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The influence of phospholipid structure on the interactions with nystatin, a polyene antifungal antibiotic A Langmuir monolayer study

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Abstract

This work presents the investigations of the interactions between nystatin, a polyene antibiotic, and phospholipids with various head groups (phosphatidylcholine and phosphatidylethanolamine) and acyl chains of different length and saturation degree. The experiments were performed with the Langmuir monolayer technique. Among phosphatidylethanolamines, DMPE, DPPE and DSPE were studied, while phosphatidylcholines were represented by DSPC and DOPC. The influence of the antibiotic on the molecular organization of the phospholipid monolayer was analysed with the compression modulus values, while the strength of nystatin/phospholipid interactions and the stability of the mixed monolayers were examined on the basis of the excess free energy of mixing values. The results obtained proved a high affinity of nystatin towards phospholipids. Nystatin was found to interact more strongly with phosphatidylcholines than with phosphatidylethanolamines. The most negative values of the excess free energy of mixing observed for the antibiotic and DOPC mixtures prove that nystatin favors the phospholipid with two unsaturated acyl chains. The results imply that nystatin/phospholipid interactions compete in the natural membrane with nystatin/sterol interactions, thereby affecting the antifungal activity of nystatin and its toxicity towards mammalian cells.

Keywords: Nystatin; Cellular membrane; Phospholipids; Interactions in mixed Langmuir monolayers

1. Introduction

Nystatin and other polyene antibiotics are amphiphilic compounds of pharmacological activity, they belong to the group of so-called surface active drugs. It is well known that the surface-active drugs may affect the biomembrane's structure, even though their site of action is not associated directly with a cellular membrane. Self-organization of drugs and their interactions with cellular membranes may lead, for example, to a change in the membrane permeability, its solubilization or in the shape deformation. These may result in lipids flip-flop and even cell lysis. Such aspects of drug effects on cellular membranes were thoroughly discussed in a review article by Schreier et al. (2000).

Interactions of nystatin, an antibiotic investigated herein with membrane components are of great impor-

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tance for the understanding of the mechanism of action of both this drug and other polyenes. Polyenes are believed to form complexes with membrane sterols. Association of the complexes into transmembrane pores allows the leakage of vital cellular components, thereby leading to the cell death (De Kruijff et al., 1974). Since both mammalian and fungi cells possess sterols in their membranes (cholesterol and ergosterol, respectively (Karp, 2004; Ribĭereau-Gayon et al., 2006)), polyenes are toxic for human cells. The interactions of polyenes with membrane sterols normally favor ergosterol over cholesterol (Vertut-Croquin et al., 1983; Milhaud et al., 2002; Founier et al., 1998; Bagiński et al., 2002; Seoane et al., 1999a,b; Saint-Pierre Chazalet et al., 1988; Fujii et al., 1997). As antifungal agents of wide spectrum of activity used in clinical therapies are these of polyenes which reveal significantly higher affinity towards ergosterol. Due to this affinity their action against pathogenic fungi and yeast prevails over their toxic effect on human cells (amphotericin B, nystatin and pimaricin (Volpon and Lancelin, 2002)).

