through the aspiration of the metaphase plate within a small amount of the surrounding cytoplasm. This is the way of enucleated oocytes (recipient cytoplast) obtainment. Recipient cytoplast is required for animal cloning and mitochondrial replacement therapy in humans. The loss of this particular portion of the cytoplasm containing metaphase plate may affect the development, because exactly in this place some reprogramming factors are concentrated, for example, MPF, ORF1 and soronin. An advantage of the laser is an ability to localize the impact exactly in the area of metaphase plate and to avoid losing of reprogramming factors.

We have shown before that we are able to perform the femtosecond laser enucleation of the oocytes with a perfect preciseness and low invasiveness (Osychenko A.A. et.al, Biomedical Optics Express, 2022). To develop our technique, we tried different laser parameters for determination of non-invasive diapason of oocyte enucleation. More, we studied the cytotoxic effect of the femtosecond laser exposure.

The range of femtosecond laser radiation parameters for local destruction of the mouse oocyte genetic material has been studied. In this work we applied the femtosecond pulse repetition rate mode of 80 MHz, 1 kHz, 10 kHz, and 100 kHz. The efficiency of the mouse oocyte enucleation was studied depending on the wavelength of femtosecond laser radiation, pulse energy, and pulse train duration. Limitations of the range of parameters in terms of the pulse energy and the duration of the trains were found, due to the speed of the procedure and the frequency of formation of unwanted vapor-gas bubbles.

The cytotoxic effect of the femtosecond laser enucleation procedure was studied by fluorescent microscopy. The fluorescence lifetime of NADH was measured by FLIM (Fluorescence-lifetime imaging microscopy) in oocytes irradiated and not irradiated (control) with femtosecond laser radiation. The results indicate an approximately equal contribution of the bound and unbound forms of NADH to the metabolism of oocytes, this ratio does not depend on the presence or absence of laser irradiation, and also does not change significantly in the presence/absence of parthenogenetic activation.

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S6.422. The effect of microenvironment dynamics of tryptophan on its fluorescence parameters at different temperatures

Gorokhov V.V.[1], Knox P.P.[1], Korvatovsky B.N.[1], Goryachev S.N.[1], Paschenko V.Z.[1]*, Rubin A.B.[1]
[1]*Lomonosov Moscow State University;*
* vz.paschenko@gmail.com

The fluorescence of tryptophanils in proteins is widely used as a natural indicator of their intramolecular conformational dynamics, which is closely related to the functional activity upon temperature changes, in particular. Temperature dependences of spectrum patterns and Trp fluorescence lifetime are the reference sources for the the intramolecular dynamics behavior of the closest environment of the excited tryptophan (Trp) molecule. The Trp fluorescence decay kinetics both in solution and in polypeptides are multicomponent. Depending on the type of solvent, pH, 2–3 fluorescence components are recorded with times of hundreds of picoseconds to about 10 ns. The nature of intramolecular microconformational dynamics of the water-protein medium, which affects the functional activity, is largely determined by the state of the hydrogen bond system in the macromolecule. Certain information for understanding mechanisms of the processes occurring in this case can be obtained, among other things, by a comparative analysis of temperature dependences of the tryptophan fluorescence parameters in solution and in the protein composition.

It is on record that an ordered system of hydrogen bonds is formed in the tryptophan molecule environment in an aqueous medium. This system is shaped as a zwitterionic complex and causes a significant effect on the excitation dynamics in the Trp molecule. Here we interrogated the change in the spectral and kinetic parameters describing the decay of tryptophan fluorescence in an aqueous medium under pulsed photoexcitation as a function of temperature in the range of -170°C to +20°C. A model is proposed and a quantitative analysis of the rates of direct and reverse electronic transitions in the tryptophan molecule from the excited state to the ground state and to the charge transfer state (CTS) is carried out. Basing on the experimental and theoretical data, three regions in the Trp fluorescence spectrum were distinguished: the short-wavelength region (300 nm < l < 386 nm), the medium-wavelength region (386 nm < l <400 nm), and the long-wavelength region (400 nm < l < 470 nm), for which temperature dependences of the CTS formation rate are different. The key role of structural transformations of hydrogen bonds in the system determining the nonlinear behavior of the change in tryptophan fluorescence parameters in the selected spectral regions is shown.

