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Biological Membrane Mimetics to Study Radiation Induced Damages by NMR Spectroscopy: Application to Study Erythrocyte Membranes

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Abstract. The role of biological membranes as a target in biological radiation damage remains unclear. In the present article, we show that the structural, biochemical and biophysical changes of a simple biological membrane, i.e. human erythrocyte membranes, which is altered after exposure, could be studied by membrane mimetics and conventional NMR spectrometers.

Keywords: membrane mimetics, bicellar systems, NMR spectroscopy, magnetic field orientation

When erythrocytes or red blood cells (RBC) are exposed to radiation, they are damaged like all other human cells. According to Bergonie and Tribondeau law [1], lymphocytes and erythrocytes as the smallest and highly specialised cells are most sensitive to radiation-induced damages.

Damage could occur both in the membrane itself, and in the protein molecules located on membrane or inside of it. Here we will consider the modern possibilities to study the influence of radiation on the membranes using artificial membranes, which mimics the natural ones, and NMR spectroscopy.

The change in structure and properties of biological membranes, and particularly RBC membranes, under UV and X-ray radiation exposure is of fundamental importance for understanding phenomena leading to radiation-induced damages in living organisms.

Natural biological membranes have a very complex composition and contain a multitude of lipids, proteins and carbohydrates unique for any given cell or organism, which does not allow understanding the detailed rule of each membrane component in radiation damaging processes. This insight can be obtained by using simplified model systems to reduce the number of studying parameters. On the other side, there is a risk of oversimplification, which can lead to loss of testing characteristics in mimetic system.

There are many physical methods allowing the study of radiation induced damages. Among them NMR spectroscopy is notable by a number of advantages that could make it as one of the main methods of such studies.

NMR spectroscopy could be effective in research using both natural membranes and membrane mimetics for the following:

- composition (components analysis to reveal their changes after irradiation);
- structure (conformation analysis of head groups including orientational order of chain groups);
- dynamics (flexibility , rotational and translational diffusion, etc.).

A variety of NMR techniques is available for such studies. Their advantages are based on the following arguments:

a) Relatively low price and the possibility to use conventional NMR spectrometers, available in many advanced chemical and biochemical laboratories.

b) The possibility to do research in solution and in biological samples, in vivo and in vitro.

c) The possibility to use for study different atoms, having magnetic nuclei (hydrogen, deuterium, carbon, nitrogen, phosphorus, fluorine, etc.) in natural abundance.

d) The existence of a lot of parameters of different physical nature, which can be measured during NMR experiment in isotropic or anisotropic solutions: isotropic parameters (chemical shifts, indirect spin-spin couplings and relaxation times), as well as anisotropic parameters (direct dipole-dipole couplings, quadrupolar couplings, anisotropies of chemical shift and indirect spin-spin couplings in anisotropic media), which can be measured only for oriented molecules.

e) The possibility to use spin labelling in selected molecules.

f) The possibility to determine structural features of membranes forming molecules and membranes itself.

g) The possibility to determine order parameters of each molecule existing in membrane. Moreover, for no rigid molecules, such as phospholipids, order parameters of rigid and flexible parts can be determined separately, as well as for each segment of side chain.

There are many mimetics modelling various features of biological membrane [2]. From those four classes of mimetics are frequently used modelling the bilayer structure of membrane: micelles, bicelles, artificial membranes of various lamellarity and vesicles.

Micelles are the smallest and the simplest objects (sometimes with only one component) among the membrane mimetics and give better NMR spectra, compared to larger ones. Unfortunately, micelles are oversimplified in several perspectives. A lack of bilayer means that micelles do not mimic biologic membranes well enough.

From different types of membrane mimetics, ***bicellar systems*** [3] are morphologically versatile lipid nanostructures that open up large possibilities to study membrane geometry changes during the irradiation (changing size, shape and structure of bicelles). Today bicelles are unique model membrane systems that have found wide applications in NMR spectroscopy and crystallography for structure determination of membrane proteins [4].

The main advantage of these systems is their ability to align in a magnetic field, which allows using unique opportunities of high-resolution NMR spectroscopy: possibility to observe anisotropic parameters, which contain direct information about the molecular structure: bond lengths and angles [5]. The degree of order in magnetic field can be controlled by lipid composition, specific dopants, etc. Very advantageous is the possibility to flip magnetically aligned bicelles to make their membrane normal line up with the external magnetic field axis by adding lanthanide ions or by using lipids with a biphenyl in one of their acyl chains. In a strong magnetic field RBCs also oriented [6], which can be used to study their properties by using spin labelled molecules as probes.

