Molecular modeling of post-diffusion stage of biotissue optical clearing under effect of iohexol aqueous solution

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Abstract. Interaction of iohexol (Omnipaque), an X-Ray contrast agent, with a mimetic peptide of collagen (GPH), as one of the main components of biological tissues has been studied with the use of methods of classical molecular dynamics (GROMACS). Complex molecular modeling of the post-diffusion stage of optical clearing allowed to evaluate such parameters as the average number of hydrogen bonds, formed between the clearing agent and collagen per unit time, and the immersion agent’s effect on changes in the collagen peptide volume. The obtained results are compared with similar results for glycerol, a polyatomic alcohol, and with the existing experimental data on the efficiency of optical clearing of these immersion agents.

1. Introduction

The application of modern methods of biomedical optics and photomedicine for diagnosis and therapy of diseases entails difficulties arising because of strong scattering in visible and near-infrared regions, inherent to skin and many other biological tissues. This scattering happens due to inhomogeneity of the refractive index at borders of such structures as cell organelles, lipid droplets, membranes and fibrillar proteins (collagen), which are primarily responsible for light scattering of skin [1]. These difficulties are usually overcome with introduction of biocompatible molecular agents into the tissue, which to some extent facilitates its optical clearing. A lot of papers are dedicated to in vivo and in vitro experimental studies on the clearing of different biological tissues, which indicates a strong interest in the issue.

It has been considered so far that the mechanism of immersion optical clearing of biotissues has three stages. The first stage is osmolar dehydration of the tissue, leading to compaction of fibers of fibrillar proteins. At the second stage, immersion agents penetrate into the tissues and partially substitute the interstitial fluid. It results in an increase in the refractive index of the medium and, consequently, in a decrease in the difference with the refractive indices of fibrillar proteins. At the third stage, immersion agents interact with fibrillar proteins, which leads to reversible dissolution of
the latter and, as a result, to a decrease in their refractive index. In other words, the process of two-sided balancing of the refractive indices between the interstitial fluid and fibrillar proteins takes place at the second and third stages, which leads to optical clearing.

The molecular modeling, conducted in [1,2] with the method of classical molecular dynamics, allowed to assume that there is correlation between the optical clearing efficiency and the time of the immersion agent being in a hydrogen-bonded state with collagen protein. Therefore, it suggests that the third, post-diffusion stage of immersion optical clearing of biotissues plays a significant part in the whole process of optical clearing. That is why theoretical studies on the molecular processes, taking place at this stage, are essential for proper understanding of the whole process of biotissue optical clearing.

A large number of substances and their combinations have been studied as immersion agents so far. Some of them demonstrated high efficiency in the process of biotissue optical clearing. All the known substances for immersion can be conveniently classified into the following groups – polyatomic alcohols, sugars, organic acids, other organic solvents, and x-Ray contrast agents. Glycerol, glucose and polyethylene glycol are the most widely used agents, especially in case of skin clearing, due to their good compatibility and pharmacokinetics in relation to biological tissues.

These substances are generally considered non-toxic; nevertheless, when living tissues are exposed to them for a long time, it may lead to such negative effects as localized hemostasis, excessive compaction of tissues and venous or arterial hyperemia. Therefore, seeking for the safest agent and choosing the optimal concentration and time of exposure remain one of the major tasks. Recent research has shown that iohexol (Omnipaque), an x-Ray contrast agent, is one of the promising substances in this regard.

The present paper dwells on molecular modeling of the processes of reversible dissolution of collagen fibrillar protein under its interaction with aqueous solution of iohexol (Omnipaque) and comparative analysis of interaction of this agent and glycerol, a polyatomic alcohol, with a collagen mimetic peptide (GPH) through methods of classical molecular dynamics.

2. Molecular modeling methods

A mimetic peptide of collagen (GPH)₃, forming the basis of a great part of regular domains of human collagen, was used as a molecular model of collagen. Such relatively small synthetic peptides are often used for collagen molecular modeling. A peptide 3D model was built according to the Protein Data Bank (PDB) data with further addition of hydrogen atoms and optimization of the structure by the molecular mechanics method. Five molecules of iohexol and glycerol were treated as immersion clearing agents.

Molecular modeling of interaction of clearing agents with collagen was carried out in several stages. At the first stage, the method DFT/B3LYP/6-311+G(d,p) [3,4] and the program Gaussian [4] were used to identify and calculate all the lowest energy conformers of the considered clearing agents in their isolated state.

At the second stage of the modeling, methods of classical molecular dynamics were used to analyze formation of hydrogen bonding between the collagen peptide ((GPH)₃), and the chosen molecular agents. Molecular modeling of interaction of these agents with collagen was carried out with GROMACS package of classical molecular dynamics with AMBER-03 force field. Before the start of each modeling, 20 molecules of the agent were distributed randomly within the cell. Initial velocities of atoms were set with the use of random-number generators of GROMACS package and had the Maxwellian distribution, corresponding to the chosen temperature (300K). The total time of modeling was 100 ps. Recorded tracks of molecular motion were processed by GROMACS tools and with the use of VMD (Visual Molecular Dynamics) program. Each system under study was modeled 30 times, and obtained results were averaged. The average number of hydrogen bonds, formed between low-molecular agents and collagen per unit time, was estimated within this stage of modeling.

