Effect of terahertz radiation on intermolecular interactions of albumin under aerobic and anaerobic conditions

E A Nemova¹, G G Dultseva², N A Nikolaev¹,³, O P Cherkasova¹,⁴
¹Institute of Laser Physics SB RAS, 15 B Academician Lavrentiev Ave., Novosibirsk, Russia
²Voevodsky Institute of Chemical Kinetics and Combustion SB RAS, 3 Institutskaya St., Novosibirsk, Russia
³Institute of Automation and Electrometry SB RAS, 1 Academician Koptyug Ave., Novosibirsk, Russia
⁴Novosibirsk State Technical University, 20 Karl Marks Ave., Novosibirsk, Russia

Corresponding author: Nemova Evgenia, email: endy@ngs.ru

Abstract. The effect of THz radiation on coupling between albumin molecules under aerobic and anaerobic conditions is assessed through an EPR-quantified procedure. Rearrangements induced by terahertz radiation are found to affect the hydrogen bonding network causing an increase in the rate of bimolecular interactions. Molecular mechanism of the observed effect is considered involving the interactions between the functional groups of albumin molecules. Irradiation causes conformational changes in albumin molecules, which involves changes in the steric states of molecules. These rearrangements hinder or simplify the adsorption of specific agents on the reactive sites of albumin molecules.

1. Introduction
The appearance of contemporary THz sources and detectors stimulates a rapid development of medical applications and causes an increased exposure of human population to THz waves [1, 2]. At the same time, the biological effects of THz radiation remain insufficiently investigated [3-7].

Intermolecular interactions are crucial for the transport functions of albumins, so interactions with different agents to be transported in vivo are the subject of extensive investigation. Albumin conformations determine the possibilities of binding with oxygen, nitrous oxide, various metal complexes of biological significance. The effect of preliminary terahertz radiation on conformation changes in albumin molecules has already been traced as changes in albumin binding with molecular oxygen, in albumin-mediated NO - NO₂ equilibria, and in the rates of interaction with ozone [8]. The possibility to assess conformation changes in globular proteins is provided by the presence of paramagnetic centers in these biopolymers: the parameters of their EPR spectra (the general structure, hyperfine splitting constants, line width, and decay kinetics) allow one to determine chemical structure, mobility, oxidation state, lifetimes of paramagnetic centers formed and decayed in various interactions [9]. Paramagnetic centers permanently existing in albumin molecules in aqueous solutions interact not only with dissolved oxygen molecules and nitrous oxide but also with the same centers of other albumin molecules. These kinds of interactions are strongly dependent on conformation states,
so it could be expected that preliminary irradiation of filmed albumin preparations in the terahertz range (before dissolution) would affect also intermolecular binding between protein molecules.

The goal of the work was to quantify the influence of terahertz radiation on coupling between albumin molecules in the presence of oxygen and in its absence. To assess the rates of intermolecular interactions, a quantitative EPR-based technique was applied in combination with high performance liquid chromatography and the methods of qualitative chemical analysis.

2. Experimental

Bovine serum albumin BSA (Sigma-Aldrich, lyophilized powder) was dissolved in distilled water to prepare a solution with the concentration 1 mg/mL. A drop of this solution (0.05 mL) was applied onto a substrate made of crystal quartz and dried in the air.

The sample was irradiated with terahertz waves using a THz-TDS described in detail in [10]. The radiation spectral range was 0.2–1.5 THz, in the pulse mode, with pulse duration about 2.5 ps and a repetition rate of 80 MHz. The estimated THz irradiance was ~10 mW/cm² and exposure time was 60 min. Then the samples were dissolved in deionized water (0.05 mL) for EPR investigation.

The solution was poured in a quartz flat cell for EPR spectroscopy of aqueous solutions. EPR spectra were recorded with a Bruker EMX spectrometer (Germany) within the X band, with amplification up to $5 \times 10^5$. Dihydropyrazine-1,4-dioxide (DPDO) was used as a spin probe precursor to examine paramagnetic centers in BSA. The actual spin probe was formed in situ as a result of the oxidation of this dinitrone compound.

Chromatographic studies were carried out with a microcolumn Milikhrom A-02 chromatograph equipped with an UV detector (Econova, Russia). Chromatograms were recorded in the direct gradient mode and in the ion pair chromatographic mode. The volume of BSA samples introduced into the chromatograph was 20 μL. Elution rate was 150 μL/min. Elution was carried out in the gradient mode: eluent A – acetonitrile (Sigma-Aldrich, for HPLC, gradient grade, ≥99.9 %,), eluent B – $2 \times 10^{-2}$ M phosphate buffer (pH=3). The initial concentration of eluent B was 3 %, the gradient: 0-600 μL – 3 %, 600-1800 μL – 30 %, 1800-2500 μl – 50 %. The ion pair chromatographic mode was used to provide separation of coupled BSA molecules held together through amino groups.

Intermolecular interactions were simulated using the CS Chem 3D Pro software, version 5.0 (Cambridge Soft Corporation, UK).

