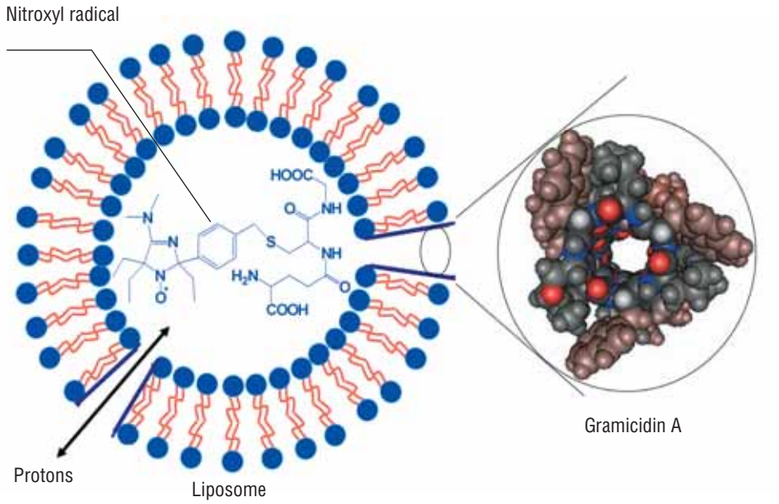
Nano-sized probes in biophysics

The Russian scientists from the Novosibirsk Institute of Organic Chemistry and International Tomography Center SB RAS and their American colleagues from Ohio, Valdosta, and North Carolina State Universities joined their efforts and created the nano-sized probes for pH measuring by the spectrum of electronic paramagnetic resonance (EPR). These probes might be used for non-invasive studying of biochemical and physiological processes in living organisms. The central element of these molecular probes is nitroxyl radicals with pH-dependant EPR spectrum.

Nitroxyl radicals (NRs, nitroxides) are the most widespread class of stable radicals. Small molecules of nitroxides with easily varied structure and simple spectrum of *electronic paramagnetic resonance* (EPR), sensitive to various parameters of its surrounding, are a unique set of *molecular probes* for studying the most complex molecular systems. An important field for using these probes is biophysics, where NRs are used to study the structure and mechanisms of biomolecular interaction and to learn about the vital functions of cells accompanied by the change of redox state, oxygen and NO concentration, pH, etc.

Acidity of the medium (pH) determined by the concentration of hydrogen ions (protons) is one of the most important and frequently measured parameters in biology, biophysics, medicine. The change of pH reflects the course of various processes in an organism and can serve as a sign of a pathology development, such as ischemia, infections, inflammations. The value of extracellular pH plays a significant role with the appearance, growth, and therapy of tumors.

Today, the most effective molecular EPR-probes for determining pH are the imidazoline nitroxyl radicals designed in the Novosibirsk Institute of Organic Chemistry SB RAS. These probes, however, have one serious drawback—they are quickly reduced in the tissues of living organisms. They may be reduced by both low-molecular cell antioxidants (first of all, ascorbic acid) and enzymatic systems. The primary products of the NR-reduction in the biological samples are the EPR-silent hydroxylamines.



A sketch of the nano-sized probe for pH detection. The inner cavity of the liposome contains the pH-sensitive nitroxyl radical. Gramicidin A incorporated into the membrane of the liposome provides for the formation of the proton permeable channels

One of the methods to stabilize the NR probes is to encapsulate their aqueous solutions into the inert nano-sized capsules or vesicles comprised, for example, of the derivatives of fatty acids-lipids, which are the basic components of cell membranes. Such vesicles, in which small volumes of aqueous solution are separated from the rest of it with lipid membrane, are called *liposomes*. Encapsulation into liposomes with semipermeable membrane can protect the NR probe from undesirable interaction with the reducing components of microenvironment on the one hand, and on the other hand, it maintains the possibility of monitoring the concentration of such small particles as protons or NO molecules.

The ability to use liposomes to increase the resistance of NRs to reduction in biological objects and the suitability of the formed nano-sized EPR-probes for *oxymetry* (determination of oxygen concentration) were first demonstrated in the early 1990s. Later, phospholipid liposomes were successfully employed for protection from the impact of biogenic reductants of extremely unstable nitronylnitroxides used for NO detecting.

To create nano-sized pH-sensitive EPR-probes the scientists from the Institute of Organic Chemistry conducted multistage synthesis of nitroxyl radicals unable to permeate through the membrane of phospholipid liposomes. Gramicidin A was incorporated into the membrane to form the proton-permeable channels. Highly hydrophilic pH-sensitive nitroxyl radical containing tripeptide glutathione moiety was placed into the liposome. The samples did not show any signs of HPs leaking out of the liposomes for several hours. At the same time the membrane of liposome protected the encapsulated nitroxide against the reduction by biogenic reductants. In a 100-fold excess of ascorbic acid, an insignificant drop of intensity of the signal of EPR of nano-sized probe was observed.

A laboratory rat in the resonator of EPR-spectrometer in the experiment of pH monitoring in a stomach

The developed probe based on nitroxyl radical was used to study the decrease in pH (*acidosis*) in the tissues of a rat's heart with artificial ischemia. The signal of EPR of the free nitroxyl probe disappeared in ten minutes. The intensity of the signal of EPR of nano-sized probe dropped only 15% in the same time, which allowed us to follow the change of pH.

Thus, the proposed concept made it possible to create the first nano-sized probe for pH detection in biological objects. The new probe dramatically exceeds the free nitroxyl radical in resistance to reduction. Taking into account the important role played by the pH parameter in various physiological processes, these nano-sized probes may become valuable analytical tools for biophysical and medical-biological research.

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