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Historical perspective

Hemolysis by surfactants — A review

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ABSTRACT

An overview of the use of surfactants for erythrocyte lysis and their cell membrane action mechanisms is given. Erythrocyte membrane characteristics and its association with the cell cytoskeleton are presented in order to complete understanding of the erythrocyte membrane distortion. Cell homeostasis disturbances caused by surfactants might induce changes starting from shape modification to cell lysis. Two main mechanisms are hypothesized in literature which are osmotic lysis and lysis by solubilization even if the boundary between them is not clearly defined. Another specific mechanism based on the formation of membrane pores is suggested in the particular case of saponins. The lytic potency of a surfactant is related to its affinity for the membrane and the modification of the lipid membrane curvature. This is to be related to the surfactant shape defined by its hydrophobic and hydrophilic moieties but also by experimental conditions. As a consequence, prediction of the hemolytic potency of a given surfactant is challenging. Several studies are focused on the relation between surfactant erythrolytic potency and their physico-chemical parameters such as the critical micellar concentration (CMC), the hydrophile–lipophile balance (HLB), the surfactant membrane/water partition coefficient (K) or the packing parameter (P). The CMC is one of the most important factors considered even if a lytic activity cut-off effect points out that the only consideration of CMC not enough predictive. The relation K.CMC must be considered in addition to the CMC to predict the surfactant lytic capacity within the same family of non ionic surfactant. Those surfactant structure/lytic activity studies demonstrate the requirement to take into account a combination of physico-chemical parameters to understand and foresee surfactant lytic potency.

Contents

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1. Introduction

Surfactants are widely employed in many cell biology processes such as protein purification or cell lysis as well as in several fields such as cosmetics for their interfacial properties; and pharmaceutics to improve for instance galenic and biodisponibility. Surfactant has also very interesting clinical applications, in particular for the treatment of lung diseases.

Hematology is an important domain of use of surfactants. Indeed, in hematological in vitro diagnostic, the counting and the identification of white blood cells (WBC) is a critical procedure. These analyses require a prior lysis of red blood cells (RBC) because erythrocytes are typically thousand folds more abundant than leukocytes. Surfactants are therefore key components of hematology reactants that are used in biology automates worldwide. The research of surfactants inducing specific lysis of RBC in a short incubation (surfactants/cells) time is consequently of great interest since it will impact the quality of the final diagnosis of blood samples. This review is focused on surfactants used for RBC lysis and their hemolysis mechanisms.

Surfactants are characterized by a structural similarity with cell membrane lipids. Lipids are water insoluble amphiphile molecules self-organized in a continuous liquid crystal bilayer delimiting the cellular compartment [1]. Surfactants are also amphiphile molecules able to interact and disturb cell membranes by modifying the lipid organization, the integral protein arrangement and more generally the cellular equilibrium. All those cell alterations can lead to the disruption of the membrane called cell lysis.

Erythrocytes have been widely studied as model cells for several reasons: (i) they are a simplified model of cell plasmic membranes due to the absence of nucleus and organites; (ii) their hemolysis can be easily monitored by spectrophotometry due to the release of hemoglobin; (iii) their large abundance; and (iv) their importance in the hematology field.

The erythrocyte membrane composition and its control in surfactant shape maintenance are described in order to get a better comprehension of surfactant–erythrocyte membrane interactions. Surfactant penetration induces a reorganization of the membrane lipids and proteins leading to changes in the erythrocyte shape and sometimes erythrocyte lysis. Different mechanisms depending on surfactant concentration are proposed in the literature such as the osmotic and the solubilization hemolysis. However, the boundary between these mechanisms remains unclear. Another specific mechanism based on the formation of membrane pores is suggested in the particular case of saponins which are the most frequently used surfactants as erythrolytic agents [2–5]. Surfactant–cell interactions are complex making difficult the hemolytic mechanism comprehension and classification.

The hemolytic potencies of surfactants have been studied regarding surfactant physico-chemical parameters and descriptors such as the critical micellar concentration (CMC), the hydrophile–lipophile balance (HLB), the surfactant membrane/water partition coefficient (K) or the packing parameter (P). Indeed, surfactant concentration as well as their structure and experimental conditions modulate surfactant–erythrocyte membrane interactions. Consequently, surfactant structure/lytic activity studies have been made to correlate these parameters with surfactant erythrolytic properties.