The revealed nonlinear nature of the temperature dependence of the decay time of the fast (t1, a few ns) and slow (t2, about 10 ns) components of the fluorescence of an aqueous solution of tryptophan molecules is explained by appearance of an additional deactivation channel Trp* - electron transfer from the indole part of the tryptophan molecule to hydrogen bonds of the aqueous environment and then to its amide groups. As a result, a state with charge transfer Trp^+R^- is formed (where R can denote both a system of hydrogen bonds in the environment of the Trp molecule and amide groups with electron-withdrawing properties associated with Trp). Deactivation of this CTS occurs both resulting the reverse transition from this state to the excited Trp state and either due to fluorescence, or during thermal relaxation to the ground state.

The spectral-dynamic approach used in this work to study the temperature dependences of the deactivation rates of Trp excited states also allowed detection of conformational (phase) transition occurrence in the system of hydrogen bonds in the environment of Trp* and CTS within the temperature range of -80 °C to 20 °C. In this temperature range, a nonlinear behavior of such parameters as t1, t2, the activation energy Ea, and the solvation shift rate of the fluorescence spectra is observed. It is significant that in the temperature range of -110 °C - -80 °C the Trp* molecule is solvated faster than CTS, while at higher temperatures (T > -80 °C) the solvation rate of the Trp^+R^- state becomes greater than the Trp* solvation rate. We explained this fact so that in the temperature range of -80°C - 20°C in the CTS environment, the structural (phase) transition captures a greater number of H-bonds than in the Trp* environment. In such temperature range of -80°C - 20°C, temperature dynamics of the excited tryptophan molecule transitions deviate from the standard Arrhenius behavior with the constant Ea value. Thus, the hydrogen bond system dynamics are decisive for the nonlinear change in parameters describing deactivation of Trp* and CTS. It is obvious that further comparison of these results with similar studies for tryptophanils in the composition of proteins will provide new information on the extent to which differences in the fluorescence parameters of tryptophan in the composition of a functional protein detected during thawing of samples cooled under different conditions, can be due to direct changes in the nature of its interaction with protein molecules and with the surrounding solvent.

S6.423. The influence of wavelength, power and exposure of laser radiation on singlet oxygen generation

Makovik I.N.[1]*, Vinokurov A.Y.[1], Eratova L.V.[1], Dremin V.V.[1]
[1]*Orel State University named after I.S. Turgenev;*
* irina.makovik@gmail.com

Singlet oxygen (SO) is a highly reactive form of molecular oxygen and plays an important role in many physical, chemical, and biological processes, as well as in the therapy of different pathologies. The growing interest in SO due to its high chemical activity has contributed to the development of various approaches to SO generation in biological systems. Thus, the mechanism of direct photosensitizer-free optical generation of the singlet form of oxygen by light at specific wavelengths has been actively studied recently. Along with the study of the effect of laser-induced SO on physiological processes at the cellular and tissue levels, approaches to its detection and quantification are being actively developed and studied. This is an important task for evaluating effective doses and searching for optimal parameters of triplet oxygen excitation. In this work, the influence of a number of wavelengths, powers and exposures of laser radiation on SO generation is studied, and the advantages and disadvantages of existing approaches to the quantitative measurement of SO formation are analyzed.

To date, due to the high reactivity of SO, there are a limited number of possible approaches to its detection. The paper considers a polarographic method using an Oxytherm+R respirometer (Hansatech Instruments, UK) and a method using a Singlet Oxygen Sensor Green (SOSG) fluorescent probe (Invitrogen, USA). The ground triplet state of oxygen has several absorption bands in the spectral range from 390 to 1300 nm, at which SO can be produced. The laser wavelengths of 1267 and 1064 nm, which have the greatest absorption by the triplet form of oxygen, are considered. In this work, 1244 and 1122 nm laser radiation sources were used as controls. According to the data from literature, they do not activate the transition of triplet oxygen to the singlet state.

The study by polarographic method included measurement of the level of dissolved oxygen in ddH2O or 5 mM L-histidine solution in ddH2O. L-histidine was used as a "chemical trap" due to its ability to interact with SO. This made possible the polarographic measurement of the decrease in the dissolved oxygen concentration. Laser-induced SO generation was carried out through the glass wall of the measuring chamber, in which the temperature was maintained at a level of 26 °C. Measurements were made using a Zeiss LSM 900 microscope (Carl Zeiss AG, Germany) with a 10× objective. To excite the fluorescence of the probe, a laser with a wavelength of 488 nm and a power of 0.1 % of the maximum was used. The delivery of laser radiation during the experiments was carried out from the opposite side of the microscope objective. The study protocol included the stages of recording the baseline signal level (3 min), the signal level in the process of SO generation by laser radiation (the duration depended on the selected dose), and recording the signal after laser exposure (6 min). The analysis of the influence of time (laser radiation exposure) and power factors on the formation of SO was carried out when exposed to different doses (50, 100, 150, 200 and 250 J/cm2) of laser radiation at a fixed power value of 50 mW, as well as for a dose of 200 J/cm2 at different powers equal to 50, 100 and 150 mW.