3. Results and discussion

3.1. Interpretation of the EPR spectra. Mobility of weak paramagnetic centers

The effects of THz radiation detected by us previously included an increase in the number of paramagnetic centers, lower mobility of these centers and increased adsorption of molecular oxygen in THz-irradiated BSA samples in comparison with non-irradiated ones [8]. A weak broad triplet ($\alpha_N = 0.94$ mT) observed in EPR spectra of the aqueous solutions in addition to rather intense signals ($\alpha_N = 1.47$ mT) related to the paramagnetic centers in BSA was attributed to a nitrogen-to-nitrogen bound cluster, because the hyperfine splitting constant for nitrogen atom has changed dramatically. The intensity of this weak triplet was found to be affected by preliminary irradiation in the terahertz range. In addition to differences in hyperfine splitting constants, the width of lines in the EPR spectra recorded in the differential form was found to decrease. This means that THz irradiation causes an increase in the mobility of paramagnetic centers in BSA. This was rather unexpected result, since the mobility of paramagnetic centers characterized by the strong EPR signal was previously demonstrated to decrease under irradiation. A possible explanation of an increase in the mobility of weak paramagnetic centers under irradiation might be their dependence on oxygen adsorption, which was proved to be stronger in irradiated BSA sample.
3.2. The dependence of the intensity of weak EPR signals on oxygen concentration in solution

To test the assumption concerning the interaction of BSA with oxygen dissolved in water, we studied the dependence of the weak EPR signals on irradiation in the absence of oxygen. Oxygen adsorption on BSA after film dissolution was found to be an equilibrium process, so oxygen was removed from solution by bubbling argon for 20 min. Oxygen concentration was measured by means of iodometric titration. The dependence of oxygen concentration $C_{ox}$ in solution on the time $t$ of bubbling with argon under our experimental conditions is presented in figure 1. This curve shows the values averaged over 10 independent measurements for each time point. The error did not exceed ±2 %. In subsequent measurements, oxygen concentration was estimated from $t$ using the curve shown in figure 1.

After the removal of molecular oxygen, the integral intensity of these weak EPR signals was detected to increase by a factor of 2.4 in comparison with non-irradiated BSA samples treated in the same way. The dependence of the integral intensity of this weak EPR signal on $t$ is shown in figure 2. To check whether these signals are due to the clusters formed by several BSA molecules interacting through the nitrogen atoms of their heterocycles or due to the interactions in which the spin probe DPDO was involved, chromatographic investigation was carried out.

3.3. Chromatographic investigation of the system BSA – DPDO: the effect of THz-irradiation

Chromatographic detection was performed by measuring the optical absorption at 230, 246 and 258 nm. An intense peak related to unbound DPDO was present in all chromatograms, though its intensity was about 20 % lower in the chromatograms of THz-irradiated BSA. Two very broad peaks with low intensity were detected in the chromatograms. Their absorption spectra were identical, but retention times differed greatly. Relying on concentration dependencies, we suppose that these peaks relate to monomeric BSA and a cluster formed by two BSA molecules held together through rather strong hydrogen bonding. Under aerobic conditions the concentration of clusters formed in non-irradiated BSA is slightly less than that in the THz-irradiated sample, while under anaerobic conditions (after removal of dissolved molecular oxygen) coupling becomes substantially stronger in irradiated BSA samples than in non-irradiated ones. Relative concentrations of clustered BSA molecules (dimers) in the aqueous solutions of THz-irradiated and non-irradiated BSA samples are shown in Figure 3.

3.4. Semi-empirical simulation of clustering in BSA solution

To determine the reasons of changes in intermolecular coupling of BSA, simulation was carried out to evaluate the strength of hydrogen bonds formed between different functional groups of all the
compounds present in the system. It was shown that oxygen molecules adsorbed on BSA molecules occupy the same positions that are involved in intermolecular coupling of BSA molecules. Simulation of the structure of a cluster formed by coupling revealed that in the presence of oxygen two BSA molecules are held together mainly through SH---O---HS bonds, while under anaerobic conditions intermolecular coupling is provided mainly by S---S interactions, with a small (about 15 %) contribution from the interactions of a nitrogen atom in the heterocycle of one BSA molecule with a hydroxyl group of another BSA molecule. So, conformational transition induced by irradiation within the THz range removes the steric hindrance for oxygen adsorption, and removal of oxygen from the solution makes these positions free for intermolecular coupling between BSA molecules. The studied effects of terahertz waves may lead to diverse biological consequences, for example, as those reported [11]: irradiation within the terahertz range affects cell adhesion, which may be a consequence of conformation changes in the structures on cell shells. A link is still to be traced from the molecular effects of terahertz radiation to its effects on much more complicated systems, such as cells, tissues, and organisms.

4. Conclusions
Irradiation of albumin, one of the representatives of biomacromolecules, within the terahertz range affects the rotational and low-frequency vibrational transitions in these molecules, thus inducing changes in their conformations. These changes affect the steric states, which simplifies or hinders the adsorption of specific agents. Under anaerobic conditions, these conformational changes enhance intermolecular coupling, which was demonstrated experimentally for albumin molecules. Conformational transition induced by irradiation within the THz range removes the steric hindrance for oxygen adsorption. The removal of oxygen from definite positions makes these positions free for intermolecular coupling between albumin molecules. The studied effects of terahertz waves may lead to diverse biological consequences, but exact mechanisms of the expected effects remain unknown and are to be studied.

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