

MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE

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**«BIOLOGY: FROM A MOLECULE  
UP TO THE BIOSPHERE»**

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again to the 80 minute of incubation, reaching a maximum. At the second stage of fluorescence increase, the percentage of sorption was 95–97 % and did not change within the course of the subsequent observation time.

The nature of ellipsoidal nanoparticles ( $8 \times 20$  nm in size) sorption was one-step, qualitatively and quantitatively different from spherical particles. Thus, the maximum for these nanoparticles type sorption by hepatocytes was already accounted for 10 minute of incubation. Herewith, the percentage of their sorption was 45–50 %, and during the subsequent 55 minutes of incubation it did not change, and from 60 minutes was gradually decreasing, reaching 40 % of the maximum percentage of sorption.

The physico-chemical and biological aspects of the observed effects are discussed.

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### **Novel heptamethine cyanine dyes as potential amyloid markers**

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The accumulation of the highly ordered protein aggregates, amyloid fibrils, in various tissues disease, systemic amyloidosis, type II diabetes, etc. One of the most powerful approaches to the detection of amyloid fibrils involves the use of fluorescent dyes. Extensive research efforts are currently focused on looking for the effective amyloid-specific fluorophores. The present was undertaken to assess the amyloid-sensing propensity of the two newly synthesized near-infrared cyanine heptamethine dyes, AK7-5 and AK7-6. To this end, we compared the fluorescence responses of the examined dyes in the presence of the native and fibrillar lysozyme.

Most cyanine dyes are known to aggregate in an aqueous solution and show the changes in the emission and absorption spectra with respect to the monomeric species. The aggregates that exhibit hypsochromically shifted band (H-band) in their absorption/emission spectrum are called H-aggregates. Recently, using the absorption spectroscopy technique we have found that both the above dyes can form non-fluorescing H-aggregates in water solution, but tend to disaggregate in the presence of protein. In an organic solvent, dimethylsulfoxide (DMSO), AK7-5 and AK7-6 monomers display fluorescence emission maxima at 835 and 839 nm, respectively, while fluorescence excitation maxima are observed at 816 nm for both dyes. In the sodium-phosphate buffer solution the examined dyes have the emission maxima similar to DMSO, but there were no bands corresponding to H-dimers or H-aggregates, suggesting that such kinds of aggregates are non-fluorescent.

The monomeric peaks of AK7-5 and AK7-6 showed the increase during the dye titration with the native lysozyme, but above a certain protein concentration the fluorescence intensity reduced to nearly initial level. This process was accompanied by the appearance of hypsochromic band at 700 nm, whose intensity increased with the protein concentration. In the presence of lysozyme fibrils, both heptamethine dyes demonstrated significant rise of monomeric bands (by a factor of 4.5/3 times for AK7-5/AK7-6, respectively) without any decrease and very weak enhancement of the hypsochromic bands as compared to the native protein. Based on these data we concluded that both the monomeric and aggregated dye forms can bind to lysozyme, but the concomitant changes in the electronic structure of H-aggregates render them capable of fluorescing. The growth of the hypsochromic bands with negligible changes of the monomeric peaks induced by the native protein

and the opposite effects brought about by the lysozyme fibrils suggest that the native lysozyme has more binding sites for the dye aggregates than fibrillar lysozyme, while the fibril grooves represent specific binding site for the dyes monomers. The changes of the monomer band observed in the presence of the native lysozyme is indicative of the disassembly of the H-aggregates in solution followed by the assembly of the H-aggregates with probably modified properties on the protein surface. At the same time, the fluorescence intensity of monomeric dyes gradually increases with the concentration of lysozyme fibrils without any re-assembly of the H-aggregates within the fibril structure.

Eventually, the revealed spectral behavior of the cyanine dyes, namely significant distinctions in the fluorescence spectra in the presence of monomeric and fibrillar forms of lysozyme, point to the possibility of using these molecules as fluorescent amyloid markers along with the classical amyloid marker Thioflavin T.

*Supervisor – Dr. Sc., Professor, Trusova V. M.*

### **Long term effects of low concentrations of Cd<sup>2+</sup> on oxidative stress parameters in rats' blood serum**

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Cadmium took a strong position in human vital activity and the effect on health, which is associated with its technical application growth. Acute intoxication with cadmium is extremely rare, during a lifetime the human body is exposed to the long-term effects of small and sub-small doses of cadmium ions.

The purpose of this study was to investigate long term effects of low concentrations of Cd<sup>2+</sup> on oxidative stress parameters in blood serum of rats, which were obtaining small concentrations of Cd<sup>2+</sup> with drinking water daily within 36 days.

Outbred white male rats aged 3 months were used in the study. Two weeks before the experiment, the animals were transferred to drinking water, tested for absence of Cd<sup>2+</sup>. The animals obtained per oral water containing Cd<sup>2+</sup> in doses of 0.1 µg and 1 µg of Cd<sup>2+</sup>/kg of body weight/day for 36 days, daily. Furthermore, individual groups of animals were administered an acute dose (1 µg<sup>2+</sup>/kg of body weight) on day 36.

Oxidative stress parameters (8-isoprostane, carbonylated proteins concentration, catalase and superoxide dismutase activity) were measured in blood serum. Superoxide dismutase (SOD) activity was determined by spectrophotometric method with the SODs Assay Kit (Cayman Chemical). Carbonylated proteins concentration was determined by immunoenzymatic assay with the Protein Carbonyl Content Assay Kit (Cayman Chemical). Catalase activity was determined by spectrophotometric H<sub>2</sub>O<sub>2</sub>/oxidoreductase method with the Catalase Assay Kit (Cayman Chemical). 8-isoprostane concentration was determined by immunoenzymatic assay with the ELISA Kit (Abcam).

Concentration-dependent induction of oxidative stress by cadmium ions was shown. Prooxidant parameters (concentration of 8-isoprostane and carbonylated proteins) increased with increasing dose of cadmium. Herewith, the activity of antioxidant enzymes (SOD and catalase) did not change. When the acute dose was administered, prooxidant effect of cadmium ions was less pronounced on 36 day, which, apparently, is a manifestation of the organism's adaptation to the action of cadmium ions.