between non-bonded atoms are modeled with a 6–12 Lennard–Jones potential and through the coulombic electrostatic interactions between the atomic partial charges.

We have used the SHAKE procedure [19] to maintain rigid bond lengths.

2.2. The system

A series of runs were done with a collagen-like peptide molecule in vacuo and with 4747 molecules of SPC/E water. For the hydrated system the average box has dimensions of 3.853 nm × 3.888 nm × 10.1759 nm. The box size was selected such as to include about five water shells around the protein and then let to adjust during the simulation at constant pressure. Periodic boundary conditions were applied.

Each system was equilibrated for 50 ps and run for 200 ps. Averages shown correspond to the last 30 ps. We have used as a starting point the crystallographic coordinates as given by Bella et al. [7] (Protein data Bank code 1CAG). Six residues were eliminated at the extremes of the molecule, including those for which the coordinates were undefined. With the obvious exception of the simulation in vacuo, we have included the crystallographic water.

3. Results and discussion

Although the crystal structure is constrained by the economy of packing of the crystal, we may expect that the molecular structure obtained from crystallography is very close to the native state. Therefore, we have compared the structures obtained from the simulations with crystallographic coordinates.

The effect of different conditions on the overall structure can be observed in Fig. 1. A quantitative comparison can be seen in Fig. 2, which shows the root mean square (RMS) departures of distances of the α-carbon atoms when com-

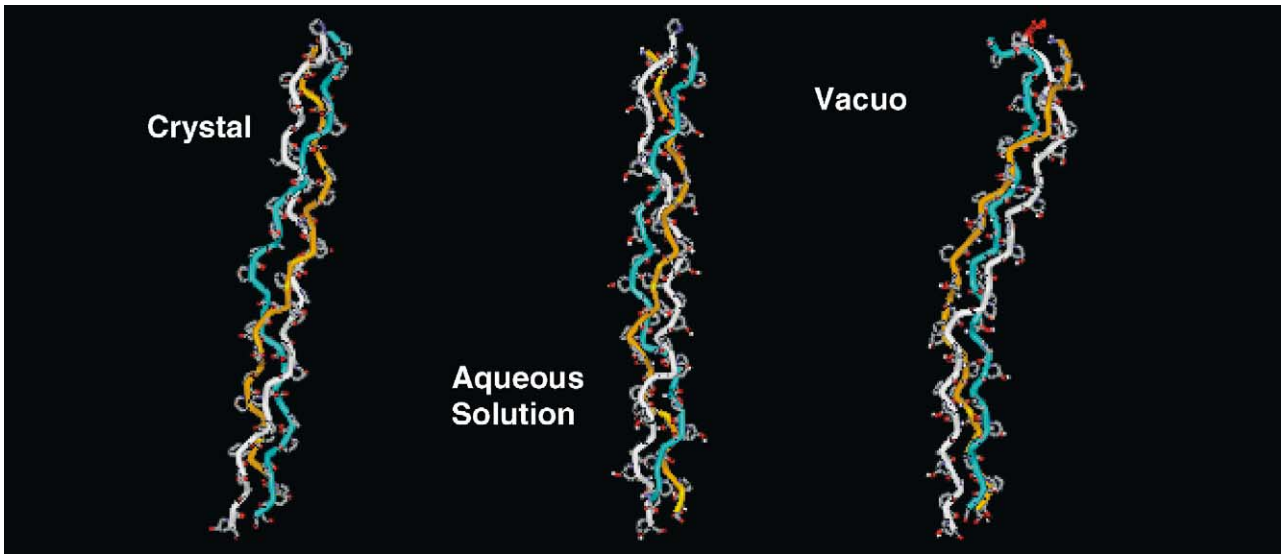

Fig. 1. Structure of the collagen-like peptide (Pro-Hyp-Gly)$_4$-Pro-Hyp-Ala-(Pro-Hyp-Gly)$_5$ as obtained by X-ray diffractions, by molecular dynamics simulation in aqueous solution and in vacuo. The three pictures are oriented in the same position to facilitate the comparison (drawn with WebLabViewer).

