Molecular modeling of the process of reversible dissolution of the collagen protein under the action of tissue-clearing agents

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ABSTRACT

The interaction of glycerol immersion agent with collagen mimetic peptide $((GPH)_9)_3$ and a fragment of the microfibril $5((GPH)_{12})_3$ was studied by the classical molecular dynamics method using the GROMACS software. The change in geometric parameters of collagen α-chains at various concentrations of an aqueous solution of glycerol is analyzed. It is shown that these changes nonlinearly depend on the concentration and are limited to a certain level, which correlates with the experimental data on optical clearing efficiency of human skin. A hypothesis on the cause of the decreased efficiency of optical skin clearing at high immersion agent concentrations is put forward. The molecular mechanism of immersion optical clearing of biological tissues is discussed.

Keywords: molecular modeling, immersion optical clearing of biological tissues, glycerol, collagen, molecular dynamics.

1. INTRODUCTION

Employing state-of-art methods of biomedical optics and photomedicine for various diseases diagnostics and treatment implies some difficulties due to the fact that the skin and many other biological tissues are characterized by significant light scattering properties in the visible and near-infrared regions. The scattering occurs due to diverse refractive index at the boundaries of structures such as cellular organelles, lipid droplets, membranes and fibrillar proteins (collagen), which are the main source for the scattering of light in the skin.[1]

These problems are usually solved by injecting biocompatible molecular agents enhancing its optical clarification to some extent[2–5] into a tissue. Quite a number of works[6–11] dwell on experimental in vivo and in vitro studies of the clearing of various types of biological tissues, indicating an active interest in this problem. A mathematical model of the light propagation in biological tissues was proposed in.[12] The effect of model diabetes mellitus on optical clearing of mouse skin is discussed in detail in.[13] In work[14] investigates the mechanism of optical skin clearing using a solution of glycerol as an clearing agent by means of the visualization of the second optical harmonic (SHG-imaging).

To date, it is believed that the mechanism of immersion optical clearing (IOC) of biological tissues has three stages. At the first stage, osmotic dehydration of tissue takes place, leading to a temporary tightening of fibers of the fibrillar proteins. At the second stage, the immersion agents penetrate tissues and partially replace the interstitial fluid. This leads to an increase in the refractive index of the medium and, the difference with the refractive indices of fibrillar proteins is decreased accordingly. At the third stage, immersion agents interact with fibrillar proteins, causing a reversible dissolution of the latter and a consequent decrease in their refractive index. In other words, the second and third stages of the process are characterized by a two-sided equalization of the refractive index between the interstitial medium and

fibrillar proteins, resulting in optical clearing. Attempts have been made to define correlations between the parameters of optical clearing efficiency (OCE) and such characteristics of immersion agents as osmolarity and the value of the refractive index in natural conditions. As shown in,[1] these attempts were unsuccessful. Molecular modeling, carried out in[1] employing the method of classical molecular dynamics, suggested the existence of the relationship between OCE and the time immersion agents remain in the hydrogen bonded state with the collagen protein. These data were confirmed in.[15] Thus, it can be concluded that the third post-diffusion stage of IOC of biological tissues plays a key role in the entire process of optical clearing. Therefore, theoretical studies of molecular processes at this stage are necessary to understand the entire process of optical clearing of biological tissues correctly.

This paper is dedicated to molecular modeling of the processes of reversible dissolution of fibrillar collagen protein when it interacts with an aqueous solution of glycerol by methods of classical molecular dynamics.