At the third stage of modeling, the study focused on the effect of aqueous solutions (40%) of immersion agents on geometric parameters of α-chains of a collagen microfibril fragment, which is an ensemble of five mimetic peptides ((GPH)₁₂). The total time of modeling at this stage was 1000 ps.
3. Findings and discussion

Figure 1a,b gives graphical representation of the low-energy conformers of the immersion agents, calculated with the method DFT/B3LYP/6-311+G(d,p). Figure 1c,d presents spatial configurations of mimetic peptides of collagen, used in the present study.

![Spatial structures of molecules of the immersion agents](image1)

**Figure 1.** Spatial structures of molecules of the immersion agents – iohexol (a), glycerol (b) and collagen peptides ((GPH)$_9$)$_3$ and 5((GPH)$_{12}$)$_3$ – (c) and (d) respectively.

To make a discussion on the qualitative experimental [5-9] and theoretical parameters of the immersion agents more convenient, all of them are given in Table 1. The table also contains the main characteristics of the substances.

| Parameter                                               | Glycerol | Iohexol |
|---------------------------------------------------------|----------|---------|
| Refraction index, n                                     | 1.474    | 1.432   |
| Osmolarity, Osm/L                                       | 10.87    | 0.465   |
| Molecular mass, g/mol                                   | 62.09    | 821.14  |
| Increasing intensity of a second-harmonic generation signal at the depth from 50 to 200 µm | 1.54     | 1.22    |
| Changes in the relation between the Raman peaks on 938 cm$^{-1}$ and 922 cm$^{-1}$ at the depth of 40 µm after 60 minutes | 0.88±0.05 | 1.34±0.03 |
| Changes in the relation between the Raman peaks on 938 cm$^{-1}$ and 922 cm$^{-1}$ at the depth of 120 µm after 60 minutes | 1.34±0.04 | 1.43±0.06 |
| Average number of hydrogen bonds, #/ps                 | 0.840 [2] | 1.797   |
Comparative analysis of structures of the agents under study clearly demonstrates that an iohexol molecule contains significantly more atoms, capable of forming hydrogen bonds with a collagen peptide molecule, than glycerol. It correlates well with the molecular modeling results. The average number of hydrogen bonds (see the Table) per time unit for iohexol molecules, obtained with the modeling, is more than twice as high as the same value for glycerol. However, no regard to the aqueous environment of collagen molecule was paid at this stage of the modeling.

The next stage consisted in molecular modeling of interaction between aqueous solutions (40%) of immersion agents and a microfibril fragment of collagen $((\text{GPH})_{12})_{3}$. The effect of immersion agents on geometrical dimensions of collagen molecules – an important parameter for evaluation of the optical clearing efficiency – was analyzed. The results, shown in Figure 2, demonstrate that the effects of both agents on changes in the structure of a collagen microfibril fragment are compatible. Only the first 130 ps demonstrate a significant difference in the velocity of changes in the geometrical dimensions of collagen molecules. It is connected with differences in the mobility of molecules, which is natural, taking into account their significant difference in geometrical dimensions and masses.

**Figure 2.** Time dependence of changes in the volume of the collagen peptide $5((\text{GPH})_{12})_{3}$ from the time of interaction with its environment: 1 – clean water; 2 and 3 – 40% aqueous solutions of glycerol and iohexol.

Experimental studies from [5-9] on the *ex vivo* effect of the immersion clearing agents, considered in this paper, on skin and its various components like collagen and water showed that, in most cases, the optical clearing efficiency of glycerol is somewhat higher than the one of iohexol. For example, methods of multiphoton tomography allowed to study the effect of introducing immersion agents on second-harmonic generation signals. The results show (see the Table) that at the depth from 50 to 200 µm, the intensity of a second-harmonic generation signal of glycerol became 20% higher than the one of iohexol. These papers also studied the effect of immersion agents on collagen hydration in deep layers of skin with Raman spectroscopy methods. The correlation between the Raman peaks on 938 cm$^{-1}$ and 922 cm$^{-1}$ was calculated to evaluate collagen hydration when applying immersion agents. This correlation is widely used as a marker of collagen hydration, indicating an increase in collagen hydration. It is clearly seen from the Table that the 60-minute exposure of glycerol led to strong dehydration down to the depth of 120 µm. At the same time, iohexol had a significantly less effect at the same depths. Therefore, despite its lower efficiency of optical clearing in comparison with glycerol, iohexol is a promising immersion agent that provides significant optical clearing of the skin cover with no noticeable effect on the tissue structure.

4. Conclusion
Some differences in evaluating the optical clearing efficiency, found during the molecular modeling and obtained in the experimental studies, presented in papers [5-9], are due to the fact that the present
paper considers only the post-diffusion stage of biotissue optical clearing with molecular dynamics methods, while it is also necessary to take into account other stages of the process in order to describe it more adequately, for example the velocity of diffusion of immersion agents. Thus conducting further research in the field of molecular modeling opens the door to a deeper understanding of the molecular processes that lead to biotissue optical clearing, which, in its turn, will allow to find efficient clearing agents with tailor-made properties.

References
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