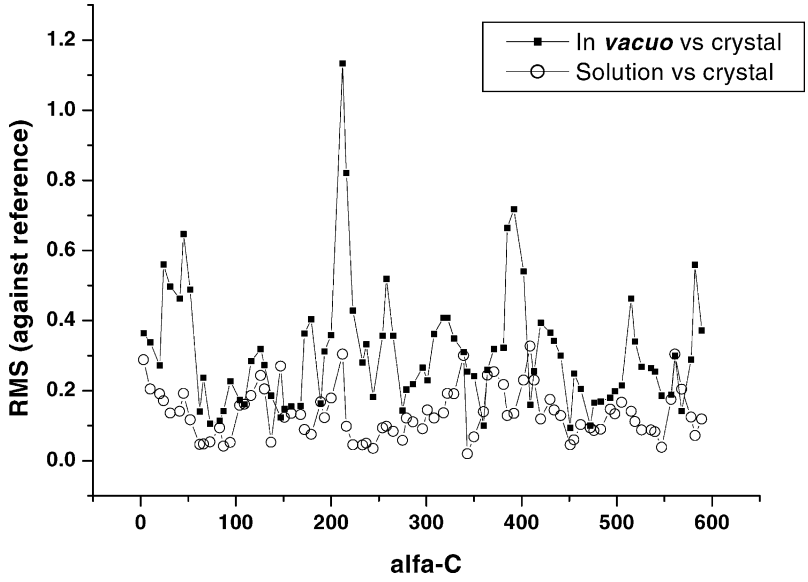

Fig. 2. Difference on the average coordinates between the simulation and the crystal structure seen as the RMS of α-carbon atoms. Simulations in vacuo and in aqueous solution.

pared with the crystallographic data for the two simulated systems. We can see that the results from the simulation in vacuo show the largest departures from the reference.

We may ask ourselves how do these results compare to the experimental data. According to the evidence [1,2] dehydration produce more rigidity on the fibers and also increase the thermal stability.

The protein mobility was analyzed from the trajectories collected along the MD runs. Fig. 3 shows the RMS for α-carbon atoms of each of the simulated systems as an average over 30 ps. We can see there that the absence of the solvent make the molecule more stiff, according to the experimental observations.

We see that around residue numbers 1, 30, 58 and 84 (around atoms 1, 225, 425 and 597, respectively) the mobility, as is seen from the simulation, is higher for all cases. This corresponds to those residues located around the beginning or the end of the chains, having therefore less constrains from the neighboring atoms.

Related to the stiffness of the molecules the most interesting data are the computations of intra-chain hydrogen bonds, as shown in Fig. 4. There the criterion for the existence of hydrogen bonds was that the angle between donor, proton and acceptor is larger than 135° and the distance between the donor and acceptor is equal or shorter than 2.0 nm. We can see that in the simulation that includes water the sys-

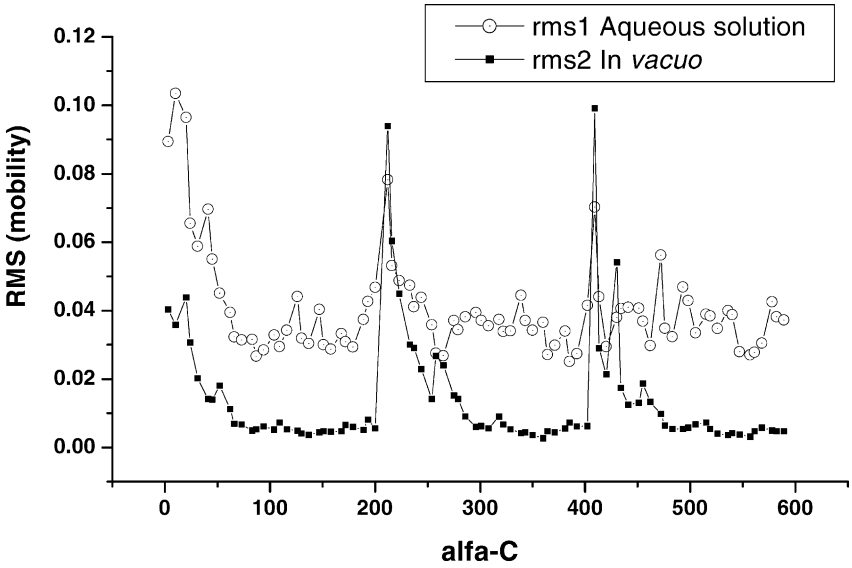

Fig. 3. Comparison between the mobility of collagen-like peptide α-carbon atoms along the simulations for the in vacuo and in aqueous solution.

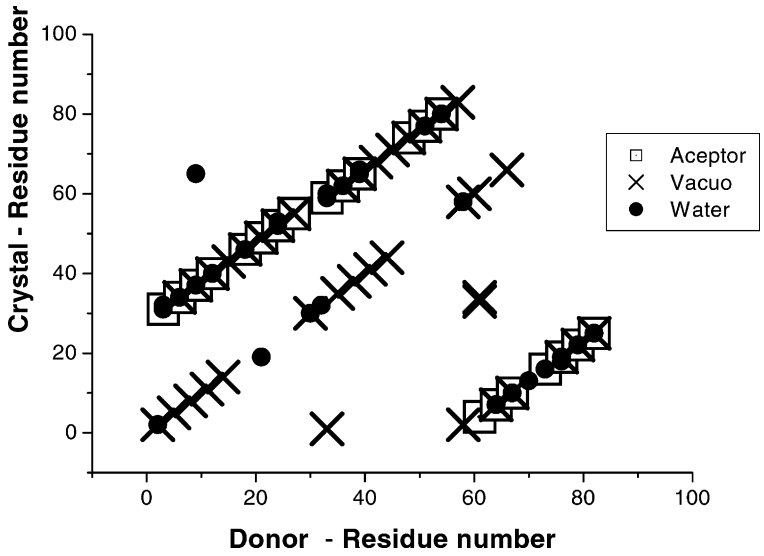

Fig. 4. Plot of the hydrogen bonds between residues determined crystallographically for the dry crystal and detected during the simulation of the system in vacuo and in aqueous solution.