An analysis of the measurement results by the polarographic method showed that the content of dissolved oxygen in the L-histidine solution in the measuring chamber after 1267 nm and 1064 nm laser exposure turned out to be lower compared to the control lasers. However, this approach is characterized by insufficient sensitivity to the detection of various doses of generated SO. In addition, the sensitivity of the polarographic method to temperature changes, despite its stabilization by the design of the respirometer, makes it impossible to analyse the change in the signal at the time of laser exposure. Therefore, only an indirect estimate can be made after calculating the final decrease in oxygen concentration compared to its initial level before exposure.

Measurements using SOSG fluorescent probe showed that 1267 and 1064 nm laser exposure leads to an increase in the green fluorescence intensity of the probe oxidized form. The increase in the signal indicates the production of SO and its selective interaction with SOSG. At the same time, the wavelength of 1267 nm has a more significant influence. An increase in fluorescence intensity was also registered under laser irradiation at a wavelength of 1244 nm. This result may indicate the formation of SO and the incorrect use of this radiation source as a control laser. An analysis of the results under the influence of different doses of laser radiation showed the sensitivity of the approach to differences in the amount of SO generated. Exposure with a power of 50, 100 and 150 mW at a dose of 200 J/cm2 revealed a greater influence of exposure time than power on the amount produced SO. This is most likely due to the short lifetime of the SO.

The considered approaches for the detection of SO generation are characterized by different sensitivity to its detection. These methods allow one to realize only an indirect estimation and cannot provide SO-generation control during the laser exposure. In the case of the polarographic method only SO generated in solution or diffused from the tissue can be measured. But the last seems impossible due to the low SO lifetime. The use of SOSG is significantly limited by its poor penetrating ability through the cell membrane. Therefore, the use of chemical traps or specialized fluorescent probes is suitable only for a narrow type of tasks. This indicates the need to develop new highly sensitive SO detection methods.

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S6.424. The mechanism of age melanin concentration decrease in the retinal pigment epithelium cells of the eye

Dontsov A.E.[1]*, Yakovleva M.A.[1], Vasin A.A.[2], Gulin A.A.[2], Aybush A.V.[2], Nadtochenko V.A.[2], Ostrovsky M.A.[1]
[1]*Emanuel Institute of Biochemical Physics, RAS;*
[2]*N.N. Semenov Federal Research Center for Chemical Physics, RAS;*
* adontsovnick@yahoo.com

It is known that in the process of aging there is a significant decrease in the number of melanosomes in the retinal pigment epithelium (RPE) cells of the human eye [1, 2]. However, the exact mechanisms of this phenomenon are unknown. Previously, we showed that the interaction of melanin melanosomes with superoxide radicals results in its oxidative destruction with the formation of water-soluble fluorescent products [3] containing highly active carbonyl compounds [4]. In the present study, using fluorescence analysis, HPLC, and mass spectrometry, it was shown that when melanolipofuscin granules isolated from human eye RPE cells are irradiated with visible light, water-soluble fluorescent products are formed. The formation of these products occurs as a result of oxidative degradation of melanin caused by superoxide radicals, which are generated by the lipofuscin part of the melanolipofuscin granule. It is important to emphasize that when the fractions of melanosomes and lipofuscin granules are irradiated, the formation of water-soluble fluorescent products does not occur. Destruction of melanosomes under the action of light is also possible; however, this requires significantly higher irradiation intensities than when the melanolipofuscin granules are irradiated. This is explained by the fact that in melanosomes, in contrast to melanolipofuscin granules, there is no lipofuscin, a light-dependent generator of superoxide radicals. Fluorescent products of light-induced melanin decay were identified both in the melanosomes and in the melanolipofuscin granules fractions. Statistical analysis by principal component analysis (PCA) for mass spectrometric data obtained by ToF-SIMS allowed us to identify the peaks characteristic of these products of light-induced melanin degradation. It was shown for the first time that water-soluble melanin degradation products caused by superoxide radicals are light-sensitive generators of reactive oxygen species. From this, it follows that the process of light-induced melanin degradation in the melanolipofuscin granule can be enhanced with the accumulation of melanin degradation