Biochimica et Biophysica Acta 1808 (2011) 332–340

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Interaction of chromium(III) complexes with model lipid bilayers: Implications on cellular uptake

Natesan Sella Raja, Kamatchi Sankaranarayanan, Aruna Dhathathreyan *, Balachandran Unni Nair *

Chemical Laboratory, Central Leather Research Institute, CSIR, Adyar, Chennai, India

ARTICLE INFO

Article history:
Received 28 June 2010
Received in revised form 9 September 2010
Accepted 20 September 2010
Available online 13 October 2010

Keywords:
Langmuir films
Phospholipids
Cr(III) complexes
Supported multibilayers
Fluorescence anisotropy
Toxicity of Cr(III)

ABSTRACT

To understand molecular cytotoxicity of chromium(III) and how it affects the stability of biological membranes, studies on the interaction of chromium(III) complexes aquapentaminechromium complex (complex I) and *trans*- [Cr(5-methoxysalcyclohex) $(H_2O)_2$] ClO_4 (complex II) with model biomembranes have been carried out. Langmuir films of dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidic acid (DPPA), dioctadecyldimethylammoniumbromide (DOMA) at air/water interface interacting with the chromium(III) complexes have been characterized using the surface pressure-molecular area (π-A) isotherms. Initial surface pressures changes for the two complexes show that the chromium(III) complexes inserted in the Langmuir films and complex I interacted strongly compared to complex II. Supported bilayers (SB) of the lipids on solid substrates formed by hydrating their Langmuir-Blodgett films (LB films) have been characterized using linear dichroic spectra, low angle X-ray diffraction and steady state fluorescence anisotropy. Depending on the geometry of the ligands and concentration, the complexes either insert in the alkyl or in the head group region of the SB and sometimes in both regions. The Supported lipid bilayers are well-layered and at low concentration, the metal complexes are incorporated near the head group region. Order and increase in lamellar spacing show stronger interaction of complex I with the lipids compared with complex II. This study provides some insights into the mechanism of chromium(III) toxicity and uptake of chromium(III) by the cells.

1. Introduction

In all living organisms, most of the biological processes take place in salt solutions. To maintain the fine balance between the different ions and the environment, biomembranes tune their interaction with different ionic species [1]. Specific ionic effects have been shown to influence growth rates of bacteria [2], fungi [3,4] and to affect the function of antibiotic channels [5]. This investigation reports on the chromium complexes aquapentaminechromium $[Cr(NH_3)_5(H_2O)](NO_3)$ (complex I) and *trans*- [Cr(5-methoxysalcyclohex) $(H_2O)_2$] ClO_4 (complex II) where 5-methoxysalcyclohex is N, N' – cyclohexane*bis*-(5-methoxysalicylideneimine) (Fig. 1a, b) and their interactions with model lipids as Langmuir films at air/water interface and as supported multibilayer films.

Chromium has been known to induce toxicity with the two commonly occurring, environmentally available oxidation states of the metal – chromium(III) and chromium(VI). Of the two, a number of work on chromium(VI) toxicity and carcinogenicity have been reported. Chromium(VI) enters into the cell by the phosphate and sulfate anion exchange pathway and is immediately reduced into stable, kinetically inert chromium(III) by the reducing agents such as glutathione, ascorbic acid and citric acid freely available inside the cell. Chromium(III) interacts with the biological macro molecules likes DNA, protein and lipids. Toxicity of the chromium(III) is debatable and evidences for both beneficial as well as the harmful nature of chromium(III) have been reported in the past few years. Chromium(III) has been classified under essential nutrient and marketed as nutritional supplement. Trivalent chromium is an essential element required for normal carbohydrate, lipid, and protein metabolism in humans and animals [6–8]. Chromium(III) is essential for the lipid and carbohydrate metabolism [9,10]. Other positive influences of chromium include induction of increased glucose tolerance and elimination of fat. Trivalent chromium deficiency has been associated with diseases such as type 2 diabetes [11], cardiovascular disease, and nervous system disorders [12]. Toxic effects of chromium(III) include induction of apoptosis through caspase activation [13], nephrotoxicity [14], reproductive and developmental toxicity in male rats [15]. Chromium(III) also has been known to interact with several components of biological media used in animal cell and tissue culture and modify their chemical properties [16]. Membrane perturbations induced by the metal complexes are important because several metal induced toxicity abnormalities such as membrane blubbing and mitochondrial damage involve plasma membranes. Further, the ability of metal complexes to diffuse into the

* Corresponding authors. Tel./fax: +91 44 24411630.
E-mail addresses: aruna@clri.res.in (A. Dhathathreyan), bunair@clri.res.in (B.U. Nair).

0005-2736/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2010.09.015

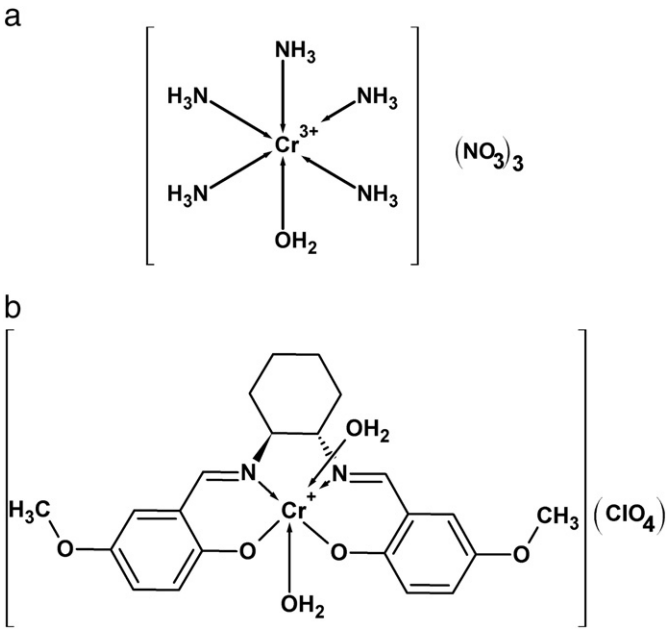

Fig. 1. (a)Structure of Complex I; (b) Structure of complex II.

cell is determined by their interaction with the membrane lipids. A number of different studies on model membranes have been carried out. Metal complexation with Langmuir monolayers of histidyl peptide lipids has been reported by Leblanc et.al [17]. SFG studies on interaction of antimicrobial peptides in the presence of metal ions with supported lipid bilayers have been carried out by Chen and Chen [18]. Separovic et.al have analyzed the effect of metal ions on the interaction of cholesterol and clioquinol with lipid bilayers and their effect on the disruption of the bilayer structure [19]. Molecular dynamics simulation of metal ions interacting with phospholipid bilayers have shown that specific ions have profound effect on the bilayer structure and in special cases even increase the order [20].

