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Structural characterization in mixed lipid membrane systems by neutron and X-ray scattering☆

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ABSTRACT

Lipids membranes, the primary component of the living cell, involve collective behaviour of numerous interacting molecules. The rich morphology and complex phase diagram of the lipid systems require different strategies in describing bio-membranes in order to capture the essential properties of self-assembly processes as well as the underling molecular collective phenomena involved in biological functions. Among the experimental methods used, the scattering techniques such as small angle neutrons and X-rays scattering (SANS and SAXS) are probably the most important experimental approaches for the structural investigation of bio-membranes and mixed lipids complex systems. In this tutorial review we describe the main approaches employed in the investigation of lipid bio-membranes by means of the neutron and x-ray scattering techniques. While introducing the main structural properties of lipid bio-membranes we highlight the important role of lipid components in different biological functions of living organisms. This article is part of a Special Issue entitled "Science for Life" Guest Editor: Dr. Austen Angell, Dr. Salvatore Magazù and Dr. Federica Migliardo.

1. Introduction

The study of the self-assembly processes of lipid bio-membranes in water environment represents a central topic for the understanding the physical and chemical bases of the biological functions in living organism [1–3]. In basic research, mixed lipid bilayers serve as models for the investigation of cell membranes and their interaction with membrane proteins [4–7]. They also serve as delivery agents for drugs, genetic material and enzymes through living cell membranes and other hydrophobic barriers in food industry, cosmetic, biotechnology and medicine [8–10]. For these reasons self-assembly properties of lipid membranes has been the object of intensive research for many decades both from the theoretical as well as experimental point of view. In both case molecular level investigation of lipid bilayers bio-membranes involve collective behavior of numberous interacting molecules, while their full description require simultaneous calculation (and simulation) of a large number of parameters due to the number of conformations present and the variety of interactions involved [11–17]. From the experimental point of view a large variety of methods were applied to study the self-assembly properties of lipid bio-membranes as well as their interaction and complex behavior in mixed systems [18–24]. Among the experimental methods used, the scattering techniques such as small angle neutrons and X-rays scattering (SANS and SAXS) are probably the most important non–destructive and widely utilized experimental approach for the structural investigation of bio-membranes, and mixed lipids systems. More specifically the formation of different structures with different topologies can be efficiently evidenced by scattering experiments thus highlighting the important role of relevant molecular conformations in many different processes of life science [24–31].

Herein, we describe the main approaches employed in the investigation of lipid bilayers by means of the neutron and x-ray scattering techniques. In the first section, we introduce the main properties of lipid bio-membranes, highlighting the important role of lipid components in different biological functions of living organisms. We then introduce the fundamental principles of scattering of neutrons and X-rays and their application in structural characterization of bio-membranes. Within each section we will present some relevant and recent examples of the efficient use of scattering techniques in the study of lipid self-assembly with emphasis on modern approaches of scattering techniques in the characterization of bio-membranes and in advanced bio-nanotechnology research.

2. Structural properties and main components of bio-membranes

2.1. Fluid mosaic model of bio-membranes

Bio-membranes, which are mainly composed by different amphiphilic molecules, contain also proteins, sugars, cholesterol, and a number of other fundamental components that control important functional processes inside the cells such as DNA synthesis, antigen–antibody

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recognition or metabolic processes. According to the *fluid mosaic model* of Singer and Nicolson [32], the bio-membranes in living organism can be represented as an highly flexible, mosaic of fluid bilayer structures of lipids were membrane *protein* are embedded. Together with the *integral protein*, which has at least one segment anchored within the lipid bilayer, the fluid bilayer contains also *peripheral proteins*, which are bound to integral proteins or to the polar headgroups of membrane lipids. Finally, the *transmembrane proteins* that span the bilayer are responsible for transporting ions or small molecules between the two sides the cell membrane (Fig. 1).

Proteins embedded in the plasma membrane facilitate cell interaction with its environment, such as carrying out nutrients across the bio-membrane, anchoring the cell in a particular location or receiving/translating chemical signals from outside the cell for intracellular processes [32,33]. Some specialized subdomains of bio-membranes called lipid rafts were proposed to mediate protein interactions. The raft hypothesis assumes that subdomain are small (<200 nm), heterogeneous and highly dynamic, liquid-ordered phases rich in sterols (cholesterol) and sphingolipids (sphingomyelin) or saturated phospholipids that spontaneously self-assemble with each other to form regions for the segregation (targeting) of specific proteins [34,35]. Although these regions are presumed to have fundamental role in specific biological functions (such as in membrane protein sorting or construction of signaling complexes), the raft hypothesis is still a subject of controversy since there is still little experimental evidences of the existence of such domains [36,37].