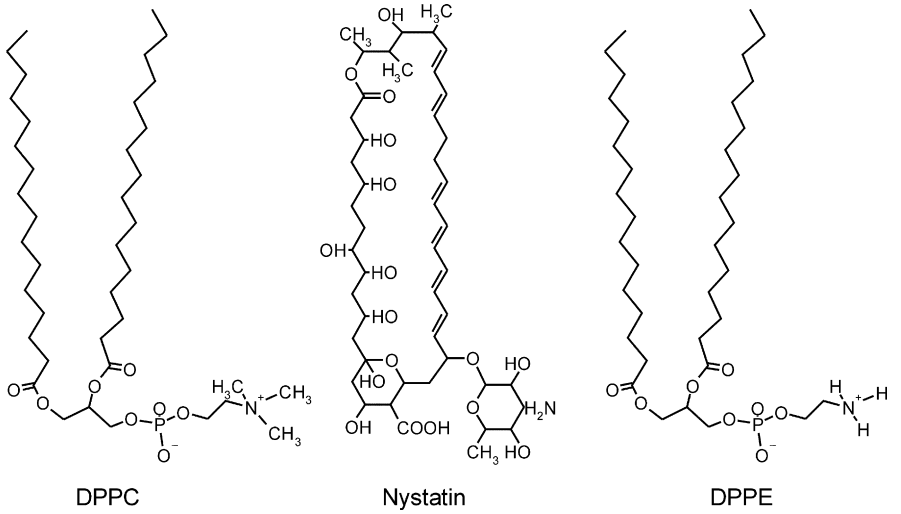
Interestingly, there are also reports that polyenes act on sterol-free phospholipid membranes (De Kruijff et al., 1974; Vertut-Croquin et al., 1983; Fujii et al., 1997; Cotero et al., 1998). However, until now the mechanism of action of polyene antibiotics has not been fully elucidated. Since the action of polyenes is on the membrane level, the role of other membrane components especially that of phospholipids should also be taken into account. Although the composition of membranes is dependent on the type of cell, phospholipids may constitute as high as 50% of lipids in a membrane. For example, in human erythrocytes ca. 45% of membrane lipids are phospholipids, while ca. 25%, cholesterol (Karp, 2004). By contrast, fungi membranes contain ca. 40% of lipids, of which ergosterol constitutes ca. 13% (Ribĭereau-Gayon et al., 2006; Barwicz and Tancrede, 1997). Although the concentration of cholesterol in mammalian membranes may be up to 50% of total lipids, however, phospholipids organized into bilayers form the framework of all of the cellular membranes. Both the human erythrocyte and fungi membranes as phospholipids contain mainly phosphatidylcholines and phosphatidylethanolamines. The phospholipids have chains of various lengths (C14–C24) and of various saturation degrees (one, two or three double bonds) (Karp, 2004). It is worth mentioning here that typically phospholipids are distributed asymmetrically within the membrane. The amount of unsaturated phospholipids increases from the outside part of the cellular membrane towards its interior (Robinson, 1975). Also, the distribution of phospholipids with different polar groups varies depending on the membrane side (Alberts et al., 1983). For example, in human erythrocytes phosphatidylcholines are present mainly in the external region of the membrane, while the majority of phospholipids with ethanolamine polar group are situated in its inner part (Guirr and James, 1980).

The majority of studies aiming at investigating the role of membrane components in the mode of action of polyene antibiotics (mainly AmB) have been focused on the interactions with sterols, i.e. ergosterol and cholesterol (Seoane et al., 1999a,b; Saint-Pierre Chazalet et al., 1988; Sykora et al., 2003, 2004). However, since phospholipids are the main membrane constituents, it is of utmost importance to study their interactions with polyenes to get an insight into the role of phospholipids in the mechanism of actions of these antibiotics. Moreover, phospholipids are the main components of liposomal and lipid-based formulations of amphotericin B and nystatin. Both antibiotics administered in these forms were found to be significantly less toxic as compared to their traditionally applied pharmaceutical preparations (for example *Fungizone*) (Johnson et al., 1998; Ghannoum and Rice, 1999; Denning and Warn, 1999; Offner et al., 2004; Carrillo-Munoz et al., 1999; Oakley et al., 1999; Arikan et al., 2002). This fact implies that phospholipids affect polyenes action, however, the exact mechanism has not been fully understood to date.

In this work the interactions between nystatin and various phospholipids in Langmuir monolayers, serving as model membranes (Maget-Dana, 1999, Brezesinski and Mohwald, 2003; Cadenhead, 1985) were studied. The phospholipids chosen for the study included distearoyl L-α-phosphatidylcholine (DSPC), dioleoyl L-α-phosphatidylcholine (DOPC), dimyristoyl L-α-phosphatidylethanolamine (DMPE), dipalmitoyl L-α-phosphatidylethanolamine (DPPE), distearoyl L-α-phosphatidylethanolamine (DSPE). They represent major phospholipids found in cellular membranes. The phospholipids possess either choline – PC (DSPC and DOPC) or ethanolamine – PE (DMPE, DPPE and DSPE) polar head, and have the hydrocarbon chains that differ in length and saturation degree. This allowed us to perform a systematic study of the influence of lipid structures on lipid–nystatin interactions. The analysis of the interactions between the antibiotic and the phospholipids enabled us to explain the effect of the polyene on the model membrane, and the role of phospholipids in polyenes antifungal activity and toxicity.

