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Short Communication

OXYETHYLENE CHAIN-CATION COMPLEXATION; NONIONIC POLYOXYETHYLENE DETERGENTS ATTAIN A POSITIVE CHARGE AND DEMONSTRATE ELECTROSTATIC HEAD GROUP INTERACTIONS

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Abstract: We report literature data indicating that the polyoxyethylene chain of polyoxyethylene detergents attracts cations via dipole-ion interactions thereby attaining a positive charge character. This implies that nonionic polyoxyethylene detergents like Triton X-100 and $C_{12}E_8$ may interact electrostatically with phospholipid head groups. We describe how a positive charge character of Triton X-100 and $C_{12}E_8$ can explain their hitherto mysterious stomatocytogenic shape altering effect in human erythrocytes.

Key Words: Erythrocyte Membrane, Shape Change, Surfactant, C_mE_n, Triton X-100, PEG, Polyoxyethylene

INTRODUCTION

The mammalian erythrocyte has been frequently used as an experimental model to study plasma membrane dynamics. Manipulations of erythrocyte shape (membrane inward and outward bending) give information about membrane physical properties which are important in different physiological processes such as endocytosis and exocytosis. Detergents (water-soluble amphiphiles) have been shown to induce either spiculated (echinocytic) or invaginated

(stomatocytic) shapes in human erythrocytes [1-5]. These shape transformations are thought to depend mainly on the distribution of the detergent in the bilayer, i.e. whether the detergent is predominantly incorporated into the outer or inner membrane leaflet, thereby expanding this leaflet relative to the other [6-9]. The distribution of charged detergents, in case they can translocate to the inner bilayer leaflet (flip), is thought to depend mainly on electrostatic interactions (attraction/repulsion) between the detergent and the negatively charged phospholipids (mainly phosphatidylserine) in the inner leaflet. At equilibrium the anionic detergents are regarded to preferentially stay in the outer monolayer, thereby being echinocytogenic, while cationic ones are thought to be trapped mainly in the inner membrane leaflet, thereby being stomatocytogenic. Although this model qualitatively explains a majority of observed shape alterations induced by a variety of detergents [1-5,9], it has been thought that it cannot account for the stomatocytic effect of certain nonionic detergents, e.g. $C_{12}E_8$ and Triton X-100, for which there should be no a priori reason to assume an asymmetrical membrane insertion on electrostatic grounds [3,10]. Thus, although detergents like $C_{12}E_8$ must be expected to easily flip in the erythrocyte membrane [see 11,12], the reason for the stomatocytogenic effect of nonionic amphiphiles like $C_{12}E_8$ and Triton X-100, as well as their transbilayer distribution (at equilibrium), has been regarded as unclear [10,13].

RESULTS AND DISCUSSION

Recent results indicate that $C_{10}E_8$ shows a weak positive charge character [14]. This observation is in line with results showing that nonionic surfactants containing polyoxyethylene (or similar) units bind cations (monovalent as well as divalent) resulting in positively charged complexes [15-17 and references therein]. The detergent-cation complex formation apparently occurs due to dipole-ion interactions [18,19]. The polyoxyethylene chain complex cations via their co-ordination to oxygen atoms resulting in a helical structure where the cation is located inside the helix formed by the polyoxyethylene chain [20,21]. Analogously to crown ethers, several oxygen atoms in the polyoxyethylene chain interact with one cation and therefore the strength of the attraction depends on the length of the polyoxyethylene chain [20,22,23]. Similar characteristics may be expected for polyethylene glycol (PEG) [20].

The results presented above may explain the stomatocytogenic effect of polyoxyethylene detergents like $C_{12}E_8$ and Triton X-100. The detergents may bind cations in the buffer solution. A positive charge of the polyoxyethylene detergent-cation complex should lead to its electrostatic attraction to negatively charged phospholipid head groups in the inner membrane leaflet and thereby to stomatocytosis in line with the bilayer couple hypothesis. Thus, it seems that oppositely to what was previously believed [3,24], polyoxyethylene detergents like $C_{12}E_8$ and Triton X-100 show electrostatic head group interactions with

phospholipids. In accordance with the results presented above, recent results from our laboratory show that the $C_{12}E_n$ oxyethylene chain must be at least five units long (n 5) for stomatocytosis to occur. A shorter oxyethylene chain may not bind the cation strongly enough. It can not be excluded that non-zero dipole moments of the polyoxyethylene chain additionally interact directly with phospholipid head groups.

There are some experimental results from our laboratory which indirectly support the assumption that $C_{12}E_8$ and Triton X-100 are predominantly accumulated in the inner erythrocyte membrane leaflet. Namely, erythrocytes treated with echinocytogenic amphiphiles (e.g. dodecylmaltoside, dodecylzwittergent and sodium dodecylsulphate) attained a stomatocytic shape upon extraction (washing) with bovine serum albumin containing buffer, thereby indicating a washing away of the echinocytogenic detergent from the outer membrane leaflet. However, erythrocytes treated with stomatocytogenic amphiphiles like $C_{12}E_8$, Triton X-100 and chlorpromazine remained stomatocytic upon washing, indicating a location of these detergents in the inner membrane leaflet from where they cannot easily be washed away [see 3].

Polyoxyethylene detergents show some specific properties among detergents. We have shown that $C_{12}E_8$ and Triton X-100 propagate transmembrane phospholipid movements exceptionally strongly [25], an observation subsequently confirmed by Pantaler et al. [26]. Furthermore, $C_{12}E_8$ induces unique torocyte-shaped endovesicles in human erythrocytes [13]. It was suggested that a specific co-operative interaction of membrane intercalated $C_{12}E_8$ with adjacent phospholipids leads to the formation of $C_{12}E_8$/phospholipid complexes. The properties of such complexes, i.e. their orientational ordering in the regions with a nonzero membrane curvature deviator, may favour the formation of torocyte endovesicles, characterised by a low average mean membrane curvature and a high average curvature deviator [13,27]. Interrelated molecular properties like a cationic charge, a relatively high lipophilicity and an appropriate effective molecular shape may be important for the above described membrane effects.

To conclude, the ability of polyoxyethylene chains to complex cations and attain a positive charge explains the for long mysterious stomatocytogenic effect of nonionic detergents like $C_{12}E_8$ and Triton X-100.

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