To gain further insights about the interaction of chromium(III) and influence of the ligand environment with the cell membranes, in the present study, three model lipid systems with different charges have been chosen. Anionic DPPA (dipalmitoylphosphatidic acid), neutral DMPC (dimyristoylphosphatidylcholine) and cationic DOMA (dioctadecyldimethylammoniumbromide) have been used as Langmuir films and also to prepare unilamellar vesicles. In this work, we have studied as a first step, Langmuir films of neutral lipid DMPC, anionic lipid DPPA and cationic lipid DOMA in the presence of complexes I and II. Langmuir-Blodgett films (LB films) of DMPC, DPPA and DOMA with the complexes have been hydrated with buffer and supported multibilayers (SMB) have been formed. These multibilayers have been studied using low angle XRD. Possible modes of interactions have been suggested from the changes in lamellar spacing. Traditionally, organized assemblies of functionalized molecules have been prepared using the method for Langmuir-Blodgett films which have found extensive applications such as energy transfer in controlled geometries, molecular electronics, nonlinear optics, coatings, sensing, to form sequential layers of thin films and as model for membranes, for template particle growth. However, for a number of characterization techniques, one or two bilayers may not provide enough material to obtain adequate signals, thus making multiple-bilayer samples a necessity. In general, the deposition of phospholipid bilayers by LB techniques is a difficult task. Some success has been achieved transferring dipalmitoylphosphatidic acid (DPPA) or mixed phospholipids films that include DPPA with calcium or uranyl ions present in the subphase [21]. Linear Dichroic measurements of the SMB used in this work gains insight into the order parameter of the SMB after incorporation of metal complexes. The order parameter 'O' of complex with reference to lipid bilayer is measured and using this information mode of interaction of the metal complexes with different lipid bilayers has been predicted and models have been represented schematically. From these models of the lipid – metal complexes estimates of lamellar spacing have been evaluated and agree with the experimental data.

2. Experimental methods

2.1. Materials

Highly purified (>99%) synthetic DMPC (1, 2-dimyristoyl-*sn*-glycerophosphatidylcholine), DPPA (1, 2-dipalmitoyltoyl-*sn*-glycerophosphatidicacid, sodium salt), and DOMA (Dioctadecyldimethylammoniumbromide) have been purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. Organic solvents are high-performance liquid chromatography grade from Aldrich (Milwaukee, WI). The two metal complexes I and II have been synthesized according to the reported procedure [22,23]. These complexes have been characterized using mass and UV-Vis spectra. The solution spectra of the two complexes are presented in Fig. 2. The lyophilized lipids have been hydrated with purified water or salt

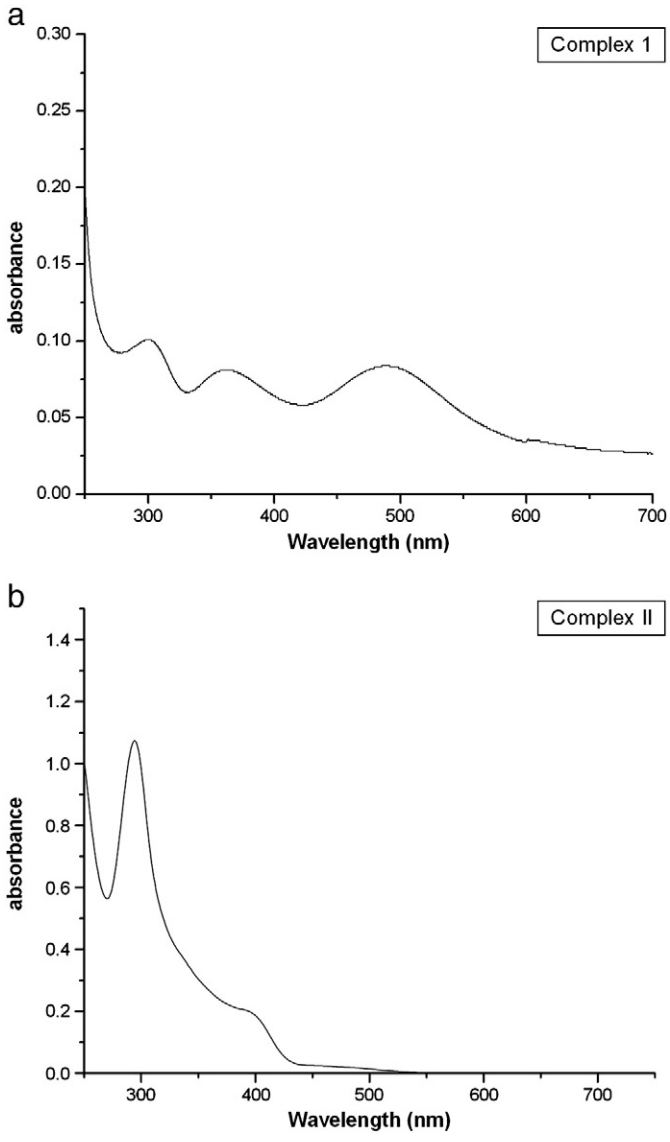

Fig. 2. (a) Solution spectra of complex I ; (b) Solution spectra of complex II.

solutions. Samples have been cycled below and above the chain melting transition temperatures, occasionally vortexed, and then typically stored for more than 48 hours at 4 °C.

2.2. Preparation of model membrane vesicles

Defined amounts of phospholipid (4 to 10 mg) have been dissolved in 100-200 μl chloroform: methanol (1:1) solution and dried under vacuum at 25 °C. For mixtures of lipids with fluorescent lipid Diphenylhexatriene (DPH) the probe to lipid ratio has been maintained at 1:200 molar ratio and a similar procedure as that of pure lipid vesicles have been followed. The residual thin film has been re-suspended in phosphate buffer (pH 7.4) and sonicated at highest power for 30 min. This solution has been subjected to 10 freeze/thaw cycles using liquid nitrogen and temperature regulated water bath to get uniform sized vesicles.

2.3. Preparation of supported multibilayers

The supported lipid bilayer (SMB) has been prepared as follows. The first leaflet of the bilayer has been transferred as a compact monolayer using the LB film technique with the polar head groups in contact with the solid substrate. In the second step a vesicle solution is contacted slowly in a controlled manner using a dip coater (NIMA) at a uniform coating speed of 5 mm^2/min with the substrate. As a result of the not so favorable thermodynamic state of the hydrophobic surface of the immobilized leaflet, lipid molecules in the vesicle would adsorb spontaneously and thus building the complete bilayer. The pure lipid bilayers DMPC, DPPA and DOMA and those containing the metal complexes have been prepared in a similar fashion.

2.4. Anisotropy measurements

Anisotropy measurements have been performed in a Cary eclipse spectrofluorometer. The instrumental parameters have been set as follows: Polarizer - on, excitation filter - auto, emission filter - open and photo multiplier tube - medium. The instrument is set in fluorescence mode and manual polarizer is attached. The excitation and emission wavelengths have been set as 358 and 425 nm, respectively. The instrument with an L shaped measuring geometry is corrected for G factor. Here G is an instrumental correction related to the ratio of intensity of perpendicular polarized light (I_{HV}) to the intensity of parallel polarized light (I_{HH}). All the experiments have been done in triplicate and average values have been taken for the analysis. The DPH incorporated vesicles solution has been used as control and chromium(III) complexes have been added at 5, 10, 20, 30, 40 and 50 micro molar concentration and average r (anisotropy) values obtained have been used to calculate the order parameter. Generally, heterogeneities seen by DPH are known to reflect the variations of the packing density of the lipids in a direction perpendicular to the bilayer surface and thus DPH has been used by a number of research groups to analyze order in vesicles [24].

2.5. LB films of the lipids and XRD

The Langmuir films of the lipids have been spread from chloroform solutions (mill molar) on the buffer subphase and on the subphase containing complexes I and II using a single barrier trough (Model 601 S) from Nima technology, UK connected to a thermostat. The Langmuir-Blodgett films have been then transferred to glass or quartz coverslips at a surface pressure $\pi = 20$ mN/m. The transfer ratio is around 0.85 and the transfer is usually Y-type. 2.2×2.2 cm^2 thin glass cover slips (ERMA, FRG) used for XRD have been cleaned with isopropyl alcohol and chloroform. The thin glass cover slip is ideal for X-raying lipid samples through the back of the glass at a 45° angle of incidence; the scattering from the sample is then totally unobstructed by the substrate. These substrates attached to the cap of a 20-mL disposable glass scintillation vial using either sticky tack or clay. This method of attachment leaves the sides of the substrate completely free, which is essential to allow surface tension and hold the solvent solution on the top of the substrate.