2. Erythrocyte membrane characteristics

Erythrocytes lysis, specifically in the presence of chemical agents such as surfactants, has been demonstrated to occur via different and complex mechanisms implying specific patterns of red blood cell membrane [6]. Consequently, erythrocyte membrane composition and its shape distortion will be discussed thereafter to enable a proper understanding of erythrocyte membrane/surfactant interactions involved in erythrocyte lysis.

2.1. Composition and function

The erythrocyte membrane is mainly composed of lipids and membrane proteins enabling its anchoring with the cell cytoskeleton. It is composed (by weight) of 52% proteins, 40% lipids and 8% carbohydrates like glycoproteins [7]. Lipid and protein cohesion is due to non-covalent interactions such as Van der Waals interactions, hydrogen bonding, electrostatic forces and hydrophobic effects [8]. Membrane lipids are mainly:

(i) Phospholipids (63%) which are insoluble amphiphile molecules [9] arranged in a bilayer with their polar residues oriented towards the bilayer-cater interface and their apolar tails into the bilayer core [1]. They are distributed asymmetrically between the outer and inner leaflets of the membrane [1] (Fig. 1). Their asymmetric organization is controlled by enzymes such as flippases, floppases, and scramblases [10]. Most abundant phospholipids are sphingomyelin and glycerophospholipids. These latter are divided into 3 main fractions with phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and several minor fractions of phosphatidic acid, lysophosphatidylcholine, phosphatidylinositol (PI) and phosphatidylinositol mono and diphosphates [11].

(ii) Neutral lipids (25%) which are almost exclusively cholesterol in erythrocytes. Cholesterol enables to maintain the membrane fluidity. Its alteration can lead to ionic and glycerol permeability modification and changes in the lipid organization [12].

(iii) Glycosphingolipids (12%).

While lipids are the fundamental structural elements of membranes, proteins are responsible for carrying out specific membrane functions. Membrane proteins are divided into two classes: integral and peripheral proteins. Integral proteins (Band 3, Glycophorin, Aquaporin, etc.) are inserted into the lipid bilayer via hydrophobic effects with the lipids. Most integral membrane proteins are transmembrane proteins, with portions exposed on both sides of the lipid bilayer. Peripheral proteins (Spectrin, Actin, Protein 4.1, Pallidin (Band 4.2), Ankyrin, etc.) are bound to the membrane indirectly by protein–protein interactions on the cytoplasmic surface of lipid bilayer and constitute the membrane skeleton. As all cell membranes, erythrocyte membrane allows the maintenance of extra and intracellular ion concentrations thanks to transmembrane transport proteins such as Na^+–K^+-ATPase, Ca^{2+}-ATPase and Na^+–K^+–$2Cl^-$-, Na^+–Cl^--, Na^+–K^+-, K^+–Cl^- cotransporters. This active transport regulates the intra-cytoplasmic viscosity related in erythrocyte cells to the hemoglobin concentration. Other membrane proteins are involved in the adhesion process (ICAM-4 and laminin proteins) and the signal transduction (transmembrane receptors) [13,14]. Moreover, the erythrocyte membrane possesses specific membrane proteins such as Band 3 (anion transporter), Aquaporin 1 (water transporter), Glut1 (glucose transporter 1), Kidd antigen protein (urea transporter), RhAG (carbon dioxide gas transporter [15]) and Gardos Channel (Ca^{2+}-dependent K^+ transporter) [14]. Other transmembrane proteins are glycophorins representing 2% of erythrocyte membrane proteins. These proteins play an important role in the regulation of RBC membrane mechanical properties and shape support [16].

The transmembrane glycoprotein Band 3 is the major erythrocyte protein with around $1.2 \cdot 10^6$ copies per erythrocyte corresponding to 25% of the total erythrocyte membrane protein amount [17]. The Band 3 protein enables the transport of Cl^- and HCO_3^- across the membrane. Anion exchange via the Band 3 protein is involved in the control mechanism of the erythrocyte shape because of its binding with spectrin filaments of the cell cytoskeleton through ankyrin proteins [13,17–20]. The cytoplasmic NH_2-terminal domain of Band 3 is acidic and therefore its conformation is pH sensitive. The Band 3 monomer has two