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# Interaction of dipyridamole with lipids in mixed Langmuir monolayers

Galina P. Borissevitch [a,1], Marcel Tabak [a,*], Osvaldo N. Oliveira [b]

[a] *Instituto de Química de São Carlos, USP, Cx. Postal 780, 13560-970, São Carlos, Brazil*
[b] *Instituto de Física de São Carlos, USP, Cx. Postal 780, 13560-970, São Carlos, SP, Brazil*

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**Abstract**

Dipyridamole (DIP), a well known coronary vasodilator and coactivator of anti-tumor activity of a number of drugs, forms stable Langmuir monolayers with the zwitterionic lipid dipalmitoylphosphatidylcholine (DPPC) and the negatively charged dipalmitoylphosphatidylglycerol (DPPG) at an air/aqueous solution interface. The drug binds to the lipid molecules and change their packing density in the monolayer in the process of compression, the effect depending on the drug location in the monolayer, protonation of the drug and also on the charge state of the lipid. The incorporation of dipyridamole (DIP) into neutral DPPC monolayers causes them to be more expanded at low DIP concentrations but more condensed at high concentrations, resembling the effect of cholesterol. Maximum expansion occurs for a DIP concentration of 2 mol%. For slightly charged DPPG monolayers spread on ultra pure water, the monolayers become increasingly more expanded with increasing DIP concentrations. For the negatively charged DPPG monolayers spread on buffer solutions, the incorporation of DIP has similar effects to that observed for DPPC monolayers. This is probably due to the interaction between the charged DPPG molecules and the protonated DIP molecules. Also, introduction of protonated DIP brings an increase in surface potential of DPPG monolayers because the negative contribution from the double layer is decreased. The results indicate that DIP molecules are located deeper in the hydrophobic region of DPPC monolayers, whereas in DPPG ones they appear to be located very close to the polar head region. Due to the electrostatic interaction of protonated DIP with the charges on the polar heads of lipids it is inclined with respect to the plane of the monolayer.

*Keywords:* Dipyridamole; Langmuir monolayer; Drug localization; Surface pressure; Surface potential

## 1. Introduction

The composition and physical properties of the phospholipid matrix of native biological membranes are very important parameters for the processing of many biological functions. To study the processes taking place in the membranes various model systems are used: phospholipid bilayers, unilamellar and bilamellar lipid vesicles, detergent micelles, Langmuir monolayers from lipids and other biologically important molecules and structures, which possess appropriate amphipathic properties, etc. A lipid monolayer at the air/water interface is considered a simple but a very efficient model of a biological membrane [1] as it can mimic the main features of many processes in native membranes.

Biologically active substances incorporated into monolayers change their characteristics: the packing density and ordering of the film-forming molecules, the electric properties of the monolayer, etc., due to specific interactions with the lipids. From the analysis of these changes it is possible to follow, in particular, the localization and orientation of these compounds within the monolayer.

The molecular mechanisms of pharmacological action of many drugs are also mediated by the membrane. This is the case of a phenomenon relevant for cancer therapy called multidrug resistance (MDR) [2] by which anti-tumor drugs are pumped away from the cell by a special P-glycoprotein (P-gp) which is present in the membranes of tumor cells. This process can be regulated by other substances called coactivators. These substances are thought to act

Abbreviations: DIP, dipyridamole, 2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine; DPPC, dipalmitoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; MDR, multidrug resistance; P-gp, P-glycoprotein; SP, surface potential; LE, liquid expanded phase; LC, liquid condensed phase.

* Corresponding author. Fax: +55 162 749163.
[1] On leave from Lomonosov State University, Moscow, Russia.


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through the alteration of the local membrane environment followed by structural and/or functional changes of the P-gp, thus suppressing its activity and decreasing the efflux of drugs. The anti-MDR activity of the coactivators seems to correlate not only with their chemical structure but also with their specific location in the lipid phase of the membrane [2]. Systematic studies of structure–activity relationships of different types of anti-MDR agents point out to the importance of two particular structural features common to most active anti-MDR pharmaceuticals, namely, a hydrophobic, conjugated planar ring and a substituted, preferably cyclic, tertiary amino group. The apparent importance of their spatial orientation was also demonstrated by studies with stereoisomers [2].