X-ray data have been recorded for the samples using Seifert JS0 Debyefllex 2002 X-ray diffractometer. The lamellar repeat distance of these phospholipid systems is measured at room temperature (21-25 °C) using Cu Kα1 radiation of $\lambda = 1.540$ Å generated at 30 kV-30 mA. All lipids except DPPA have been used above their hydrocarbon chain phase transition temperatures. Each scan covered θ from 0.5° to 5° with step size 0.005°, one second per step. The humidity/temperature chamber enclosed the goniometer head and is insulated from room temperature. The temperature of the sample has been monitored by a Pt-100 thermo resistor and controlled to ±0:025 °C via a computer based feedback system. The chamber is connected to a water source whose temperature is adjusted to vary the relative humidity in the immediate vicinity of the lipid sample. A combined thermometer and hygrometer (accuracy 0.1 ± C and 0.1% RH for RH<» 98%, respectively) is positioned next to the sample.

2.6. Absorption and LD measurements

Absorption and LD measurements have been recorded using Cary Bio 050 UV-Vis spectrophotometer on SMB of the lipids with metal complexes I and II at room temperature. A prism polarizer placed in the path of the incident light is mounted on a graduated rotating base that could be dialed to generate plane-polarized light at various angles relative to the substrate of the sample. Polarized absorption spectra have been taken at angles of 0° and 90°. An order parameter from Linear Dichroic (LD) measurement of the lipid bilayers have been used to analyze the orientation of the incorporated metal complexes. Here R the ratio between absorbance for p and s-polarized light LD is used to calculate the order parameter which is defined as

$$\text{Order parameter} = (R-1)/(R+2)$$

The polarized absorption spectra have been corrected for scattering by using a simple algorithm that removed scattering so that the correction satisfied two criteria. First, the scattering correction had to satisfy the simple formula $a + b\lambda^4$, where a and b are independent variables. We made the further assumption that the scattering correction is identical (isotropic) for both polarization angles. This prevents the scattering correction from having an influence on the calculation of the transition dipole.

3. Results

3.1. Surface pressure – area isotherms

Fig. 3(a), (b) and (c) show the surface pressure – area isotherms of the lipid monolayers spread over phosphate buffer (10 mM, pH = 6.5) and on subphase containing the two complexes. The pure lipid monolayers spread on buffer without complexes at pH 6.5 did not show any deviation in surface area from the surface area of the lipid monolayers spread on water. The change in the surface area ΔA at any constant pressure for pure monolayers was around 2 to 3% duration of the experiment. Compressibility values were used to identify the various states of the monolayer phases of the different monolayers. Table 1 shows the average area/molecule and the maximum collapse pressure for the three lipids and lipids + complexes spread on buffer obtained from the isotherms. From the changes in area it is seen that interaction of complex I is stronger with anionic lipid DPPA and to some extent with the neutral DMPC. The strength of the interaction is manifested by the maximum change in area for DPPA + complex I films. In a 2-dimensional Langmuir film where $\pi A = kT$, increase in area should be accompanied by a decrease in pressure. The larger the

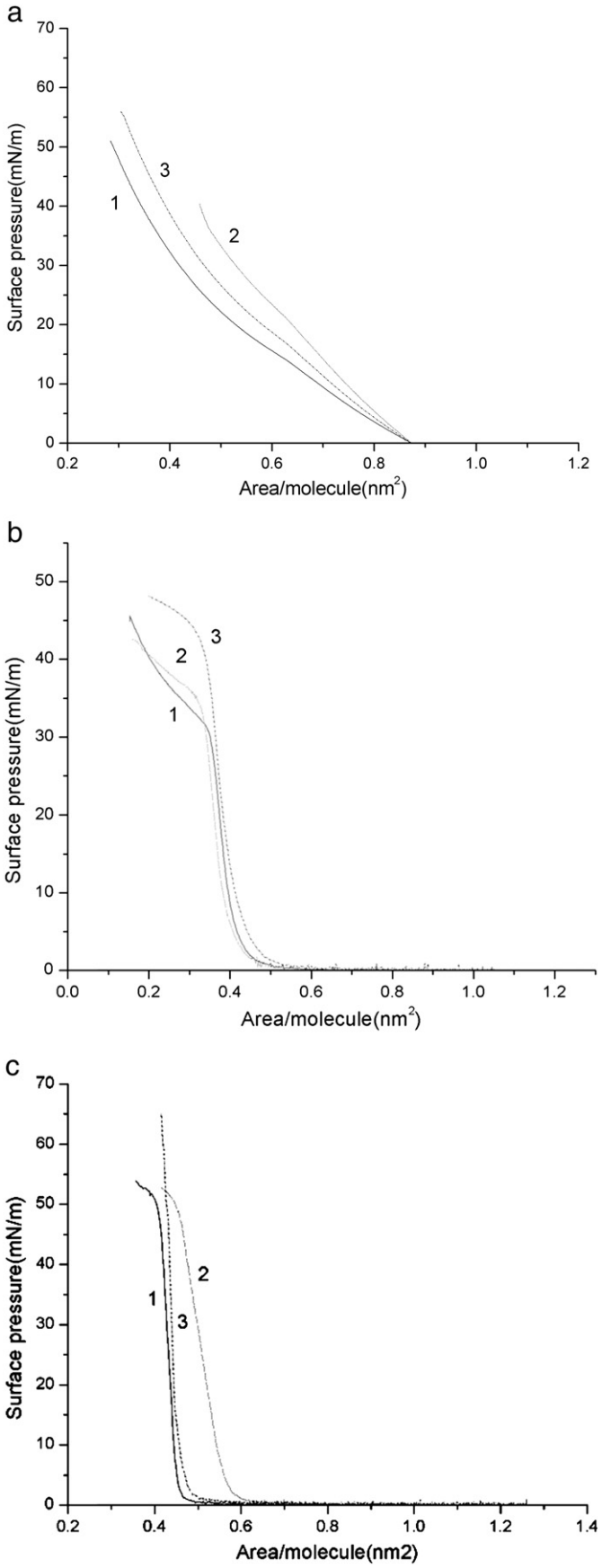

Fig. 3. Plot of Average molecular area vs. surface pressure of (a) DMPC in 1-Buffer, 2-Complex I + Buffer, 3-Complex II + Buffer ; (b) DOMA in 1-Buffer, 2-Complex I + Buffer, 3-Complex II + Buffer and (c) DPPA in 1-Buffer, 2-Complex I + Buffer, 3-Complex II + Buffer (pH6.5, T = 22 °C).

Table 1
Average area/molecule at 20mN/m and the maximum collapse pressure for the DPPA, DMPC and DOMA alone and after they were incubated with the two chromium(III) complexes.

Monolayer	Average area/molecule(nm^2) at π = 20mN/m	Max.Collapse pressure(mN/m)
DPPA	0.52	51.04
DPPA + complex I	0.63	39.93
DPPA + ComplexII	0.58	54.68
DMPC	0.45	31.80
DMPC + complexI	0.56	34.25
DMPC + complexII	0.54	42.61
DOMA	0.54	45.55
DOMA + complexI	0.56	42.75
DOMA + complexII	0.57	48.05

interaction followed by insertion into the headgroup plane, lower is the collapse pressure. The increase in area upon addition of the complexes may correspond to head group orientations extending more upright from the plane of the lipid bilayer. Such changes have been previously reported for heterocyclic compounds interacting with the lipid molecules [25-27].