2. Experimental

Nystatin dihydrat was purchased from Fluka, 99%. The phospholipids, distearoyl L-α-phosphatidylcholine



Scheme 1. The chemical structure of nystatin and selected phospholipids, representing phosphatidylethanolamine and phosphatidylcholines.

(DSPC), dioleoyl L-α-phosphatidylcholine (DOPC), dimyristoyl L-α-phosphatidylethanolamine (DMPE), distearoyl L-α-phosphatidylethanolamine (DSPE) (purity >99%) were from Sigma, and dipalmitoyl L-α-phosphatidylethanolamine (DPPE) from Fluka. The chemical structures of nystatin, phosphatidylcholine and phosphatidylethanolamine are presented in Scheme 1. The spreading solutions were prepared by dissolving the investigated compounds weighed to an accuracy of 0.01 mg, in the mixture of freshly distilled chloroform and methanol (p.a., POCh, Poland) (4:1, v/v) except for nystatin that was dissolved in DMF. Mixed solutions were prepared from the respective stock solutions. Spreading solutions were deposited onto the water subphase with the Hamilton micro syringe, precise to 2.0 μL. After spreading, the monolayers were left to equilibrate for ca. 5 min before the compression was initiated with a barrier speed of 20 cm^2/min. π–A isotherms were recorded with a NIMA (UK) Langmuir trough (total area = 300 cm^2) placed on an anti-vibration table. Surface pressure was measured with the accuracy of ±0.1 mN/m using a Wilhelmy plate made of filter paper (ashless Whatman Chr1) connected to an electrobalance. The subphase temperature (20 °C) was controlled thermostatically to within 0.1 °C by a circulating water system.

3. Results

In the first step of our investigation, the surface pressure (π)–area (A) isotherms for pure nystatin, pure phospholipids and for nystatin–phospholipids mixtures were recorded. The isotherms are shown in Fig. 1. The film-forming properties of nystatin and the characteristics of its monolayers were presented previously in one of our papers (Hąc-Wydro and Dynarowicz-Łątka, 2006a). Nystatin was found to form Langmuir films of a liquid-expanded character. The plateau region observed in the course of the isotherm was ascribed to a change in orientation of the molecules, upon compression, from a horizontal to the vertical position (Hąc-Wydro and Dynarowicz-Łątka, 2006a). By contrast, the π/A isotherms for the investigated phospholipids demonstrated significantly higher condensation of the monolayers as compared to that for nystatin. The compression modulus values (C_S^{-1}), calculated according to the formula (Davies and Rideal, 1963)

$$C_S^{-1} = -A\left(\frac{d\pi}{dA}\right) \quad (1)$$

revealed that the saturated phospholipids: DPPE, DSPE and DSPC ($C_{S\,max}^{-1} \approx 260$, 230, 290 mN/m, respectively) formed more condensed monolayers than DOPC ($C_{S\,max}^{-1} \approx 105$ mN/m) that possesses two unsaturated chains. For a DMPE monolayer, the plateau region on the isotherm at $\pi \approx 10$ mN/m represents the phase transition from a liquid expanded to a liquid condensed state.