tem presents almost the same number and kinds of hydrogen bonds than in the crystal, while in the simulation in vacuo the number of hydrogen bonds increases. Striking are the large number of bonds near to the diagonal of the graph. The appearance of this line is considered in other proteins a signature of the presence of α-helix, while the lines perpendicular to it represent β-sheets [20]. The α-helix is not a motif present in native collagen, which has a left-handed helix. These extra bonds are neither observed in the crystallographic structure nor in those corresponding to the simulation in presence of water. However, for the structures corresponding to the simulation in vacuo, several regions are formed where this bonds are present. Irrespective of that, it is also seen that the number of hydrogen bonds is larger for the dehydrated systems, with the consequent increase in thermal stability; the structure thus stabilized, however, is non-native.

The existence of water bridges, early suggested by Ramachandran and Chandrasekran [21] and confirmed by NMR [4], is also seen in the simulations and clearly

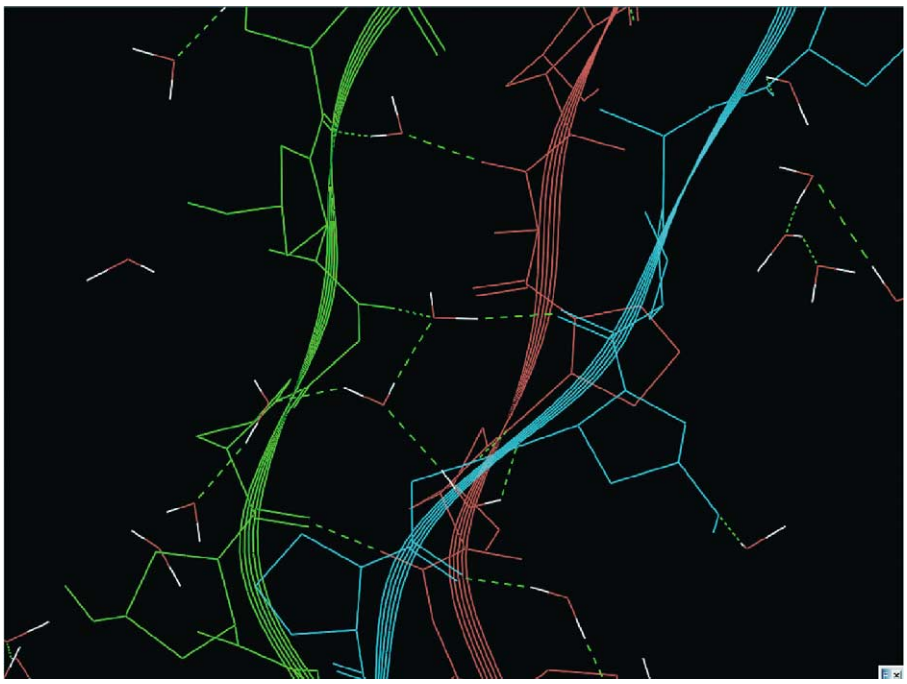

Fig. 5. Detail of the collagen structure obtained by simulation in which two inner-chain water bridges can be observed, as well of some hydration water. For the sake of clarity not all the water present in the system are shown (drawn with WebLabViewer).

contribute to the stabilization of the molecules. The more recent results both by X-ray diffraction [7] and by NMR [6] shown that water bridges are not only inter-chain but also intra-chain. Fig. 5 shows two inter-chain water bridges (a detail obtained from a snap shot of the configurations obtained in the MD run). Note that these water bridges may be connected to the rest of the water network.

It is striking, however, to see the that the conformation remains quite stable even in the extremes, where the occurrence of water bridges is low. This suggests that the overall hydration around the molecule may contribute through its network to help on the stabilization process. According to Bella [7], this hydration shell forms a cylindrical clatharate like the structure around the collagen. In spite of the fact that the exchange rate of the water molecules participating in the hydration shell is relatively high, this *quasi* clatharate structure persist [6]. As a consequence, the non-specific hydration contribution to the stability of the collagen may not be only to provide an appropriate environment to keep the specific hydration molecules (forming the bridges) but also to help in a direct way.

As we have mentioned before, severe dehydration produces irreversible changes. Even under rewetting, the rupture of the extra hydrogen bonds formed is unlikely, producing an increase in the thermal stability, when compared to the native state, even if the measurements are done immersed in water. Notwithstanding that, we are not in presence of a native state.

4. Conclusions

The molecular dynamics simulation of a collagen-like peptide shows, in accordance with the experiments, that the presence of water is essential to keep the native structure of collagen-like molecules. The absence of water induces changes in the structure and increases the rigidity of the molecules. The concept of thermal stability is not by any means an appropriate test for checking the stabilizing effect of the media on the *native* structure.

Acknowledgements

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