2. METHOD OF MOLECULAR MODELING

Collagen mimetic peptide ((GPH)$_9$)$_3$ was used as a molecular model of collagen,[16] as it forms the basis of the majority of the regular domains of human collagen (Fig. 1a). These relatively small synthetic peptides are often used as a basis for molecular modeling. A fragment of microfibril collagen (Fig. 1b), which is an ensemble of five mimetic peptides ((GPH)$_{12}$)$_3$ was also studied.[17] Three-dimensional models of peptides were built on the basis of the data from Protein Data Bank (PDB), then hydrogen atoms were added and structures were optimized by the molecular mechanics method.[18] We considered the triatomic alcohol – glycerol, as an immersion clearing agent (Fig. 1c), its experimental data on the parameters of optical clearing of the human skin are available in.[19,20]
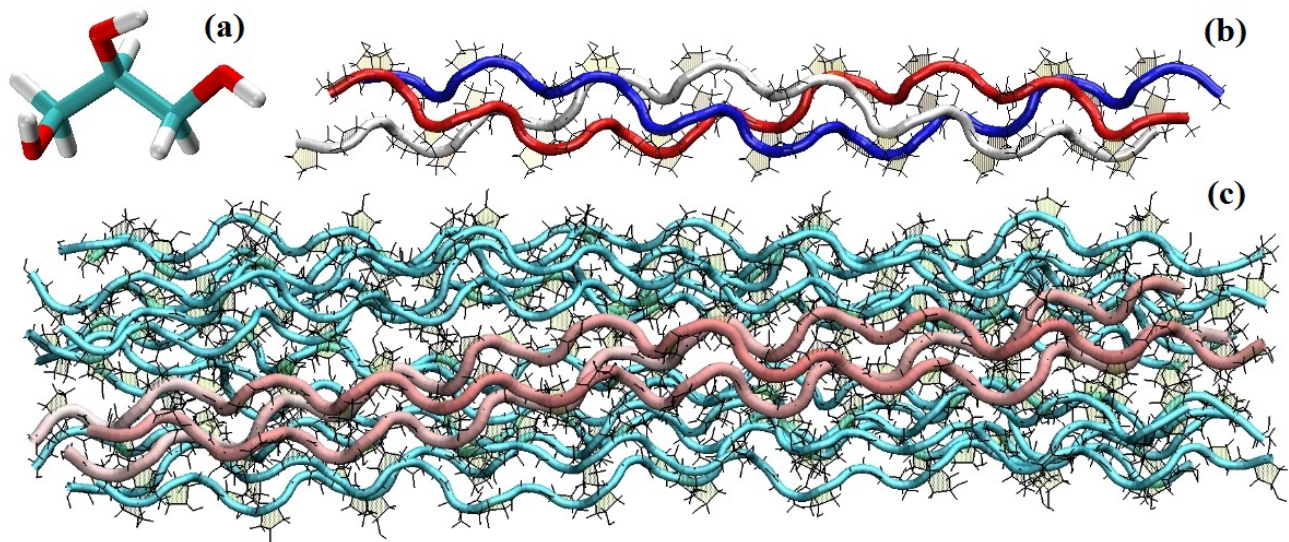

Figure 1. Spatial structures of a clearing agent molecule: glycerol (a), as well as collagen peptides ((GPH)$_9$)$_3$ and 5((GPH)$_{12}$)$_3$: (b) and (c), correspondingly.

Molecular modeling of the interaction of glycerol with collagen encompassed two stages. At the first stage, B3LYP/6–311+G(d),[21,22] a method of quantum mechanics, was implemented to calculate the lowest-energy conformation of a glycerol molecule in an isolated state using the Gaussian software.[23] The calculated geometric parameters and the Mulliken atomic charges were subsequently used in modeling the immersion agent within the framework of classical molecular dynamics.

At the second stage of modeling, the classical molecular dynamics method was implemented with the use of the GROMACS software[24] to study the effect of an aqueous solution of glycerol on the geometric parameters of the α-chains of the collagen peptide (Fig. 2) within the AMBER03 force field.[25] The model scene was a three-dimensional space cell shaped as a rectangular parallelepiped with the sides of the following length: $3 \times 9 \times 3$ nm for peptide ((GPH)$_9$)$_3$ and $5 \times 13 \times 5$ nm for peptide 5((GPH)$_{12}$)$_3$; the corresponding collagen peptide was placed in the center of the cells. The rest of the space was filled with an aqueous solution of glycerol, with the concentration varying from 0% (pure water solvent SPC/E model[26]) to 60% in 10% increments. The boundaries of the cell were set to be periodic (in the case of a collision