Fig. 4(a) and (b) show the interaction of the metal complexes I and II for different surface densities obtained by compressing the films to different initial surface pressures. After spreading and compression of the monolayer to different surface pressures (5, 10, 15 mN/m…), the surface area has been kept constant. Changes of the surface pressure have been followed over time after injection of the metal complexes into the subphase. It is seen that as the initial surface pressure is increased the interaction of the complexes with the lipid monolayer decreases showing that insertion followed by interaction is lowered for both the complexes. Thus in the liquid expanded phase of all the lipids the interaction of the complexes are strong. However for complex I the maximum change in initial pressures has been observed, especially with DPPA followed by DOMA and DMPC. In the absence of a lipid monolayer no pressure change has been detected after complex injection, demonstrating that the metal complexes are by themselves not surface active.

From thermodynamics, in a monolayer at air/water interface, an enrichment induced by physisorption or chemisorption is usually coupled with an increase in lateral pressure that can be described by the Gibbs adsorption equation. A molecular interpretation suggests that normally the binding of the metal complex enlarges the area occupied by the head group of the lipid and induces an increase in pressure. From Fig. 4(a, b) it has been observed that complex 1 shows larger Δπ than complex II and it clearly indicates that complex I interacts more favorably with the three lipid monolayers compared with the complex II. Also depending on the overall charge on the metal complex (+3 for complex I and +1 for complex II) the interaction with the cationic lipid DOMA is least. In case of neutral lipid DMPC, the interaction lies between that of DPPA and DOMA suggesting that possibly the interaction of the metal ions with the phosphate moieties of the head group could also compete with the charge-charge interactions.

3.2. Anisotropy measurements

Fig. 5 shows the variation of fluorescence anisotropy of DPH with DMPC, DPPA and DOMA vesicles with increasing concentrations of complex I and II respectively. The concentration of complexes has been varied between 0 to 50 μM, keeping the lipid concentration constant at 0.5 μM. Fluorescence spectroscopy is the technique, which has the highest sensitivity for the study of lipid vesicles, biomembranes, and lipid/surfactant interactions. Since lipids are not

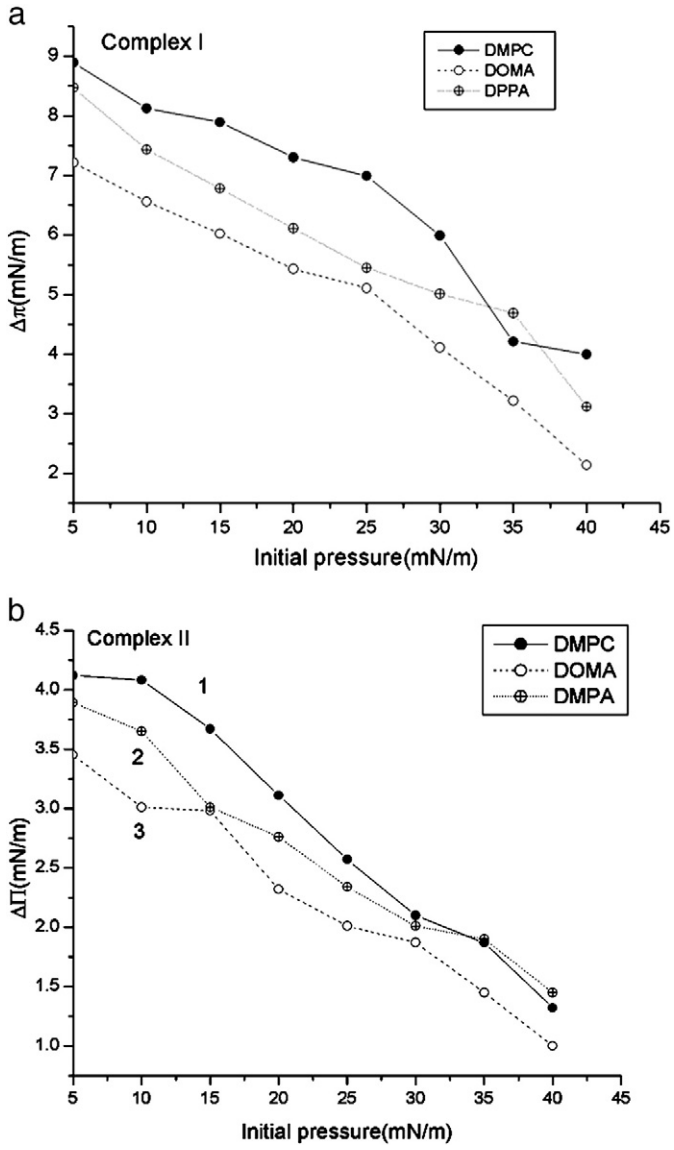

Fig. 4. Interaction of the metal complexes I and II for different surface densities of lipid films- (a) Initial surface pressure versus change in the surface pressure for complex I, (b)Initial pressure versus change in the surface pressure for complex II. These plots have been obtained by injecting complexes of different concentration under the lipid films.

fluorescent, fluorescence studies of lipid vesicles are made possible by introducing a fluorescence probe into the lipid environment.

The dye DPH exhibits a hydrophobic nature, with low solubility and fluorescence in water. In this work, DPH fluorescence anisotropy has been studied in mixed lipid/metal complex systems with several concentration ratios of the lipids DMPC, DPPA and DOMA and the complexes I and II. DPH is a rod like molecule, whose absorption and emission dipoles are collinear and lie along the principal molecular axis, which is perpendicular to the membrane plane. The probe is located deep within the hydrophobic core region of the phospholipid bilayer. Thus any modification in the movement of the chains or packing is sensitively reflected in the fluorescence anisotropy of DPH. DMPC vesicle shows the highest anisotropy (on an average 0.7) with complex I and the lowest (average value 0.1) with complex II suggesting that the local ordering of the lipid with complex I is better compared with complex II. Fig. 5 reveals that initial addition of Complex I to the DMPC vesicles gives rise to an abrupt increase in the fluorescence anisotropy from approximately 0.1 to 0.85, indicating higher lipid microviscosity. The increased microviscosity is most likely ascribed to the incorporation of the metal complex molecules, in a relatively large quantity, within the phospholipid domains, thereby reducing the rotational reorientation of the DPH probes. Reduced fluidity of biological membranes has been previously observed upon similar association with membrane proteins [26]. However for both complexes I and II there is no appreciable change in the anisotropy in the lipid vesicles for increasing concentrations. Fluorescence anisotropy measurements show that distinct changes occur in the "microviscosity" [or order-parameter] of the lipid domains after addition of the complexes I and II respectively. With both DOMA and DPPA there is no appreciable change in anisotropy with increasing concentration of the complexes. DOMA being a cationic lipid is not expected to interact very strongly with the complexes. Thus no appreciable change in anisotropy is to be expected. In case of DPPA, the strong interaction with complex I may result in compact structure and thus reducing the anisotropy.

3.3. Linear Dichroic (LD) measurements

Table 2 presents the order parameter obtained for complexes 1 and 2 in the different SMBs. The order parameter is highest for complex I with DPPA confirming the strong interaction of the complex and overall homogeneity or lack of aggregated structures of DPPA in the presence of the complex I. In case of complex II the order parameter with DPPA is very low. For the neutral SMB with DMPC, the order parameter is higher for complex II compared with complex I. This is possibly due to the metal-phosphate interaction competing with the charge-charge interaction and this is in agreement with the anisotropy studies. For DOMA for both complexes I and II the order parameter is low indicating the poor interaction.

