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The Effect of Terahertz Radiation on the Transport Characteristics of Albumin: Binding with Metal Ions

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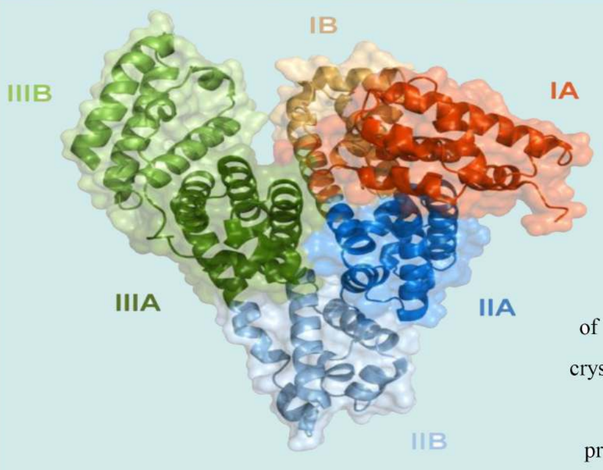
Effects of THz radiation on biological systems are extensively studied over a broad range of biological systems from cell cultures to entire organisms. However, the molecular effects of THz radiation on the molecules of biological significance remain poorly understood yet. Previously we detected structural rearrangement in bovine serum albumin under THz irraidation, manifesting itself as changes in oxygen adsorption, interaction with ozone**,** and NO binding [E.F. Nemova, O.P. Cherkasova, N.A. Nikolaev, G.G. Dultseva, Biophysics **65**, 410 (2020)].

GOAL: to investigate changes in metal binding with albumin as a chemical consequence of the structural rearrangement caused in filmed albumin preparations by irradiation within the terahertz range.

Object: bovine serum albumin, BSA



Terahertz Radiation:

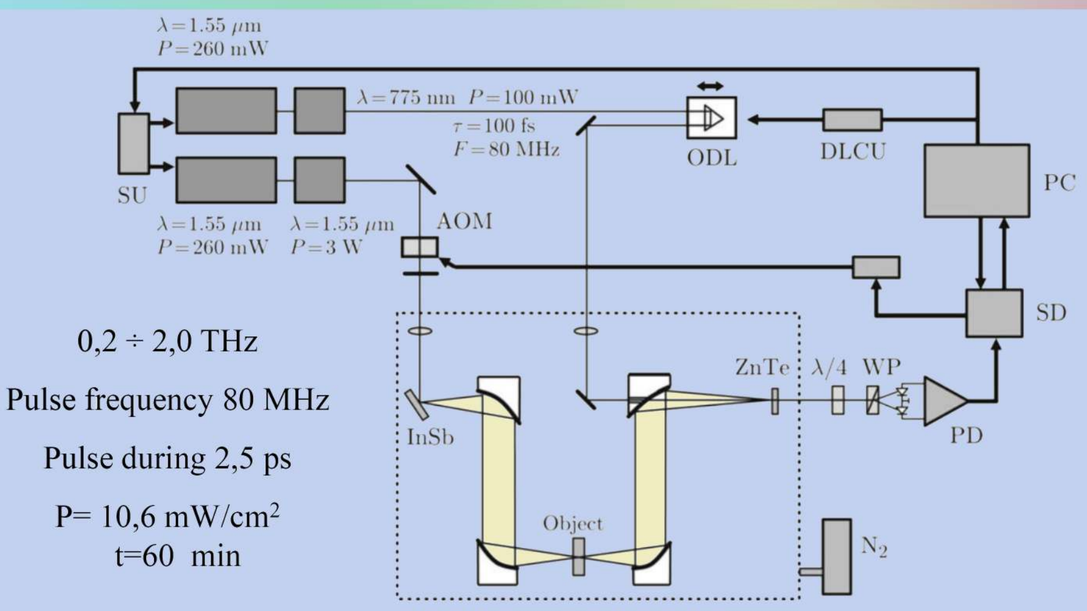
* Frequencies: 0.1 – 10 THz
* Wavelengths: 0.01 – 3 mm
* Energies: 0.4 – 40 10^{-3} eV
* Energy of rotational transitions: 10^{-5} – 10^{-3} eV

BSA films were prepared from the solution with the concentration of 1 mg/ml by applying 50 µl of the solution on a substrate made of crystalline quartz. The film was dried in air flow at room temperature. Freshly prepared films were irradiated. Reference samples were film preparations kept close to the experimental ones but not irradiated.

BSA is composed of three structurally similar helical domains (I, II, and III) arranged in a heart-shaped molecule. Each domain can be divided into two subdomains (A and B) The helical content is 74% .
BSA has 17 conserved disulfide bonds and a free thiol group associated with Cys34.

Previous results: THz irradiation of filmed BSA samples causes an increase in the amount of paramagnetic centers and a decrease in their mobility; O_2 adsorption on BSA increases.