with the boundary, a molecule passes through it and appears from the opposite boundary side). The initial velocities of the atoms were randomly generated by means of GROMACS package and had the Maxwell distribution corresponding to the set temperature. To model the system, the Berendsen thermostat and barostat[27] were used to ensure the convergence of the temperature and pressure of the system to the set values of $T_0 = 300$ K and $P_0 = 1$ bar. The model had a time step of 0.0001 ps, and total time of the model was 5 ns. The state of the system was recorded every picosecond. The recorded molecular trajectories were processed by means of the GROMACS package and the VMD software (Visual Molecular Dynamics).[28] To carry out comparative analysis of each system, the period from 4 to 5 ns was set as control period of time. The average of the results obtained at the control period of time was calculated. The calculation of standard deviation of the arithmetic average was also performed.
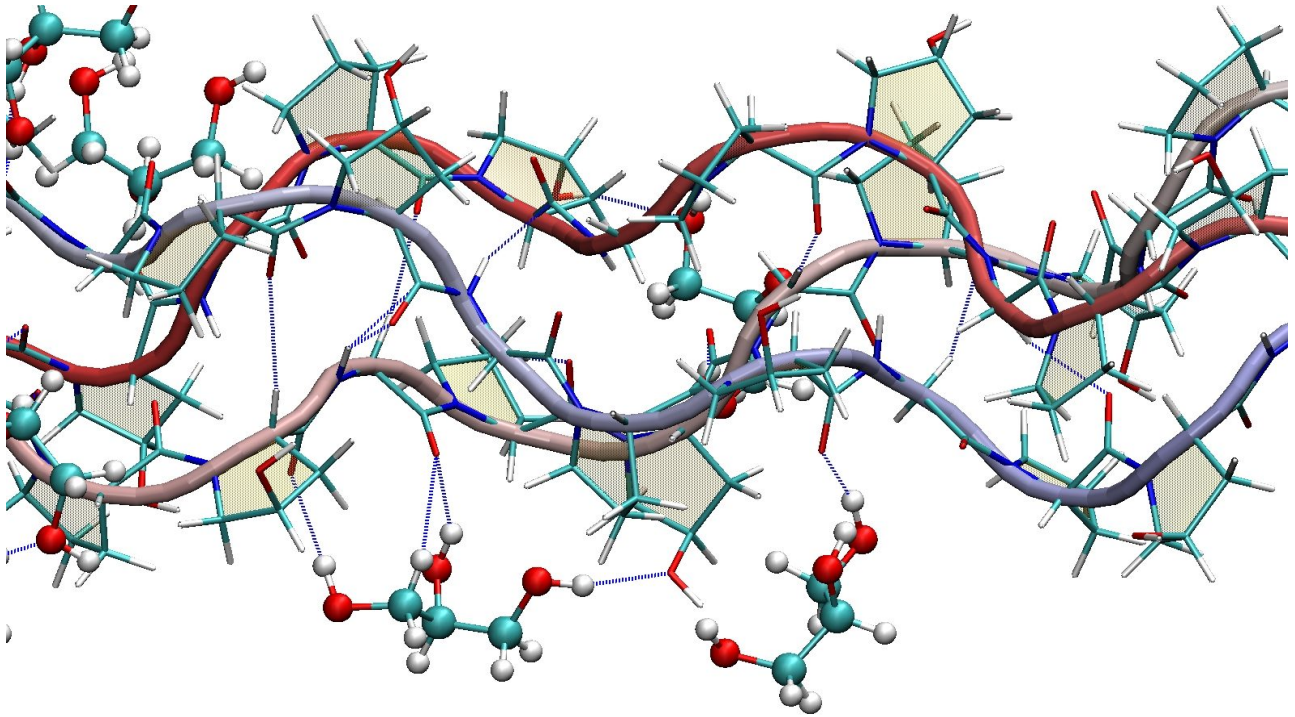

Figure 2. Spatial structure of hydrogen-bonded complex of glycerol and collagen peptide $((GPH)_9)_3$ molecules, obtained within the classical molecular dynamics. The Figure shows the hydrogen bonds that were formed.