3.4. XRD studies of supported bilayers

In order to understand the effect of incorporation or localization of the complexes in the vesicles, low angles XRD of supported multibilayers (SMB) of these systems have been studied. A number of papers have appeared in the literature reporting on the changes in interbilayer forces due to the presence of monovalent ions. The Ion-lipid interactions have been analyzed by solid-state NMR spectroscopy [28], electrophoretic mobility [29–31], and monolayer surface pressure [32].The interlamellar ('d'-spacing) spacing in these systems is plotted as function of concentration of the metal complexes at a constant osmotic pressure. X-ray diffraction of the different multibilayer films of lipids and lipids with complexes I and II give several sharp reflections, suggesting that the structures of these lipid-water systems could have single lamellar phases. Fig. 6 presents the lamellar spacing for complex I and II with the different lipid multibilayers. The interlamellar spacing for the DMPC bilayers shows marginal change from 55 Å (pure hydrated lipid bilayer) to about 58 Å for the highest concentration of complex I. With complex II the lamellar spacing does not show any dramatic changes indicating that even at high concentration of the complex, it is possibly incorporated only in the non-polar region and this may not necessarily alter the lamellar packing. In case of DPPA bilayers a sharp increase in the lamellar spacing is observed for both complex I and II with increasing concentration. The lamellar spacing shifts from about 61 to 70 Å for complex I and 61 to 68 Å for complex II. This increase could be attributed to a charge – charge interaction where, the two complexes can enter into the interlamellar space of the anionic DPPA bilayers and reorient themselves. Ligand structure around the metal complex is bulkier than the simple metal ions and they push the two layers to get accommodated in the interlamellar space and this is evidenced by the increase of the lamellar spacing of bilayers with the increase in the concentration of the complexes.

For cationic DOMA multibilayers there is a contraction in 'd' spacing from about 64 Å for the pure hydrated layer to about 54 Å for

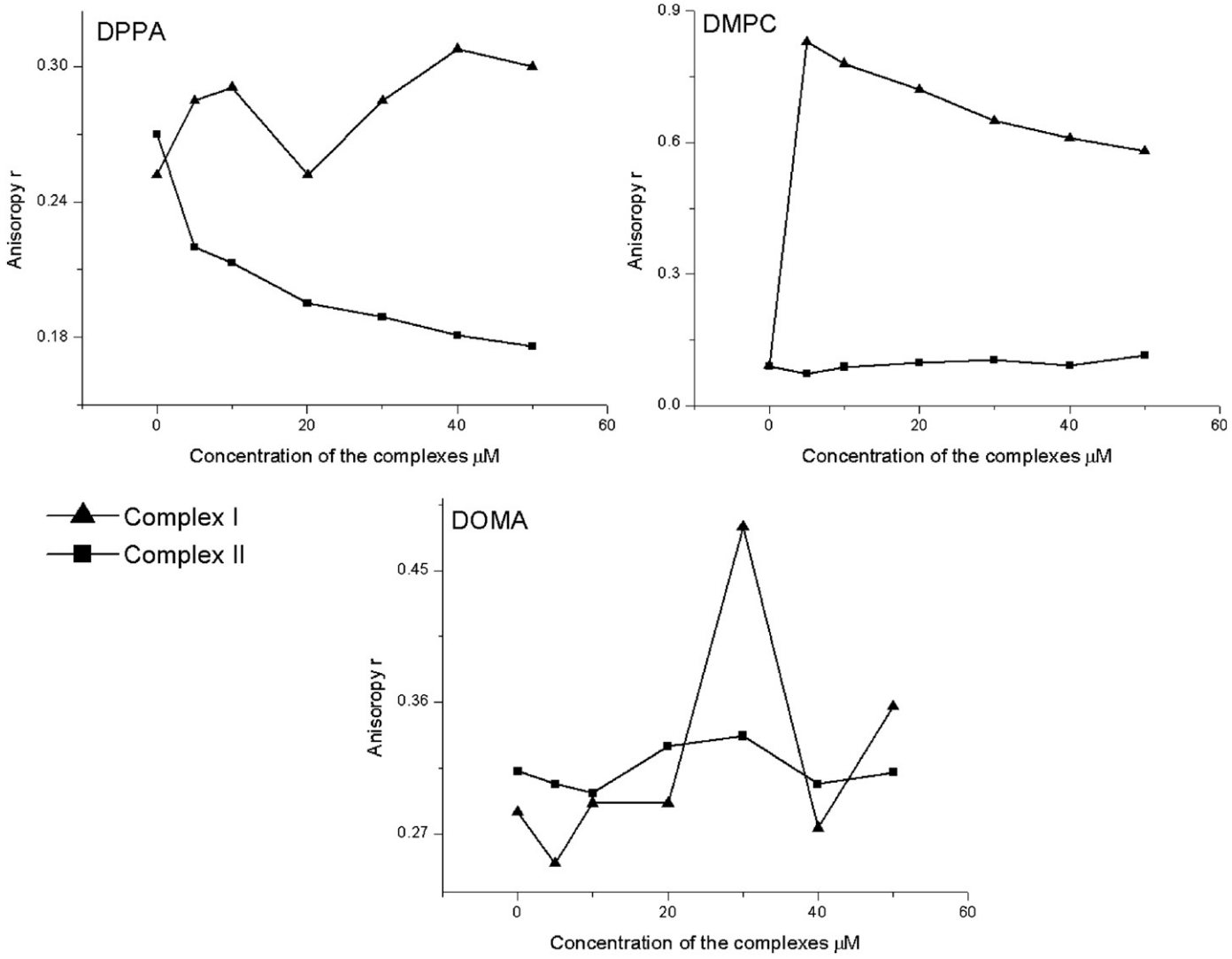

Fig. 5. Fluorescence anisotropy changes in the DPH incorporated vesicles in the presence of increasing concentrations of complex I and complex II.

complex I incorporated layer. Complex I is polar and carries positive charge. Non polar alkyl groups of these bilayers already repel each other but are held together by the hydrophobic forces. Presence of complex I destabilize the fine balance and possibly cause a collapse in the structure with subsequent decrease in the lamellar spacing. In the case of complex II there is a slight increase in the lamellar spacing to about 68 Å before leveling to 64 Å. The complex II is comparatively less polar than the complex I. At lower concentrations therefore it orients itself towards tail portion of the lipids and increases lamellar spacing. At higher concentration probably the threshold limit to maintain the bilayer architecture is lost and contraction in lamellar spacing observed. This kind of concentration depended changes in the ordering and changes in the thickness is observed lipid vesicles incorporated with curcumin [33]. Since there exists a dielectric gradient at the membrane solvent interface, there can arise an attractive force due to an induced electromagnetic field in a neighbouring membranous region and vice versa. This Van der Waals' force leads to formation of myelin sheets in vivo and multilamellar vesicles in vitro. A scheme for possible modes of interactions has been made using ACD Chemsketch and the structures are shown in Fig. 7 (a1, a2, a3), (b1, b2, b3) and (c1, c2, c3). The calculated lamellar spacing ('d' – values) agree to a high degree with the experimental values. Using small angle X-ray scattering it has been established that the interlamellar spacing of neutral membranes in water is of the same order as that of the membrane thickness itself. [34–36]. These spacings change as the surrounding solvent environment changes [37–39]. Thus any alteration of the repeat spacing with different ions or ionic species in the solvent is an indicator of a shift in the balance of repulsive and attractive forces in membranes. The results obtained for Cr (III) interaction with model lipid membranes reported here for the first time, should contribute significantly to the literature on interaction of different metals with the lipid bilayers reported earlier [40–45]. In most of

Table 2
The order parameter obtained for complexes 1 and 2 in the different SMBs using linear dichroism measurements. The complex showing high order parameter has stronger interaction with the SMB.