Dipyridamole (DIP), 2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine, which has already been widely used in medicine as a vasodilator, i.e., has been accepted as an official pharmaceutical, possesses rather high anti-MDR activity [2–5]. This has stimulated the investigation of its interaction with lipids in different organized model systems. Studies on charged detergent micelles have shown that the molecular mechanisms imply both nonpolar and polar interactions of DIP with these systems [6,7], which determine its localization. Evidence has been gathered that the polarizable heteroaromatic cycle of DIP tends to be situated at the border of polar heads and hydrophobic tails, and the aliphatic side groups introduced into the hydrophobic phase [7–9]. These conclusions arise from: (a) the dependence of the DIP association constants on pH of the medium and the charge state of the micelle; (b) the $^1$H-NMR spectra of the DIP side chains; and (c) the DIP fluorescence quenching by quenchers with known position in the lipid moiety. However, in these experiments the properties of DIP molecules were monitored, but their effect upon the lipid phase was not investigated.

In the present work we analyze the DIP influence upon phospholipids in highly organized systems-floating Langmuir monolayers at the air/water interface.

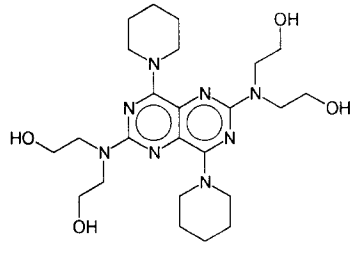
## 2. Materials and methods

Dipyridamole (DIP), dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG) were purchased from Sigma and chloroform from Merck and used without further purification. Monolayers were fabricated using a KSV 5000 Langmuir trough mounted in a clean room by spreading 100 $\mu$l of fresh chloroform solutions of phospholipids mixed with the desired amounts of the DIP chloroform solution onto the surface of an aqueous subphase. Ultra pure water from a Millipore system (pH 5.9) and phosphate buffer (5 mM $NaH_2PO_4$ + 2.5 mM $Na_2HPO_4$) at different pH values were used as the subphase. The necessary pH values in the subphase were obtained by adding HCl or NaOH solutions of analytical grade. The surface pressure vs. area per molecule ($\pi$–$A$) isotherms were registered while compressing the floating monolayer with a constant speed of 2 mN m$^{-1}$ min$^{-1}$. Surface pressure was measured to an accuracy of 0.1 mN m$^{-1}$ using a Wilhelmy plate connected to an electrobalance. The surface potentials (SP) were measured with a vibrating plate (frequency 300 Hz) located ca. 2 mm above the water surface. The amplitude of the plate vibrations was insufficient to disturb the floating monolayer. Both the vibrating plate and the reference electrode, immersed into the subphase, were made from platinum foil. All the experiments were performed at 20 ± 1°C.

Because DIP on its own would not form stable Langmuir monolayers, for it lacks the required amphipathic properties, use was made of mixtures of DIP and phospholipids dissolved in chloroform to form stable monolayers. The resulting films then had DIP molecules embedded in a phospholipid matrix. Our attempts to prepare stable mixed monolayers from a chloroform/alcohol solution which is often used for better spreading were not successful as in the presence of alcohol DIP did not bind exclusively to the lipids but was also dissolved in the subphase.

We have studied seven relative concentrations of DIP (per molecule of lipid) in the film varying in the range from DIP1 = 0.1 mol% up to DIP7 = 20 mol%. Possible DIP dissolution in the aqueous subphase was checked using fluorescence measurements. They have shown that even for the highest concentration used the amount of DIP dissolved during several compression-expansion cycles (up to 10) did not exceed 10% of its quantity in the film. The presence of such amounts of DIP in the subphase had no effect upon the monolayers as it was demonstrated in special subsidiary experiments with introduction of DIP into the subphase.

The chemical structure of dipyridamole is shown below.




## 3. Results and discussion

### 3.1. Interaction of DIP with DPPC monolayers

Incorporation of DIP into the DPPC monolayer modifies its structure, which is manifested in noticeable changes of both pressure–area ($\pi$–$A$) and surface potential–area (SP) isotherms. The details of these changes depend on the pH and on the ionic strength of the medium, which

determines the charge state of DIP bound to the lipid moiety. Even though the p$K$ for DIP in a DPPC monolayer is not known, it is ~ 4.8 for DIP, embedded into lysophosphatidylcholine micelles, (unpublished results of our group).

