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Coordination forces between lipid bilayers produced by ferricyanide and Ca^{2+}

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ABSTRACT

Attractive forces usually invoked to take place in membrane–membrane contact in aggregation are hydrogen bonding cross-linkings and hydrophobic interactions between opposing surfaces. However, little is known in relation to the presence of coordination forces in the membrane–membrane interaction. These are understood as those that may be favoured by the formation or the participation of coordination complexes between surface specific groups. In this work, we have analyzed the formation of this type of aggregates between phosphatidylcholine vesicles mediated by a coadsorption of ferricyanide and Ca^{2+} ions to the interface. The results obtained by surface potential measures, optical and electronic microscopy, FTIR and ^1H NMR spectroscopies indicate that ferricyanide $[Fe(CN)_6]^{3-}$ but not of ferrocyanide $[Fe(CN)_6]^{4-}$ can form the complex when Ca^{2+} has been adsorbed previously to the membrane surface. In this condition, the anion is likely to act as a bridge between two opposing membranes causing a tight aggregation in which geometry and the polarizability of the ligands to Fe^{3+} play a role.

1. Introduction

Forces at the surface of a membrane control the ordering and the packing of adsorbing molecules in a two dimensional arrangement. In addition, intermolecular repulsion and attractive forces have been postulated to take place in the interplanar membrane–membrane contact in aggregation and fusion [1].

Repulsions hindering the membrane–membrane contact are identified with charge repulsion and non electrostatic forces including hydration (dipole potential), undulations and steric forces [2,3]. The attractive forces usually invoked are hydrogen bonding crosslinkings and hydrophobic interactions between opposing non polar surfaces.

The role of electrostatic forces in the interactive process is mainly ascribed to the screening of the surface charge repulsion, not to a net attractive force [4]. Electrostatic repulsions are drastically reduced by the high ionic strength at physiological salt concentrations. Thus, the reduction of this repulsion is relevant in the way that it makes possible the interaction between surfaces via short range attractive forces, such as van der Waals and H bonds.

One exception to this rule seems to be Ca^{2+} and others high density charge ions (high charge to radius ratio) ions, such as La^{3+}, that strongly bind to phosphate groups of zwitterionic and negative phospholipids causing changes in packing and polymorphic arrangements of lipid molecules [5]. Calcium ions bind naturally to negatively charged phospholipids as phosphatidylserine [6], phosphatidylinositol [7] and phosphatidylglycerol [8] but rather weakly to zwitterionic lipids as phosphatidylcholine (PC) and phosphatidylethanolamines (PE) [9–11]. However, Ca^{2+} affects the band absorption of asymmetric and symmetric phosphate stretching modes as measured by the FTIR spectra, which has been ascribed to the dehydration caused by the interaction [11–13].

The binding constants of Ca^{2+} ions to pure PC bilayers may differ from those corresponding to mixtures of PC with another kind of phospholipids, e.g., phosphatidylglycerol (PG) [14]. In general, the stoichiometry of the Ca^{2+}/PC binding is 1:1 in the fluid state and 1:2 in the gel state [15]. In chemically synthesized phospholipids like dipalmitoylphosphatidylcholine (DPPC) different changes in the state of assembly at their phase transition temperatures have been reported [16]. Faure et al. [16] found that the tilt angle of DPPC molecules was 30° in the gel phase and 0° in the fluid phase while the geometrical parameters of the head group remained constant throughout the different phases [16].

Among the techniques to detect Ca^{2+} adsorption on suspended particles, measure of the zeta potential of PC liposomes in ionic solutions is a direct one [10,17]. With this technique also anion adsorption can be detected.

Anions may also adsorb to the lipid membrane due to the increased polarizability of their electronic clouds following the Hoffmeister series [18–20]. In this case, the surface acquires a negative surface charge density. Ferricyanide is not included in the Hoffmeisster series [20]. However, due to the presence of CN^- ligands, ferricyanide and ferrocyanide anions should be in the end

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near ClO_4^-. The corresponding radii are 400 and 450 pm according to Marcus while Ca^{2+} is 100 pm [21].

The titration of sonicated vesicles of egg phosphatidylcholine with $[Fe(CN)_6]^{3-}$ in the presence of Ca^{2+} results in the formation of aggregates [22]. The turbidity increase caused by these aggregates cannot be reversed by EDTA treatment. In addition, no rearrangement of the bilayer structure has been found in this process. This was deduced from the absence of leakage of a dye trapped in sonicated vesicles parallel to a turbidity increase [22]. The aggregation seems to be dependent on the Ca^{2+} content of the vesicles, the outer Ca^{2+}, the $[Fe(CN)_6]^{3-}$ concentration and the order of addition of Ca^{2+} and $[Fe(CN)_6]^{3-}$. The results were explained by a specific adsorption of $[Fe(CN)_6]^{3-}$ to bilayers of sonicated vesicles, in contrast to other anions of a similar radius but with a higher charge such as ferrocyanide $[Fe(CN)_6]^{4-}$ that were unable to produce aggregation in the presence of Ca^{2+}. This was indicative that the charge is not a determinant property in the aggregation phenomena.

In order to have an insight on the forces and structural arrangements, conveying to the formation of aggregates as a function of the nature of the complex, the aim of this article is to study the binding of $[Fe(CN)_6]^{3-}$ and Ca^{2+} to dipalmitoylphosphatidylcholine (DPPC) layers in the fluid and gel states by electrophoretic mobility, light scattering measurements FTIR and ^1H NMR spectroscopies. Optical and electronic microscopy provided a direct visualization of organization, or lack thereof, and can thus provide critical information about the mechanisms of aggregation.