At the second stage of the modeling, the authors chose the following geometric parameters of the α-chains of the peptide (Fig. 3): L, the length of the helix, and R, its radius (calculated as the average distance from the Z axis to C_α - atoms of the chain); Δ, its shift, and ω its rotation (implying the distance between two residues projected to the axis of the helix and the angle between them); the angles φ and ψ (characterize the rotation about the single bonds C_α-N and C_α-C, respectively); the value of the two-edged angle θ (it is formed by two half-planes $C_{\alpha1}$-N-C and N-C-$C_{\alpha2}$) – to analyze the effect of the clearing agent on the tertiary and quaternary structure of the collagen. The change in the distance between different chains of the collagen peptide was also calculated (by observing the changes in the sum of the distances between C_α atoms of identical residues of different α-chains). Since the tertiary structure of the collagen peptide is stabilized by intra-peptide hydrogen bonds, and hydrogen bonds formed between the tertiary structures add up to the stability of its quaternary structure, so the average time of each amino acid residues remaining in the hydrogen-bonded state can be used as an additional parameter describing the stability of the system. The analysis of the changes in all the parameters described above makes it possible to enhance understanding of the mechanism of the immersion agent influence on collagen structure. However, the volume of a molecule seems to be the best parameter connecting the change in the structure of collagen peptide under the influence of the clearing agent and optical clearing of the medium, since it is the change in the geometric dimensions of the scatterers, as well as the change in their shape and refractive index, that has a maximum effect on the optical parameters of a medium.
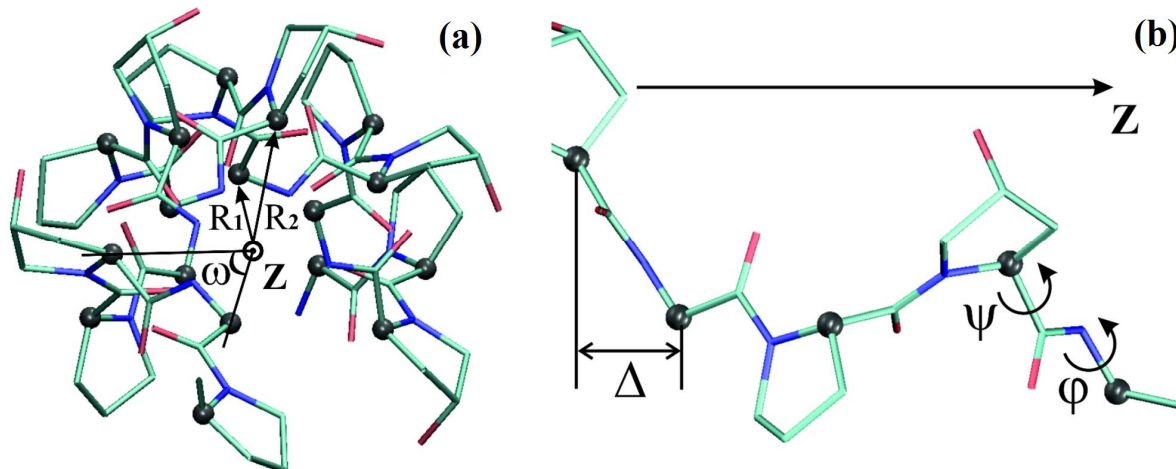
Figure 3. Geometrical parameters of collagen peptide α-chains. A single α-chain is depicted in frontal (a) and lateral (b) projections.

All the parameters listed above were assessed using standard procedures included in the GROMACS package: the geometric parameters of α-chains, the distance between them and the volume of the collagen molecule were calculated using the procedures gmx_helix, gmx_distance and gmx_freevolume,[29] respectively. The hydrogen bond analysis was carried out using the hydrogen_bonds procedure, which is part of the VMD package. It is considered that a hydrogen bond is formed between the atoms if the following geometric criteria[30] are met: $R \leq 3.5$ Å and $\gamma \leq 30$ °, where R is the distance between the donor atom A, covalently bound to the hydrogen atom H and the acceptor atom B of another molecule (or functional group of the same molecule), and γ is the angle formed by the bonds AH and AB.