Bicelles are a mixture of aliphatic long chain lipids (from 12 to 18 carbons) and short chain lipids (6–8 carbons). Their morphology is fairly versatile depending on composition, temperature and hydration. The most recognized organization is a nano-disc structure with the majority of long chain lipids in the disc plane and the short chain lipids mainly distributed in the torus of the disc.

Although, bicelles were discovered more than twenty years ago, they are used mainly for 3D-structure determination of biological macromolecules, particularly globular and membrane proteins [7-9]. Application of bicellar systems in other type of biochemical and biophysical studies are also reported [10-11].

The simplest way is the use of bicellar solution as aligning media for other dissolved molecules. Due to structural and diamagnetic anisotropy of bicelles they can easily align in magnetic field and create the conditions for non-free rotation and tumbling of the other molecules presented in the solution. By this way, more than hundreds of globular proteins' structures have been determined.

Another way is embedding understudy molecules or proteins into bicelles. This possibility is very important for the membrane proteins enabling now their study by solution-state or solid-state NMR spectroscopy methods.

Bicelles were originally made by mixing lecithin, which is a mixture of long-chained lipids, phosphatidylcholine (PC), phosphatidylethanolamin (PE) and phosphatidylinositol (PI), and bile salts. The most commonly used bicelles nowadays are prepared by mixing lipids such as

dimyristoylphosphatidylcholine (DMPC) with edge-stabilizing detergents (i.e., CHAPSO) or short chain lipids such as dihexanoylphosphatidylcholine (DHPC) in 4:1 to 1.5:1 lipid: detergent molar ratios. These edge stabilized planar bilayered assemblies present several advantages over traditional mixed micellar or lamellar mimetic systems: (1) bicelles represent a more native-like environment for structural studies of membranes and membrane proteins, (2) the effects of membrane curvature may be less pronounced than seen in pure detergent micelles, (3) for NMR studies, bicelle aggregate sizes are sufficiently small and they can be aligned in a magnetic field, and (4) for crystallization trials, bicelles are easy to manipulate and the crystals produced from them can be easily isolated and mounted for diffraction.

Irradiation can cause physical and chemical changes of biological membranes, relating to changes of composition, structure and dynamics. It can lead to changes of NMR parameters of ^{1}H, ^{2}H, ^{13}C, ^{15}N, ^{31}P and other nuclei existing in different parts of phospholipids as well as their counter ions. In order to define the behavior of individual components of biological membranes, which are absent in the model membrane, they can be included in model membranes.

Radiation can directly damage membrane molecules or produce reactive species in water leading to lipid peroxidation and damaging (increased permeability of the lipid bilayer, decreased fluidity or lowered electric resistance of membranes). Oxidation process can be initiated by the reactive oxygen species, such as the superoxide anion O^{-}_{2}, hydroxyl radical OH or singlet oxygen $^{1}O_{2}$.

Other type of membrane mimetics, like ***artificial model membranes***, have bigger size with complicated structural features, less homogeneity and alignment in magnetic field.

Vesicles are closed spherical bilayers made from lipids. Vesicles with only one bilayer are called unilamellar vesicles. Depending on the size, they can be divided into three categories: small unilamellar vesicles, which have diameters of 25-50 nm, large unilamellar vesicles (with diameters of 50-200 nm and giant unilamellar vescicles, which are in the size range of cells. Vesicles with many bilayers, i.e. multilamellar vescicles, first form after dissolving lipids in water and vortexing the samples. Sonication or freeze-thaw cycles can decrease the lamellarity. To ensure uniform size in the sample, ultracentrifugation or extrusion is required. Compared with bicelles, vesicles are even more native-like as membrane mimicking systems due to the small curvature and large bilayer surfaces. However, the large size causes slow tumbling leading to poor quality of NMR spectra, and zero or low diamagnetic anisotropy does not allow their alignment in magnetic field.

There are other membrane mimetic media like nanodisks, amphipols, reversed micelles and supported lipid membranes. Within the same size range, nanodisks are similar to bicelles. Instead of detergents, nanodisks have a lipid bilayer stabilized by apolipoprotein, the length of which is used to control the size of the nanodisk. Amphipols are made of designed short amphiphilic polymers carrying many hydrophobic chains.

These systems have another disadvantage – they are very different from the biological membranes and could not be used as appropriate mimetics for all types of natural membranes including RBC's membranes.

In conclusion, bicelles and lamelles are appropriate mimetics for the study of RBC’s membranes, and NMR spectroscopy is a valuable method for study the influence of radiation on the structure, dynamics and functions of membranes and encapsulated or incorporated molecules.

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