A model of the interlamellar structure is proposed on the base of these results.

2. Materials and methods

2.1. Lipids and chemicals

Egg phosphatidylcholine (eggPC) and dipalmitoylphosphatidylcholine (DPPC) were obtained from Avanti Polar Lipids Inc. (Alabaster, AL). The purity of the lipids was checked by running the FTIR spectra of lyophilized samples and by differential scanning calorimetry (DSC) and by thin layer chromatography (TLC), using the solvent mixture corresponding to each type of phospholipid. Single spots after exposure to the suitable developers were obtained. Peroxidation levels in unsaturated phospholipids (eggPC) were checked by ultraviolet spectroscopy, and it was found negligible under the conditions employed.

Calcium chloride ($CaCl_2$) was obtained from Sigma Aldrich Chemical Co. Inc. (St. Louis, MO) and $[Fe(CN)_6]^{-3}$ from Analytical reagent – Mallinckrodt.

All other chemicals were of analytical grade and ultrapure water (conductivity 0.09 μS/cm, pH 6 ± 0.3) was obtained in an Osmoion equipment (Apema, Buenos Aires, Argentina).

$K_3[Fe(CN)_6]$ and $CaCl_2$ stock solutions of a concentration 10 and 8 mM, respectively were prepared in tridistilled water and used as a blank.

2.2. Light scattering measurements

Lipids in chloroform solution were dried to form a film under a stream of nitrogen. Then, they were rehydrated in solutions of $K_3[Fe(CN)_6]$ and $CaCl_2$ heating above the transition temperature with gently agitation during 15 min to produce multilamellar vesicles (MLV's) [23]. Large unilamellar vesicles (LUV) of DPPC were prepared by extrusion of MLV's through Nuclepore® membranes of 400 nm pore diameter using an Avanti Mini-Extruder.

In all cases, the relation lipid to water was maintained in order to make the preparation more reproducible.

In order to investigate aggregation effects, turbidity changes were followed by adding known amounts of $K_3[Fe(CN)_6]$ and $CaCl_2$ to LUV's DPPC dispersions.

The turbidity changes at 25 °C were followed by measuring the changes in the absorbance at 450 nm in a Hitachi U-2000 spectrophotometer by adding known amounts of the stock $K_3[Fe(CN)_6]$ and $CaCl_2$ solutions to a measured volume of water or LUV's DPPC vesicles.

2.3. Optical microscopy

Morphological evaluation of LUV's and multilamellar liposomes with the addition of 10 mM $[Fe(CN)_6]^{-3}$ and/or 8 mM $CaCl_2$, prepared as were described below was conducted using an inverted microscope CKX41 (Olympus, Japan), and were visualized by digital Olympus QColor3-RTV-R (Olympus). Finally images were analyzed by software Q-Capture Pro (Q-imaging, Surrey, BC Canada).

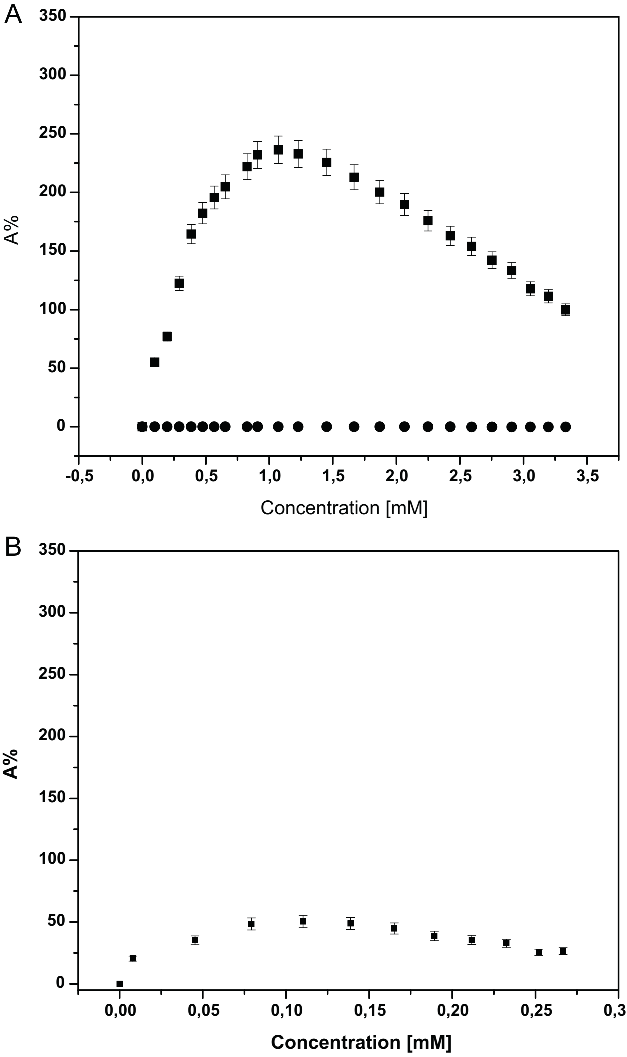

Fig. 1. Aggregation of LUV's induced by $[Fe(CN)_6]^{3-}$ and Ca^{2+}. (A) Turbidity changes induced by $[Fe(CN)_6]^{3-}$ on (●) LUV's DPPC vesicles without Ca^{2+} in the suspension media and, in the presence of 8 mM Ca^{+2} (■). (B) Turbidity changes of LUV's DPPC vesicles titrated with an equimolar solution of Ca^{+2} and $[Fe(CN)_6]^{3-}$·($Ca^{2+}/[Fe(CN)_6]^{3-}$ ratio = 0.8).