Fig. 1 shows the DPPC + DIP $\pi$–$A$ isotherms on the ultra pure water subphase (pH 5.9). Curve 1 corresponds to the pure DPPC and is essentially the same as in previous works [10,11]. At low concentrations (up to 2 mol%) DIP induces a rise and a shift towards larger areas per molecule in the plateau that characterizes the liquid–expanded (LE) phase. Changes are also observed in the liquid condensed (LC) region, the area for close packing increasing with the DIP concentration. The maximum effect, i.e., the maximum expansion of the monolayer, takes place at a concentration around 2 mol% (curve 2). Above this concentration, the monolayers become increasingly condensed (Fig. 1, curves 3, 4). In order to ensure that this trend was correct we measured other intermediate values of DIP concentration which indeed confirmed the results in Fig. 1. These curves are not shown to avoid overcrowding of the figure.

Mixed DPPC + DIP monolayers were also spread on phosphate buffer solutions of different pH values (ionic strength 10 mM): 5.9 (same as pure water), 4.3 and 7.2. The monolayers are generally more expanded than those on pure water because of the substitution of protons by larger counterions in the buffer. For pH values 4.3 and 5.9 when DIP is, respectively, protonated and partially protonated, changes in the isotherms are similar to those observed for monolayers on ultrapure water. This is illustrated in Fig. 2A where the area at which the LE plateau starts ($A_{LE}$) is plotted against the DIP concentration. In the same way as it happens for the areas of closely packed monolayers, $A_{LE}$ increases at low DIP concentrations, goes through a maximum and then decreases. The effect is

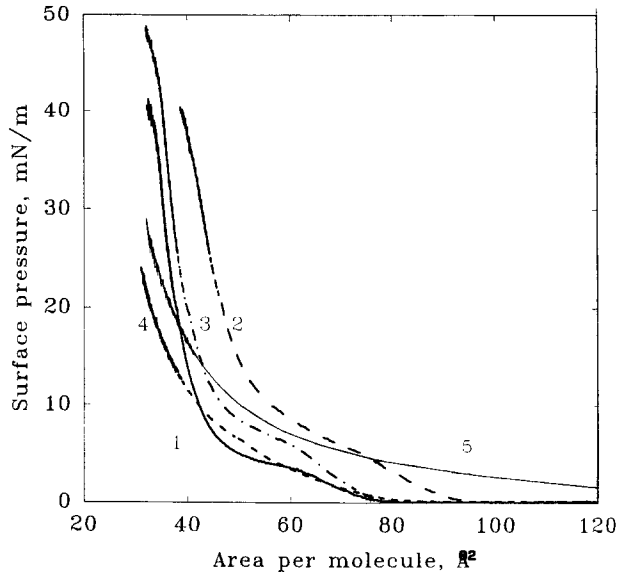



Fig. 1. Surface pressure vs. area per molecule ($\pi - A$) isotherms for mixed DPPC + DIP Langmuir monolayers on water subphase. 1, pure DPPC; 2, DIP/DPPC = 2 mol%; 3, DIP/DPPC = 4 mol%; 4, DIP/DPPC = 20 mol%; 5, DPPC on DIP subphase ($2 \cdot 10^{-5}$ M). $T$ = 20°C, pH 5.9.