3. RESULTS AND CONSIDERATIONS

Table 1 shows the geometrical parameters for three α-chains of peptide $(GPH_9)_3$ obtained by means of the molecular modeling. As Table 1 demonstrates, glycerol added to water medium has an impact on the majority of the α-chains geometrical parameters, describing the rotation of amino-acid residues in a chain with respect to each other: angles φ and ψ. In contrast, changes are far less significant in the α-chains geometrical parameters describing the shift of amino-acid residues in a chain with respect to each other, namely L and Δ. This is due to the following: when hydrogen bonds are formed during interaction between collagen peptide and glycerol molecules, it is more energetically feasible to unfold a group of atoms directly involved in interaction, than to dislocate it. As all three chains are wound into a single helix, the parameters, such as R and ω reflect alterations not only within a specific chain, but in the entire tertiary structure of a collagen molecule. Due to the above, they are more sensitive to all alterations in a collagen molecule special structure.

Table 1. Influence of adding glycerol to water medium of collagen molecule on the geometrical parameters of three α-chains of peptide $(GPH_9)_3$.

α-chains parameter	0 % glycerol solution			40 % glycerol solution			Relative difference, %		
	Chain #1	Chain #2	Chain #3	Chain #1	Chain #2	Chain #3	Chain #1	Chain #2	Chain #3
R, nm	0.3	0.4	0.4	0.3	0.3	0.4	7.0	-8.1	8.6
L, nm	7.3	7.5	7.2	7.4	7.1	7.2	1.0	-4.5	0.7
Δ, nm	0.3	0.3	0.3	0.3	0.3	0.3	0.8	-0.9	0.8
ω, deg.	25.1	19.3	22.4	24.5	26.4	17.4	-2.3	36.5	-22.4
θ, deg.	-89.3	-90.2	-88.9	-90.2	-92.1	-90.0	1.1	2.1	1.3
φ, deg.	-63.5	-65.7	-65.7	-70.2	-70.1	-70.0	10.5	6.7	6.4
ψ, deg.	97.9	114.8	99.5	119.8	116.8	118.2	22.3	1.8	18.8

As follows from the analysis of distances between different chains in collagen peptide $(GPH_9)_3$, if the immersion agent is added to the system, they do not change significantly, which means that internal peptide hydrogen bonds are not weakened. The exception is the least stable molecule areas located at their ends.

Table 2. Influence of adding glycerol to water medium of collagen molecule on the geometrical parameters of three α-chains of microfibril-peptide $(GPH_{12})_3$.

α-chains parameter	0 % glycerol solution			40 % glycerol solution			Relative difference, %		
	Chain #1	Chain #2	Chain #3	Chain #1	Chain #2	Chain #3	Chain #1	Chain #2	Chain #3
R, nm	0.3	0.4	0.5	0.3	0.5	0.4	2.0	28.9	-7.2
L, nm	10.5	10.3	10.4	10.5	10.4	10.4	0.0	0.9	0.3
Δ, nm	0.3	0.3	0.3	0.3	0.3	0.3	-0.1	1.0	0.5
ω, deg.	21.0	16.4	13.3	17.1	11.5	11.4	-18.8	-29.8	-14.1
θ, deg.	-95.9	-93.8	-95.2	-94.5	-95.2	-94.2	-1.5	1.5	-1.0
φ, deg.	-72.4	-67.7	-68.9	-71.1	-65.3	-69.6	-1.8	-3.7	0.9
ψ, deg.	125.0	133.4	133.8	134.1	130.3	130.6	7.3	-2.3	-2.4

Table 2 shows the corresponding results gained for three α-chains of peptide $(GPH_{12})_3$ from collagen microfibril fragment. The comparative analysis of the corrseponding results in Table 2 and Table 1 demonstrates that this structure is more resistant to external impacts. The α-chains geometrical parameters describing the rotation of amino-acid residues in a chain with respect to each other are 3-4 times less in absolute values than the same values for peptide $(GPH_9)_3$. The α-chains geometrical parameters describing the shift of amino-acid residues in a chain with respect to each other don't change significantly as the previous modeling results showed as well. Since this modeling structure shows spatial structure elements of a higher order (five triple spirals $(GPH_{12})_3$ also wound in a single spiral), such parameters as R and ω influence not only tertiary collagen structure but quaternary one as well.