To verify the influence of nystatin on the state of phospholipid monolayers, the compression modulus values for all of the investigated mixed systems were calculated according to Eq. (1). In Fig. 2 the maximal values of the compression moduli $C_{S\,max}^{-1}$ are presented as a function of the mole fraction of nystatin. As can be seen, the incorporation of nystatin into the monolayers of phospholipids

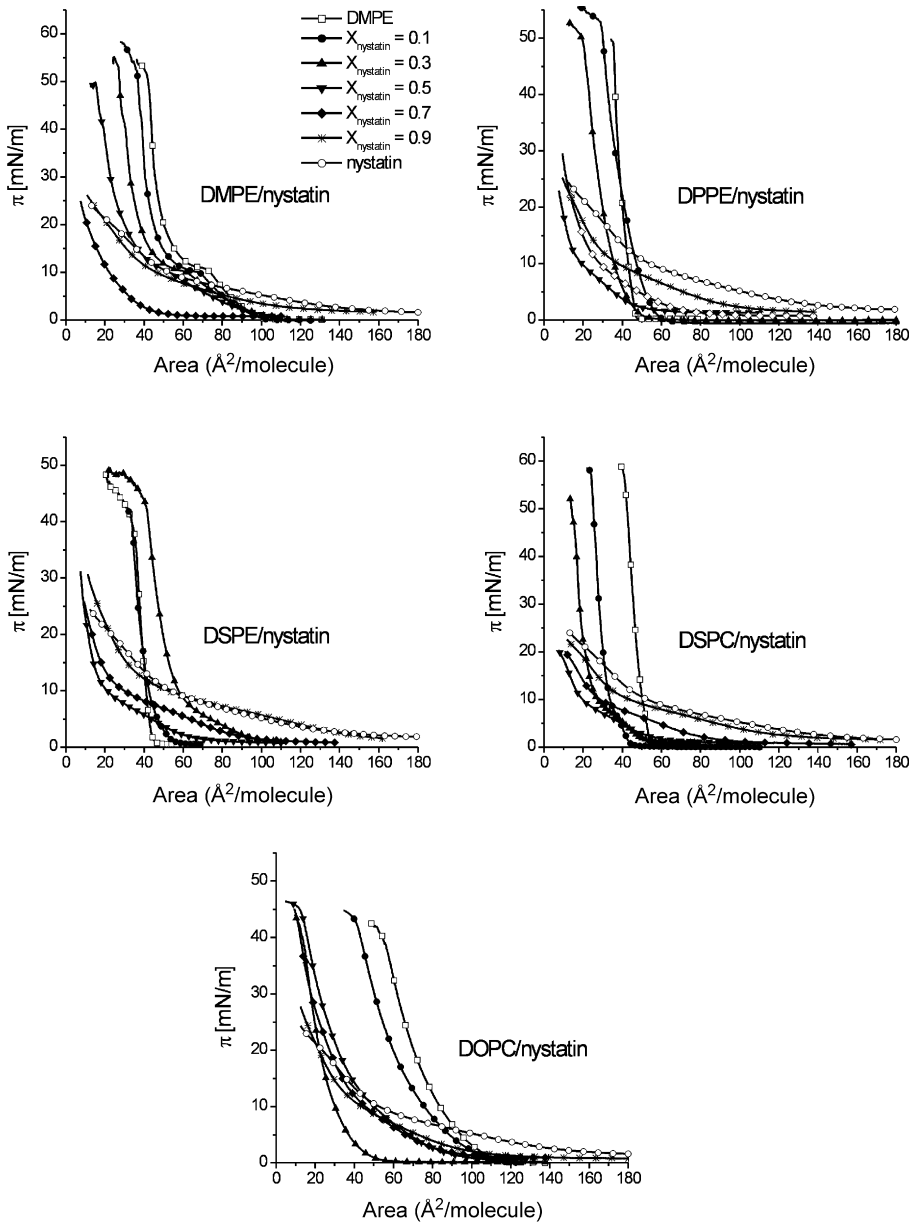


Fig. 1. The surface pressure (π)–area (A) isotherms of mixed monolayers formed at the air/water interface by nystatin and phospholipids (DMPE, DPPE, DSPE, DSPC and DOPC).

causes a decrease in $C_{S\,max}^{-1}$ value, and the monolayers become less condensed. Such a decrease is especially marked for the films of $X_{nystatin} = 0.1$–0.6. Interestingly, further addition of the antibiotic practically does not affect the maximal values of the compression moduli.