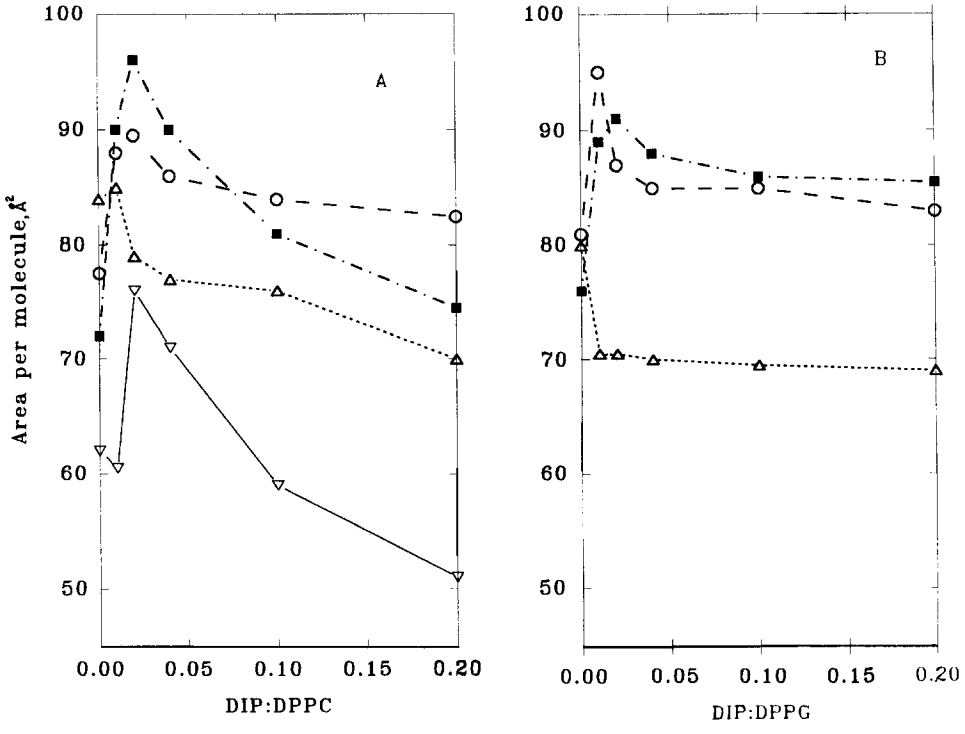



Fig. 2. The dependence of $A_{LE}$ on DIP concentration for: (A) mixed DPPC + DIP monolayers on water, pH 5.9 (solid lines) and buffer subphases (pH 5.9, dashed lines; pH 7.2, dotted lines; pH 4.3, dash-dotted lines). (B) Mixed DPPG + DIP monolayers on buffer subphase (pH 5.9, dashed lines; pH 7.5, dotted lines; pH 4.0, dash-dotted lines).

more pronounced for protonated DIP at pH 4.3. For monolayers spread on a pH 7.2 subphase, on the other hand, the incorporation of non-protonated DIP causes smaller changes without a maximum as shown also in Fig. 2.

The presence of DIP in the monolayer affects also the monolayer surface potentials (SP) which depend basically on the normal component (perpendicular to the water surface) of dipole moments of the film-forming molecules and on the contribution from the electric double-layer formed when the monolayer is at least partially ionized [11–14]. Therefore, the packing density and the orientation of the molecules appear essential parameters for understanding the surface potential results.

Fig. 3 shows SP curves for a pure DPPC monolayer (curve 1) and that with the highest DIP concentration (DIP7 = 20 mol%) (curve 2) on a pure water subphase (A) and on the buffer subphase at pH 5.9 (B). Data for lower DIP concentrations and other pH values are not shown. The curve for pure DPPC starts off at zero surface potential for large areas per molecule and increases abruptly at a given critical area. This rapid increase has been attributed, for monolayers of phospholipids and other aliphatic compounds, to the decrease in the effective dielectric constant at the monolayer/water interface, which is believed to occur when a critical packing density is achieved [11].

The introduction of DIP into the DPPC monolayer formed on the pure water and the buffer subphase at pH 5.9 generally causes an overall decrease in surface potential. The only exception occurs for DIP3 = 1 mol% which is close to the maximum expansion of the monolayer (DIP4 = 2 mol%) and for which a small increase in potential is observed. The dependencies are shown in Fig. 3D for the relative change in potential of the mixed films with respect to pure DPPC, denoted by $(SP_i - SP_0)/SP_0$(curves 1 and 2).