Table 3. Average time (in %) of peptide collagen α-chains remaining in hydrogen-bonded state for one amino-acid residue.

Glycerol concentration	Peptide $(GPH_9)_3$	Peptide $5(GPH_{12})_3$
0%	9.9	22.3
10%	8.8	21.4
20%	11.0	19.8
30%	10.6	19.8
40%	8.2	22.6
50%	8.5	20.5
60%	8.9	24.2

Besides, to make a comparative assessment of how stable the collagen peptides used in molecular modeling are, the time of amino-acid residues remaining hydrogen-bonded to each other was used (Table 3). The Table shows that a peptide assembly of five collagen peptides maintains the hydrogen bond more than two times longer than an individual peptide on average. This is caused hydrogen bonds in peptide $5(GPH_{12})_3$ formed both between α-chains inside a single peptide and between lateral chains of different peptides. The Table also shows that there is no clear correlation between the time of peptides remaining hydrogen-bonded and glycerol concentration. Moreover, this parameter does not take in account the hydrogen bond force.

The most informative parameter to ascertain correlation between immersion agents interacting with collagen protein and optical clearing efficiency is the protein volume change. Currently a lot of experimental data assessing biological tissue optical clearing efficiency with glycerol are available.[19,20] It is worth mentioning that different authors use different parameters to assess optical clearing efficiency. For instance,[19] uses collimated light transmission coefficient in the medium as an assessment parameter, while[20] uses the volume of light that enters into the analyzed medium and the signal penetration depth measured by optical coherence tomographer.

Within the framework of molecular modeling we examined the relation between collagen peptides volume change and glycerol concentration (Fig. 4). Figure 4 shows that this relation is nonlinear: its maximum is reached at medium-range concentration (40%). Experimental data analysis of human skin optical clearing by means of glycerol,[19,20] shows that the optical clearing efficiency is at its maximum at higher immersion agent concentration (60-70%). This difference can be explained in the following way: when this interaction is molecularly modulated, the glycerol concentration is set inside the intercellular space, while the experimental data indicate glycerol concentration prior to skin application. Since the tissue already has some amount of liquid inside, it can be expected that the glycerol concentration inside the tissue will be smaller than the initial one.
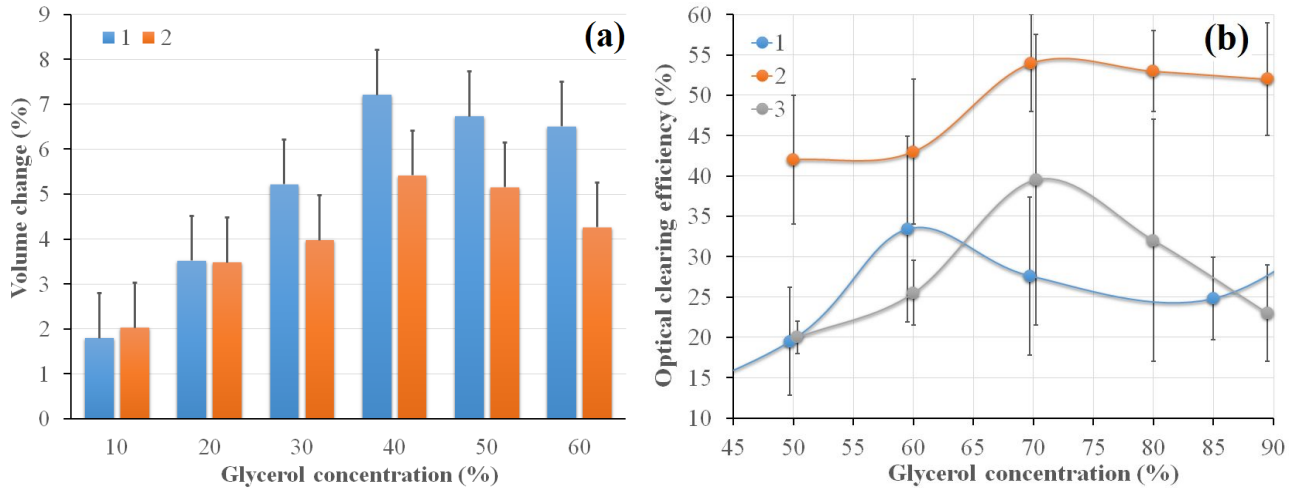
Figure 4. Dependence of collagen peptide volume change on glycerol concentration (a). The experimental data of the dependence of the value of optical clearing efficiency on glycerol concentration (b): 1 is the coefficient of collimated transmission in the medium,[19] 2 and 3 are the volume of light that enters into the analyzed medium and the signal penetration depth measured by optical coherence tomographer.[20]