Together with lipid and protein, *Carbohydrate* represent the third main component of bio-membranes. As an example in human erythrocytes the lipids, proteins, and carbohydrates are present in the ratio of 43, 49 and 8% respectively. As schematically represented in Fig. 1 bio-membrane carbohydrates are covalently bound either to proteins (*glycoproteins*) or to lipids (*glycolipids*), thus increasing with their hydrophilic character the stability of both lipids and proteins. More specifically glycolipids, which are more abundant in the outer surface (noncytosolic monolayer) of all eukaryotic cell membranes, realize different spatial combinations of sugars (such as mannose or galactose) that result in a variety of different surface markers or antigens which play important roles in in cell-recognition within the plasma membrane. Finally *Cholesterol* (and other steroids) are found in most bio-membranes, and has the main effect of stiffens the bilayers making them more rigid and less sensitive to lysis.

2.2. Lipid composition in bio-membranes

As previously stated, lipids are the main components of bio-membranes. More specifically the phospholipids, which are the main category of lipid molecules of cell membranes, are amphiphilic molecules with hydrophobic hydrocarbon (fatty acid) tails linked to a variety of hydrophilic, polar headgroups. Mixed lipids configurations in the bilayer is mainly determined by the steric and electrostatic interactions in the headgroups region and degree/number of unsaturation of the tails chains, due to the presence of one or more C═C double bonds. More specifically the presence of double bonds in the tail chains decreases the cooperativity of the chain interactions by restraining the gauche-trans isomerisation thus interfering with the regular hydrocarbon chain packing.

As reported in Table 1, the six major types of lipids which are mainly present in the in the plasma membrane of many mammalian cells are respectively phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), sphingomyelin, cholesterol and glicolipids. Other phospholipids, such as cardiolipin and inositol, although present in smaller quantities, play very important roles in specific

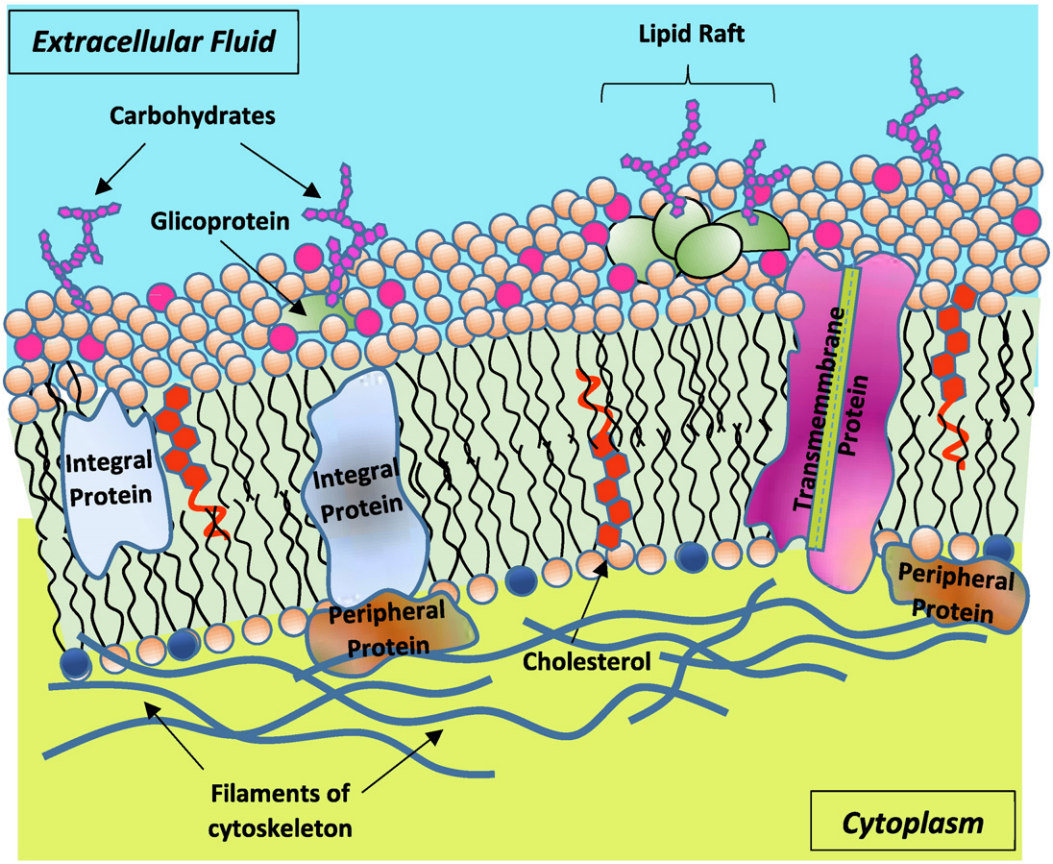

Fig. 1. Schematic representation of the fluid bilayer model bio-membranes in living organism (fluid mosaic model).