Complex I	DPPA	DMPC	DOMA
Low Concentration	0.79	0.17	-0.38
High Concentration	0.50	0.18	0.02
Complex II			
Low concentration	0.05	0.32	0.23
High Concentration	0.03	0.5	0.21

Chromium(III) complexes interact with supported lipid bilayers leading to changes in their structural organization . These changes are determined by both ligand structure and the charge of the chromium(III) complex.

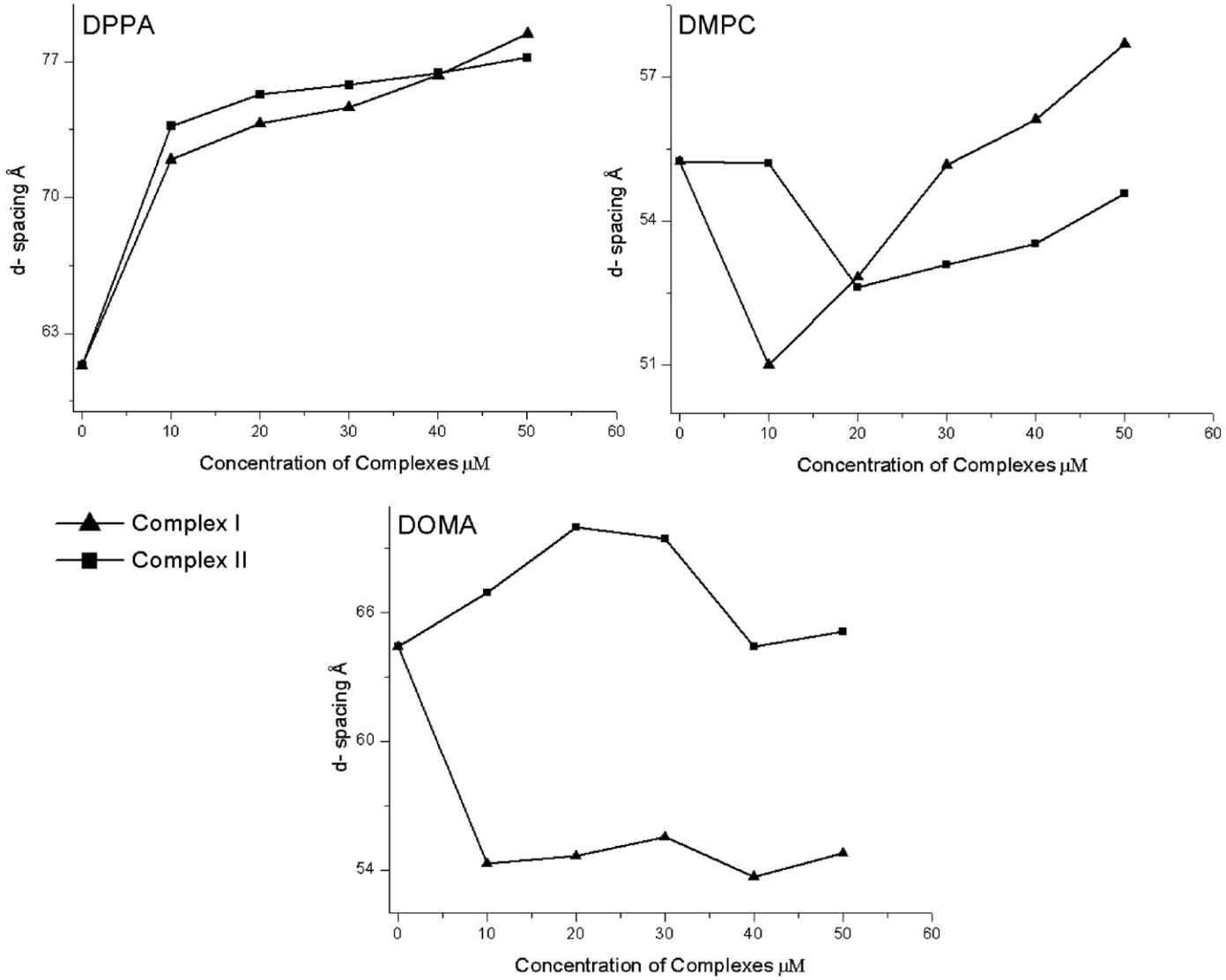

Fig. 6. Changes in the 'd' spacing of the bilayers in the presence of increasing concentrations of complex I and complex II.

these studies perturbation of the membrane structure and changes in fluidity and order have been reported.

4. Discussion

Chromium(III) has to cross the plasma membrane to gain entry into the cell [46]. Therefore, studies on interactions between the membrane lipids and chromium(III) is essential to understand the mechanism of chromium toxicity. In contrast with the commonly held notion that chromium(VI) is more reactive than chromium(III) a study has shown that chromium(III) imparts greater structural changes than chromium (VI) [47]. Unlike regular chromium(III) complexes, the complexes of salen type ligands are known to undergo facile aqua ligand substitution [48,49]. Chromium(III) can form complexes with inter and intra cellular molecules. Reactivity and interactions of chromium(III) complexes are largely determined by the ligand environment. The results of this study show the clear role of ligand environment of chromium complexes and their influence on the lamellar space of model membranes. Langmuir films of the lipids spread on subphase containing complexes I and II show that they adsorb at the interfacial region of monolayers and push the head groups laterally to create space. Both complexes seem to form ordered structure with lipid bilayers arranged in stacks. At low concentrations complex II stacks in between the polar head groups and at higher concentrations is sandwiched between the long alkyl regions. These concentration dependent changes are not observed in the case of complex I. Our results indicate that in SMBs, both complexes strongly get bound in the interfacial region of anionic DPPA thereby inducing a condensing 'effect' on the bilayer that is strongly reflected in the increase in the lamellar spacing. The presence of complexes in SMBs and their influence on the geometry of the SMB have been demonstrated by the fluorescence anisotropy and Linear Dichroism measurements. Increase in the order of the SMB is followed by an increase in lamellar spacing by complex I compared to complex II suggest stronger interaction by complex I. These experiments clearly demonstrate ability of chromium(III) to interact and modify the structure of lipid bilayers. This study also shows that uptake of chromium(III) can be regulated by the ligand structures. This study is helpful in understanding of chromium(III) toxicity and development of chromium(III) supplements.

Acknowledgement

Author NSR thanks CSIR, Govt. of India for a research fellowship.