BSA irradiation within the terahertz range was carried out using the experimental set-up:



0,2 ÷ 2,0 THz

Pulse frequency 80 MHz

Pulse during 2,5 ps

P= 10,6 mW/cm^2
t=60 min

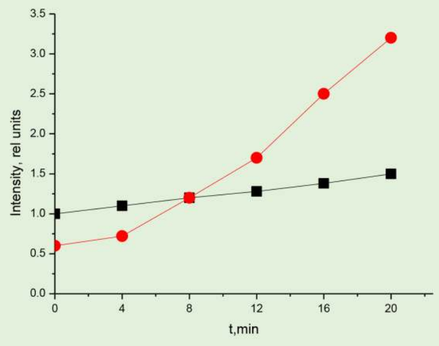
Circuit of the terahertz spectrometer with the following components: Wollaston prism (WP), delay line control unit (DLCU), synchronization unit (SU), synchronous detector (SD), and photodetector (PD).

V.D. Antsygin, A.A. Mamrashev, N.A. Nikolaev, O.I. Potaturkin , "Small-size terahertz spectrometer using the second harmonic of a femtosecond fiber laser", *Optoelectronics, Instrumentation and Data Processing,* vol.46 (3), pp. 294-300, 2010.

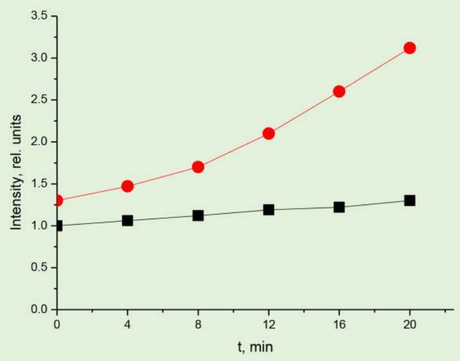
Results and discussion

EPR data: a weak triplet signal with a_N = 0.94 mT is due to the formation of a cluster (dimer) with –N·····N– bonding. The line width of this signal decreases under THz irradiation (mobility increased).
Chromatographic data: oxygen removal causes an increase in the concentration of BSA dimers; this increase in rather small (about 30 %) in the non-irradiated BSA sample, while in the THz-irradiated BSA sample in amount of dimers increases more than by a factor of 3.

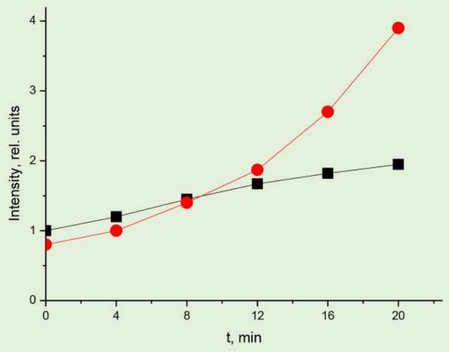
Kinetics of oxygen concentration decrease during degassing the aqueous solution of BSA by Ar bubbling for 20 min



Intensity of the weak signal in the EPR spectra of BSA: THz-irradiated (red) and non-irradiated (black)



Relative concentrations of the free (unbound) *in situ* spin probe (1,,4-dihydropyrazine 1,4-dioxide) in the aqueous solutions of BSA: THz-irradiated (red) and non-irradiated, **chromatographic data**



Relative concentrations of clustered BSA (dimers) in the aqueous solutions of THz-irradiated (red) and non-irradiated BSA samples, **chromatographic data**

Under anaerobic conditions, intermolecular BSA coupling increases sharply as a result of THz irradiation because the positions occupied previously by adsorbed O_2 become free for coupling.
These positions also become available for metal binding. The transport of metal ions (mainly calcium, magnesium and copper) is one of the most biologically significant functions of albumin molecules *in vivo*, in order to reveal the molecular effect underlying THz-induced changes, we studied albumin binding with metal ions. **Metal ions chosen for investigation for binding with BSA: Ni^{2+}, Cu^{2+}, and a larger ion, Cd^{2+},** as probes to test the steric settings at the binding sites.
Modeling data: O_2 is adsorbed mainly on the same positions in BSA that are involved in the formation of BSA dimers :

O_2 adsorption: -S-H·····O_2·····H-S- =S·····O_2·····S= >N·····O_2·····N<
Intermolecular BSA coupling -SH·····SH- =S····S= >N····N< >N·····H-O-

Binding with metals: Ni2+, Co2+ >C=O (carboxyl) -OH (hydroxyl) -SH (thiol) – Changes in binding extent inversely correlate with oxygen-binding capacity (a competition for BSA binding sites)
Cd2+ >C=O (carboxyl in β-position to aromatic ring) – Changes in binding extent are caused by elimination of steric hindrance due to rearrangement caused by THz irradiation
Ion radius: Cd2+ - 0.095 nm Co2+ - 0.074 nm Ni2+ - 0.069 nm

CONCLUSIONS

Metal ion binding with albumin has been accessed experimentally by means of EPR and HPLC, as well as through a simple semi-empirical simulation to reveal functional groups that are directly responsible for binding with metals. It is determined that THz irradiation of albumin molecules partially removes steric hindrance for binding large metal ions (for example, cadmium, which is extremely toxic), and this may lead to significant biological consequences. The discovered change in BSA binding characteristics caused by irradiation within THz region provides one more example that **a low-energy action may cause observable chemical consequences**. This phenomenon is to be studied in more detail because of the importance of the processes under investigation for protein-based transport *in vivo*.