Maximum value occurring in collagen molecule volume change at medium-range glycerol concentration can be explained in the following way. Firstly, collagen surface has a finite number of seats, i.e. "molecular pockets" suitable for efficient attachment of glycerol molecules. Secondly, the molecular modeling reveals that the higher the concentration of glycerol is, the more probable is the formation of hydrogen-bound self-associates (Fig. 5). With time these self-associates form cluster structures, which leads to nonuniform impact on different areas of collagen molecules. In its turn, this decreases the destruction rate of water sheath covering collagen and, subsequently, decreases glycerol influence on collagen.
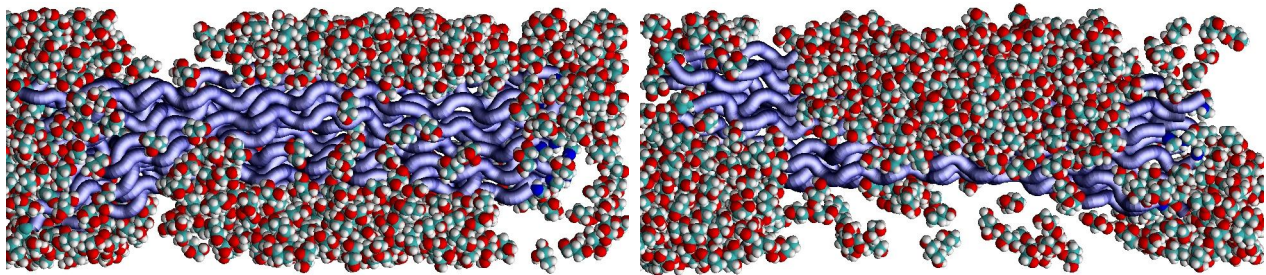

Figure 5. Spatial distribution of glycerol molecules around collagen peptide $5(GPH_{12})_3$ during 5 ns of observation in two different lateral projections.

Based on the performed molecular modeling, the mechanism of fiber swelling under glycerol influence can be described as follows: when attached, glycerol molecules of the greatest affinity to collagen push out the water bound to it. This disrupts the net of hydrogen bonds between collagen fibrils and leads to fibril protein swelling. The disruption of hydrogen bonds net occurs because glycerol molecules bound to collagen by alcohol groups, "stick out" their hydrophobic parts (CH_2-groups) and prevent formation of new hydrogen bonds.

4. CONCLUSIONS

Employing the methods of classical molecular dynamics, the molecular modeling of interaction of glycerol clearing agent with collagen fibril protein was performed.

Nonlinear relation between collagen volume change and glycerol concentration in an aqueous solution was established, which correlates well with experimental data on human skin optical clearing efficiency.

Theoretical explanation of molecular mechanism for the third (post-diffusion) stage of human skin optical clearing and its efficiency in relation to glycerol concentration in a solution is given.

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