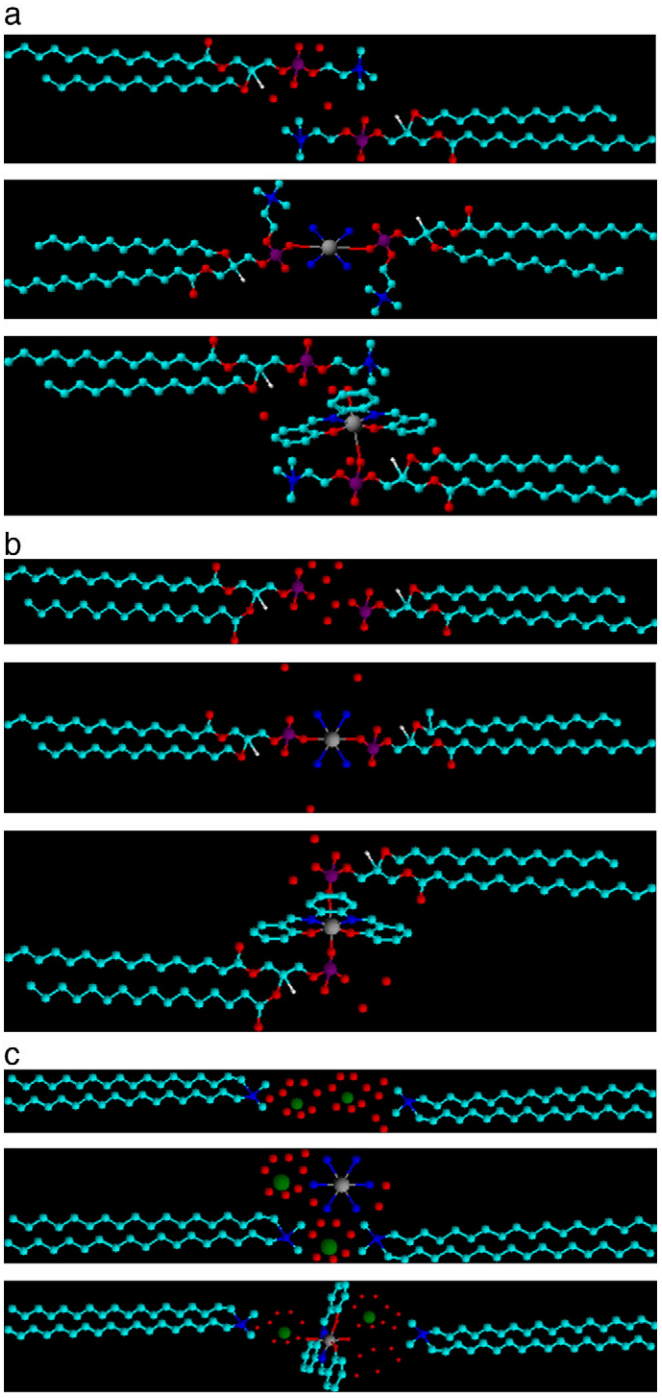

Fig. 7. Figures show the 'd – space' calculated from the end-to-end distance of the lipid bilayer. The calculated values have been compared with the experimental values of lamellar spacing from XRD. Primary energy minimized structures of (a) DMPC interacting with complex I and II. (i)54.841 Å DMPC pure, (ii)57.723 Å DMPC-complex I, (III)52.328 Å DMPC-complex II; (b) DPPA interacting with complex I and II (i)59.825 Å DPPA pure, (ii)61.489 Å DPPA -complex I, (III)52.627 Å DPPA -complex II (c)DOMA interacting with complex I and II (i)62.125 Å DOMA pure,(ii)53.848 Å DOMA -complex I, (III)65.147 Å DOMA -complex II.

References

[1] J.A. Reynolds, Are inorganic cations essential for stability of biological membranes? Ann. NY Acad. Sci. 195 (1972) 75–85.

[2] P. Lo Nostro, B. Ninham, A. Lo Nostro, G. Pesavento, L. Fratoni, P. Baglioni, Specific ion effects on the growth rates of Staphylococcus aureus and Pseudomonas aeruginosa, Phys. Biol. 2 (2005) 1–7.

[3] Y. Guillen, A. Machuca, The effect of copper on the growth of wood-rotting fungi and a blue-stain fungus, World J. Microbiol. Biotechnol. 24 (2008) 31–37.

[4] S. Jackson, I. Heath, Roles of calcium-ions in hyphal tip growth, Microbiol. Rev. 57 (1993) (1993) 367–382.

[5] B. Lakatos, K. Kaiserova, M. Simkovic, J. Orlicky, V. Knezl, L. Varecka, The effect of boromycin on the Ca^{2+} homeostasis, Mol. Cell. Biochem. 231 (2002) 15–22.

[6] R. Anderson, N. Bryden, C. EvockClover, N. Steele, Beneficial effects of chromium on glucose and lipid variables in control and somatotropin-treated pigs are associated with increased tissue chromium and altered tissue copper, iron, and zinc, J. Anim. Sci. 75 (1997) 657–661.

[7] R. Anderson, Chromium, glucose intolerance and diabetes, J. Am. Coll. Nutr. 17 (1998) 548–555.

[8] W. Campbell, L. Joseph, R. Anderson, S. Davey, J. Hinton, W. Evans, Effects of resistive training and chromium picolinate on body composition and skeletal muscle size in older women, Int. J. Sport Nutr. Exercise Metab. 12 (2002) 125–135.

[9] S. Lewicki, D. Rattman, T. Kuryl, M. Snochowski, B. Debski, The effect of chromium(III) on fatty acid metabolism and insulin path related gene expression in mouse myocytes cell line c2c12, ZYWNOSC-NAUKA TECHNOLOGIA JAKOSC 16 (2009) 183–194.

[10] G. Pattar, L. Tackett, P. Liu, J. Elmendorf, Chromium picolinate positively influences the glucose transporter system via affecting cholesterol homeostasis in adipocytes cultured under hyperglycemic diabetic conditions, Mutat. Res. 610 (2006) 93–100.

[11] Z. Wang, J. Qin, J. Martin, X. Zhang, O. Sereda, R. Anderson, P. Pinsonat, W. Cefalu, Phenotype of subjects with type 2 diabetes mellitus may determine clinical response to chromium supplementation, Metab. Clin. 56 (2007) 1652–1655.

[12] F. Calevro, S. Campani, M. Ragghianti, S. Bucci, G. Mancino, Tests of toxicity and teratogenicity in biphasic vertebrates treated with heavy metals (Cr^{3+}, Al^{3+}, Cd^{2+}), Chemosphere 37 (1998) 3011–3017.

[13] K. Balamurugan, R. Rajaram, T. Ramasami, Caspase-3: Its potential involvement in Cr(III)-induced apoptosis of lymphocytes, Mol. Cell. Biochem. 259 (2004) 43–51.

[14] P. Dartsch, S. Hildenbrand, R. Kimmel, F. Schmahl, Investigations on the nephrotoxicity and hepatotoxicity of trivalent and hexavalent chromium compounds, Int. Arch. Occup. Environ. Health 71 (1998) S40–S45.

[15] K. Di Bona, N. Rhodes, D. Mcadory, F. Brigg, M. Bailey, A. Wilson, P. Nordan, J. Rasco, J. Vincent, R. Hood, Potential of Chromium(III) Picolinate for Reproductive or Developmental Toxicity Following Exposure of Male CD-1 Mice Prior to Mating, Birth Defects, Res. Part A Clin. Mol. Teratol. 85 (2009) 452-452.

[16] A. Nguyen, I. Mulyani, A. Levina, P. Lay, Reactivity of chromium(III) nutritional supplements in biological media: An X-ray absorption spectroscopic study, Inorg. Chem. 47 (2008) 4299–4309.

[17] Q. Huo, G. Sui, Y. Zheng, P. Kele, R.M. Leblanc, T. Hasegawa, J. Nishijo, J. Umemura, Metal Complexation with Langmuir Monolayers of Histidyl Peptide Lipids, Chem. Eur. J. 7 (2001) 4796–4804.