For pH 4.3, where DIP in the DPPC monolayer is protonated, the decrease of SP with DIP concentration is monotonic (Fig. 3D, curve 3). Nonprotonated DIP (pH 7.2) does not change the surface potential at large areas per molecule and only at small ones (in the LC state) a very small decrease is observed. Such a behavior appears to support the view that the packing changes caused by the introduction of DIP also affect the surface potential by modifying the normal component of the dipole moment. The decrease in potential at high DIP concentrations cannot be explained in terms of a double-layer contribution,

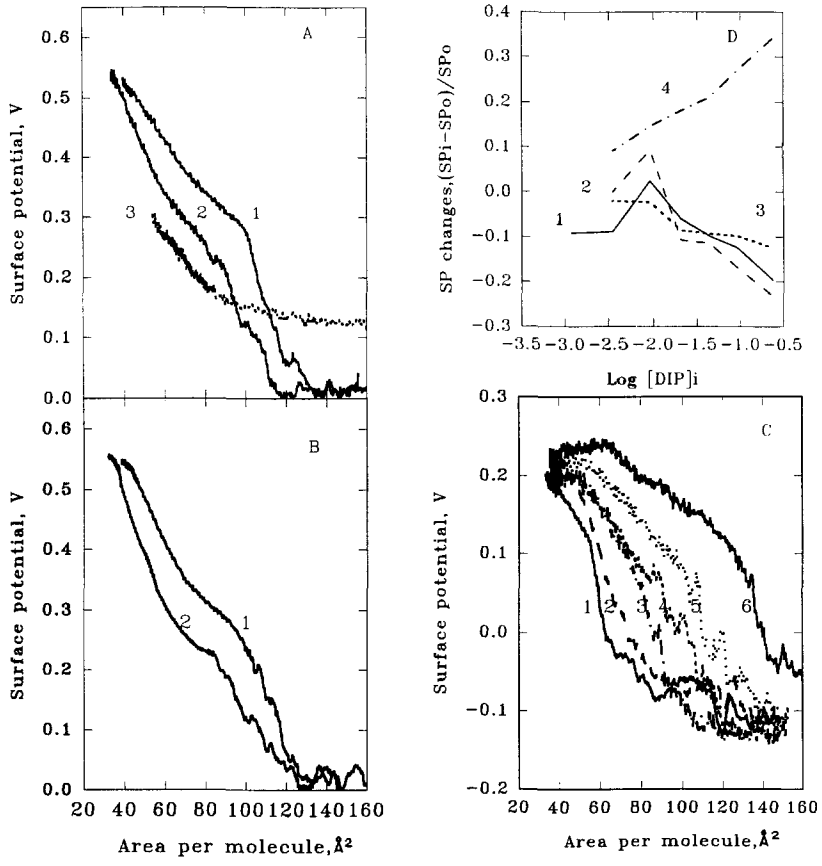



Fig. 3. Surface potential curves for mixed Langmuir monolayers: (A) DPPC + DIP on water subphase: 1, pure DPPC; 2, DIP/DPPC = 20 mol%; 3, DPPC on DIP subphase ($2 \cdot 10^{-5}$ M). (B) DPPC + DIP on buffer subphase: 1, pure DPPC; 2, DIP/DPPC = 20 mol%. (C) DPPG + DIP on water subphase: 1, pure DPPG; 2, DIP/DPPG = 1 mol%; 3, DIP/DPPG = 2 mol%; 4, DIP/DPPG = 4 mol%; 5, DIP/DPPG = 10 mol%; 6, DIP/DPPG = 20 mol%. (D) The dependence of relative changes of surface potential, $(SP_i - SP_0)/SP_0$, upon the log of DIP concentration: 1, DPPC + DIP monolayers on water (pH 5.9); 2, DPPC + DIP on buffer, pH 5.9; 3, DPPC + DIP on buffer, pH 4.3; 4, DPPG + DIP on water (pH 5.9).

because DPPC molecules are neutral both on pure water subphase and at the other pH values investigated. Moreover, any double-layer contribution would be expected to be positive for protonated DIP, but on the contrary, the incorporation of DIP generally causes the surface potential to decrease.

The binding of DIP onto DPPC molecules prevents them from close packing and this is reflected in the more expanded monolayers of mixed DPPC + DIP as compared with the pure DPPC. As one should expect, this effect increases with increasing DIP content in the monolayer which is indeed observed for low concentrations. However, the effect neither increases indefinitely nor saturates at a given concentration. Instead, there is a certain range of concentrations where the expansion of the monolayer is maximum. It is unlikely that the number of bound DIP molecules would decrease after any given concentration. Furthermore, as mentioned above in subsidiary experiments we ruled out the possibility of a considerable number of DIP molecules being dissolved in the water. A more plausible explanation may be that the DIP and DPPC molecules are able to form domains, which is a necessary step in monolayer formation [15], that are different for the DPPC + DIP mixture and the pure DPPC. If, for instance, clusters of DPPC are formed including DIP molecules, the resulting structure may be sufficiently organized to allow a more condensed packing at higher DIP concentrations. In other words, the disrupting effect of DIP molecules on the monolayer structuring would cease when the DIP concentration is high enough for a new arrangement between DIP and DPPC molecules to occur. As the results show, the new arrangement is very efficient in that close packing is nearly restored. The main difference that exists is the smaller slope of the LC phase for the DIP-containing monolayers even though the extrapolated areas to zero pressure are almost identical.

The analysis of changes in molecular packing and their effects on the monolayer surface potentials may help one to throw some light into the possible localization of DIP molecules. The smaller effects from DIP on $\pi$–$A$ and SP isotherms occurred for the pH 7.2 subphase when DIP is not protonated. The small changes in area per molecule may be explained by the fact that DIP has a cross section of about 45–50 $Å^2$ close to that of the DPPC molecule. Therefore, DIP molecules in this case appear to be located closer to the hydrophobic region of the monolayer with their heterocycles parallel to the film plane. Protonated DIP molecules cause larger effects, on the other hand, because they seem to be pulled closer to the polar region of the DPPC monolayer, probably with their heterocycles protruding from the water interface due to their interaction with the lipid headgroup charges which lie practically parallel to the plane of the monolayer [16]. The negative charge attracts the protonated DIP and pulls it into the polar region and the positive one repels the protonated DIP. As a result the heterocycle should be inclined, while the positive end of the polar head should move downward. Molecular packing is therefore affected which, in turn, causes the normal component of the dipole moment of film-forming molecules to decrease. The more protonated the DIP molecules the larger these effects are, which explains the larger changes with DIP concentration for monolayers spread on acidic, pH 4.3 subphases.

Having concluded that DIP molecules do interact with DPPC monolayers, we wondered whether this interaction would still be strong when DIP was incorporated into the subphase. Experiments were therefore carried out with pure DPPC monolayers spread on DIP-containing aqueous subphases. For subphases with low DIP concentrations no effect was observed. This was the case, for instance, for $5 \cdot 10^{-8}$ M DIP subphase which corresponds to the transfer of all the DIP molecules from the film with DIP7 = 20 mol% into the water. The effects were negligible even when concentrations were increased by two orders of magnitude. On the other hand, DPPC monolayers spread onto a saturated water solution of DIP (ca. $2 \cdot 10^{-5}$ M at pH 5.9) displayed isotherms drastically different from those of both pure DPPC and DPPC + DIP. As can be seen in curve 5 of Fig. 1, the LC region disappeared completely and the isotherm is typical of incompressible substances. These drastic changes should be attributed not only to the incorporation of DIP into the monolayer but also to the monolayer interaction with a different subphase which contains large charged molecules. Similarly the surface potential curves are also completely different for DPPC monolayers spread on a saturated DIP subphase (Fig. 3A, curve 3). It is lower and, most importantly, the sharp increase in potential at a critical area is absent. The latter observation could be related with the amount of impurities introduced into the subphase, as pointed out by Taylor et al. [17].

### 3.2. Interaction of DIP with DPPG monolayers

Fig. 4 shows the $\pi$–$A$ isotherms for DPPG + DIP monolayers in the case of the ultra pure water subphase (pH 5.9). Curve 1 corresponds to the pure DPPG monolayer and curves 2–6 to increasing DIP concentrations from DIP3 = 1 mol% up to DIP7 = 20 mol%, respectively. Upon increasing the DIP contents the monolayer becomes increasingly more expanded but the extrapolated area (at zero pressure) is close to that of pure DPPG monolayers. At DIP7 = 20 mol% the isotherm acquires a vast LE phase and lacks the LC one (Fig. 4, curve 6). DIP does not prevent DPPG molecules from close packing though it makes the process more difficult: the surface pressure necessary for the LC phase formation increases with the increase of the DIP concentration.