[18] X. Chen, Z. Chen, SFG studies on interactions between antimicrobial peptides and supported lipid bilayers, Biochim. Biophys. Acta, Biomembr. 1758 (2006) 1257–1273.

[19] D. Tong-Lay Lau, John D. Gehman, John Wade, Colin L. Masters, Kevin J. Barnham, Frances Separovic, Cholesterol and Clioquinol modulation of Aβ(1–42) interaction with phospholipid bilayers and metals, Biochim. Biophys. Acta, Biomembr. 1768 (2007) 3135–3144.

[20] Ulf R. Pedersen, C. Leidy, P. Westh, G.H. Peters, The effect of calcium on the properties of charged phospholipid bilayers, Biochim. Biophys. Acta 1758 (2006) 573–582.

[21] G. Fanucci, R. Backov, R. Fu, D. Talham, Multiple bilayer dipalmitoylphosphatidylserine LB films stabilized with transition metal ions, Langmuir 17 (2001) 1660–1665.

[22] M. Mori, J.C. Bailar Jr., J.W. Pankay (Eds.), Preparation of aquopentaammine and acidopentaamminechromium(III) salts, Inorganic Syntheses, vol. V, McGraw-Hill, New York, 1957, pp. 132–133.

[23] F. Hein, S. Herzog, in: G. Brauer (Ed.), 2nd edit, Handbook of Preparative Inorganic Chemistry, vol. 2, Academic Press, New York, 1965, p. 1301.

[24] U.A. van der Heide, G. van Ginkel, Y.K. Levine, DPH is localised in two distinct populations in lipid vesicles, Chem. Phys. Lett. 253 (1996) 118–122.

[25] J.S. Santos, D.K. Lee, A. Ramamoorthy, Effects of antidepressants on the conformation of phospholipid headgroups studied by solid-state NMR, Magn. Reson. Chem. 42 (2004) 105–114.

[26] F.J. Kleima, M. Wendling, E. Hofmann, E.J.G. Peterman, R. van Grondelle, H. van Amerongen, Peridinin chlorophyll a protein: relating structure and steady-state spectroscopy, Biochemistry 39 (2000) 5184–5195.

[27] M. Rikukawa, M.F. Rubner, Fabrication of Langmuir-Blodgett films of poly(3-hexylthiophene) and metal-substituted pthalocyanines, Langmuir (1994) 519–524.

[28] M.F. Brown, J. Seelig, Ion induced changes in the head group conformation of lecithin bilayers, Nature 269 (1977) 721–723.

[29] M. Eisenberg, T. Gresalfi, T. Riccio, S. McLaughlin, Adsorption of monovalent cations to bilayer membranes containing negative phospholipids, Biochem. 18 (1979) 5213–5223.

[30] S.A. Tatulian, Fluidity-dependence of membrane adhesiveness can be explained by thermotropic shifts in surface potential, Biochim. Biophys. Acta, Biomembr. 901 (1987) 61–65.

[31] B.E. Cohen, G. Lee, N. Arispe, H.B. Pollard, Cyclic 3'-5'-adenosine monophosphate binds to annexin I and regulates calcium-dependent membrane aggregation and ion channel activity, FEBS Lett. 377 (1995) 444–450.

[32] A. Aroti, E. Leontidis, E. Maltseva, G. Brezesinski, Effects of Hofmeister anions on DPPC Langmuir monolayers at the air-water interface, J. Phys. Chem. B 108 (2004) 15238–15245.

[33] J. Barry, M. Fritz, J.R. Brender, P.E. Smith, Dong-Kuk Lee, A. Ramamoorthy, Determining the effects of dipophilic drugs on membrane structure by solid-state NMR spectroscopy: The case of the antioxidant curcumin, J. Am. Chem. Soc. 131 (2009) 4490–4498.

[34] R.P. Rand, V.A. Parsegian, Hydration forces between phospholipid bilayers, Biochim. Biophys. Acta 988 (1989) 351–376.
[35] R.V. McDaniel, T.J. McIntosh, S.A. Simon, Nonelectrolyte substitution for water in phosphatidylcholine bilayers, Biomembranes 731 (1983) 97-10.
[36] F.R. Keppler, V. Eiden, J. Niedan, Pracht, and H. F. Scholer, Halocarbons produced by natural oxidation processes during degradation of organic matter, Nature 403 (2000) 298–301.
[37] B. Poolman, J.J. Spitzer, J.A. Wood, Bacterial osmosensing: roles of membrane structure and electrostatics in lipid-protein and protein-protein interactions, Biochim. Biophys. Acta 1666 (2004) (2004) 88–104.
[38] P.A. Grigorjev, S.M. Bezrukov, Hofmeister effect in ion transport: reversible binding of halide anions to the roflamycoin channel, Biophys. J. 67 (1994) 2265–2271.
[39] M.E. Loosley-Millman, R.P. Rand, V.A. Parsegian, Effects of monovalent ion binding and screening on measured electrostatic forces between charged phospholipid bilayers, Biophys. J. 40 (1982) 221–232.
[40] M. Suwalsky, V. Novoa, F. Villena, C.P. Sotomayor, L.F. Aguilar, A. Ronowska, A. Szutowicz, Structural effects of Zn^{2+} on cell membranes and molecular models, J. Inorg. Biochem. 103 (2009) 797-780.
[41] M. Suwalsky, B. Ungerer, F. Villena, B. Norris, H. Cardenas, P. Zatta, Interactions of Al(acac)(3) with cell membranes and model phospholipid bilayers, J. Inorg. Biochem. 75 (1999) 263–268.
[42] P. Vernier, M. Ziegler, R. Dimova, Calcium binding and head group dipole angle in phosphatidylserine — phosphatidylcholine bilayers, Langmuir 25 (2009) 1020.
[43] M. Suwalsky, P. Fierro, F. Villena, C. Sotomayor, Effects of lithium on the human erythrocyte membrane and molecular models, Biophys. Chem. 129 (2007) 36–42.
[44] M. Suwalsky, F. Villena, B. Norris, M.A. Soto, C.P. Sotomayor, L. Messori, P. Zatta, (2005) Structural effects of titanium citrate on the human erythrocyte membrane, J. Inorg. Biochem. 99 (2005) 764–770.
[45] M. Nussio, M. Sykes, J. Miners, J. Shapter, Kinetics Membrane Disruption Due to Drug Interactions of Chlorpromazine Hydrochloride, Langmuir 25 (2009) 1086–1090.
[46] M. Pesti, Z. Gazdag, J. Belagyi, In vivo interaction of trivalent chromium with yeast plasma membrane, as revealed by EPR spectroscopy, FEMS Microbiol. Lett. 182 (2000) 375.
[47] M. Suwalsky, F. Villena, C. Sotomayor, S. Bolognin, P. Zatta Human, cells and cell membrane molecular models are affected in vitro by chlorpromazine, Biophys. Chem. 135 (2008) 7–13.
[48] D. Rajendra Prasad, T. Ramasami, D. Ramaswamy, M. Kanthimathi, Kinetics and mechanism of the equlibriartion reactions of Diaquochromium(III)Schiff base derivatives, Cr(Schiff base)$(H_2O)^+$ and their conjugate bases with thiocyanate, azide, imidazoel, pyridine and nicotinic acid as ligands, Inorg. Chem. 21 (1985) 850–854.
[49] M. Kanthimathi, B.U. Nair, T. Ramasami, T. Shibara, T. Tada, Preparation, charecterization and reactivities of chromium(III) complexes of a homologus series of Schiff-base ligands, Proc Indian Natl Sci Acad –Chem sci. 109 (1997) 235–248.