When a buffer is employed, however, the $\pi$–$A$ isotherms have the LE plateau, analogous to the one for DPPC. The changes in $A_{LE}$ again obey the pattern obtained for DPPC + DIP monolayers. Fig. 2B shows that for low DIP con-

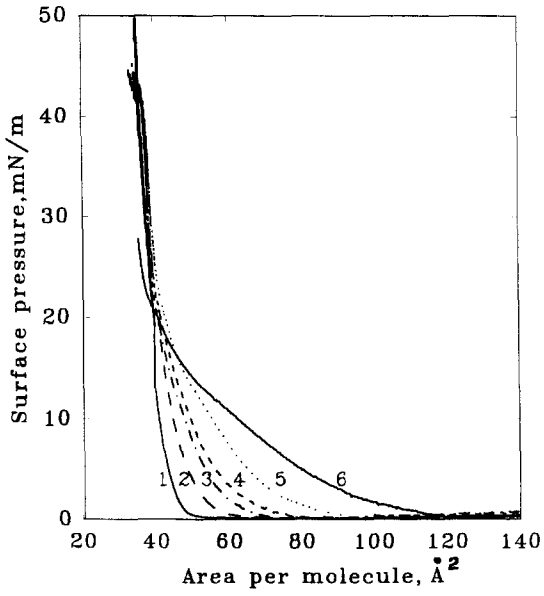



Fig. 4. Surface pressure vs. area per molecule ($\pi - A$) isotherms for mixed DPPG + DIP Langmuir monolayers on water subphase. 1, pure DPPG; 2, DIP/DPPG = 1 mol%; 3, DIP/DPPG = 2 mol%; 4, DIP/DPPG = 4 mol%; 5, DIP/DPPG = 10 mol%; 6, DIP/DPPG = 20 mol%. $T$ = 20°C, pH 5.9.

tent $A_{LE}$ increases with concentration, passes through a maximum and then decreases at high concentrations. This occurs for DPPG + DIP monolayers spread on buffers of pH values 5.9 and 4.0. For pH 7.5 where DIP is non-protonated only small changes are observed in $A_{LE}$. The area for closely packed monolayers at this pH is very little affected.

The surface potential (SP) of DPPG monolayers spread on ultrapure water (Fig. 3C) is seen to increase monotonically with the DIP concentration, even though the increase is rather small for closely packed monolayers. This is well explained in terms of the model of conformational changes in the glycerol backbone of the phospholipids in monolayers at the transition from the LE to the LC state [18]. According to this model in the LC state the negative charge of the DPPG moves closer to the positive charge of DIP introduced into the polar layer of the film and the double-layer effect decreases. The SP curve for pure DPPG starts from a negative value of about −100 mV, rises abruptly at the critical area of 80 Å$^2$ and reaches the positive value of about +200 mV. The introduction of DIP into the film induces a gradual increase in the potential with simultaneous shift of the critical area to larger values (curves 2–6 in Fig. 3C). This trend is maintained for monolayers spread on buffer solutions of pH values 4.0 and 5.9, but again changes are small for pH 7.5 at which DIP molecules are non-protonated.

In the case of DPPG monolayers, changes in SP may be associated with variations in the contribution of the Gouy-Chapman double layer that exists in (at least partially) ionized monolayers [11–14,19,20]. DPPG molecules have a p$K$ of 2.9 [20] and therefore DPPG monolayers are expected to be negatively charged at the pH values investigated and possess a degree of ionization, $\alpha$, given by

$$pK = pH_i - \log(\alpha/(1-\alpha)) \quad (1)$$

where $pH_i$ is the pH at the air/water interface which is determined from

$$pH_i = pH_b + e\Psi_0/(2.3kT) \quad (2)$$

where $pH_b$ is the bulk pH (pH of the subphase), $e$ is the elementary charge, $k$ is the Boltzmann constant, $T$ is the temperature, and $\Psi_0$ is the potential difference across the double-layer. According to the Gouy-Chapman theory, which has been found to be valid for the limit of small surface charges and ionic strengths [13,20], $\Psi_0$ is given by

$$\Psi_0 = (2kT/e)\sin h^{-1} e\alpha/A(5.88 \cdot 10^{-7} c\varepsilon T)^{1/2} \quad (3)$$

where $A$ is the area per molecule, $c$ is the ionic strength in the subphase (in mol/l), and $\varepsilon$ is the dielectric constant of the medium.

Therefore, $\Psi_0$ increases with the degree of ionization of the monolayer, $\alpha$, but decreases with increasing ionic strength, $c$. Because of the low ionic strength in ultrapure water, $\Psi_0$ is expected to be larger than for a DPPG monolayer spread on a buffer, pH 5.9 solution, even though $\alpha$ is smaller for ultrapure water (owing to a lower $pH_i$ (see Eq. (2)). Monolayers spread on buffers are expected to be fully ionized. Indeed, pure DPPG monolayers possess higher surface potentials on buffer solutions than their counterparts on pure water, because of the smaller $\Psi_0$.

The effects from introducing DIP into DPPG monolayers may be summarized as follows. For the slightly ionized DPPG on pure water, DIP causes a gradual expansion of the monolayer. For the completely non-protonated DPPG (spread on buffers), DIP causes the changes in the LE plateau similar to those for DPPC monolayers. The areas for closely packed monolayers are also affected. It means, on one hand, that the charged DPPG molecules attract the protonated DIP molecules which are then located closer to the hydrophilic headgroups than in the case of DPPC monolayers. This is consistent with the results from fluorescence quenching of DIP embedded into charged detergent micelles [9]. On the other hand, the decrease in area per DIP molecule means that it is inclined in the monolayer due to the interaction with the negative charge of the polar head.

As for the surface potential results, the incorporation of protonated DIP molecules into the monolayer made the negative contribution from the Gouy-Chapman double layer to decrease. The overall result was a systematic increase in SP with increasing DIP concentrations. The only exception was observed for DPPG + DIP monolayers spread on pH 7.5 buffer, for which changes in SP were very small since the DIP molecules were non-protonated.

## 4. Conclusion

The incorporation of dipyridamole (DIP) into DPPC monolayers causes the monolayers to be expanded at low DIP concentrations but more condensed at high ones probably because DIP and DPPC molecules assume a different molecular arrangement at these latter concentrations. This non-linear effect, which is maximum for DIP concentrations in monolayer in the range of 1–2 mol%, is similar to what occurs upon introduction of cholesterol into lipid bilayers. The surface potentials of the neutral DPPC monolayers are generally decreased due to packing changes arising from introduction of DIP. That the effects from DIP depend on the charge state of the monolayers was demonstrated in the results with DPPG + DIP monolayers. On ultrapure water, when DPPG is only slightly negatively charged, the monolayers become increasingly expanded with increasing DIP concentration, even though the extrapolated area to zero pressure remains practically constant. For the negatively charged DPPG monolayers spread on buffer solutions, the incorporation of DIP has similar effects upon the $\pi$–$A$ isotherms as those observed for DPPC monolayers. They are mainly due to DIP interference with the packing order at the border between the glycerol backbone and the acyl chains of the lipid. Introduction of protonated DIP brings an increase in surface potential of DPPG monolayers because the negative contribution from the Gouy-Chapman double layer is decreased.

The results discussed here may help one to speculate over the possible localization of DIP molecules in the lipid monolayers. As shown in Fig. 5 for the zwitterionic DPPC (sketch A) the DIP molecules are located deeper in the hydrophobic region of the monolayer than for DPPG (B). In the latter case, owing to the electrostatic interaction with the negatively charged DPPG, DIP molecules appear to be located very close to the polar head region, if not pulled into it, especially when they are protonated.

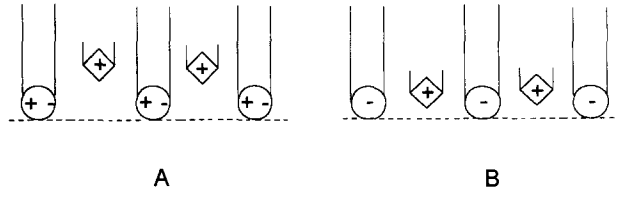



Fig. 5. A sketch of DIP localization in the Langmuir monolayers from: (A) DPPC; (B) DPPG. Circles denote polar heads of lipids, diamonds denote DIP molecules.

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