The interactions between components in the mixed monolayers were analysed in terms of their miscibility. It is known that if the mixed monolayer components are miscible and the mixed film shows a non-ideal behaviour resulting from the molecular interactions, then the film properties or simple functions, e.g. the mean area per molecule (A_{12}) versus film composition ($X_{nystatin}$) $A_{12} = f(X_{1,2})$ show deviation from linearity. On the other hand, if two components are immiscible or ideally miscible, the dependence of $A_{12} = f(X_{1,2})$ is linear (Gaines, 1966).

For the ideal mixing, the mean area per molecule, A_{12}, is defined as follows (Eq. (2)):

$$A_{12} = A_1 X_1 + A_2 X_2 \quad (2)$$

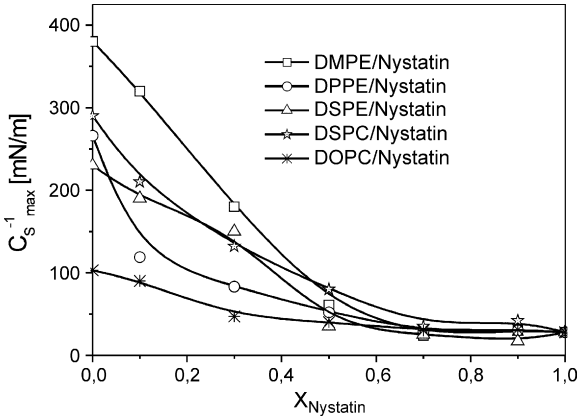


Fig. 2. The maximal values of compression modulus $C_{S\,max}^{-1}$ for all of the investigated mixed nystatin/phospholipid monolayers vs. monolayer composition.

where A_1 and A_2 are the molecular areas of single components at the same surface pressure, and X_1 and X_2 are the mole fractions of components 1 and 2 in the mixed film.

The A_{12} values for the mixed phospholipid/nystatin monolayers calculated at constant surface pressures, corresponding to the pre-plateau, plateau and post-plateau regions in the nystatin isotherm ($\pi = 2.5$, 5, 10 and 15 mN/m) are presented as a function of film composition (X_{nystatin}) in Fig. 3. The $A_{12} = f(X_{\text{nystatin}})$ plots show deviations from linearity for all of the investigated mixed systems. This proves miscibility of the film components and their mutual interactions in the mixed monolayers. The deviations are negative for the majority of the systems studied, and only for DPPE and DSPE monolayers containing a small mole fraction of nystatin, positive deviations are observed. The composition of the monolayer at the minimum in $A_{12} = f(X_{\text{nystatin}})$ plots corresponds to the isotherm for which the strongest contraction of the area appears, and indicates the mixed systems of the strongest attractions (or the weakest repulsions) between molecules as compared to the pure monolayers. This occurs for the equimolar mixtures of nystatin and DPPE, DSPE and DSPC, while for DMPE/nystatin and DOPC/nystatin the maximum of contraction appears for the mixed films of $X_{\text{nystatin}} = 0.7$ and 0.3, respectively. However, for the mixed monolayers of the antibiotic and DPPE, or DSPE containing nystatin in a low proportion ($X_{\text{nystatin}} = 0.1$ for DPPE and 0.1–0.3 for DSPE, respectively), positive deviations are observed.

From the thermodynamic point of view, the interactions between the phospholipids and the antibiotic were analysed basing on the excess free energy of mixing (ΔG^{Exc}) values. These were evaluated directly from the π/A isotherms using Eq. (3) (Gaines, 1966; Dynarowicz-Łątka and Kita, 1999)

$$\Delta G^{\text{Exc}} = N \int_0^{\pi} (A_{12} - X_1 A_1 - X_2 A_2) \mathrm{d}\pi \tag{3}$$

The excess free energy of mixing for the investigated mixed monolayers were calculated as the mean area per molecule at the same surface pressures ($\pi = 2.5$, 5, 10 and 15 mN/m). The values of the ΔG^{Exc} are presented as a function of monolayer composition in Fig. 4 for phosphatidylethanolamnines-containing films and in Fig. 5 for phosphatidylcholines-containing monolayers. The values of the ΔG^{Exc} for DPPC/nystatin mixed systems were calculated basing on the results presented in our previous paper (Hąc-Wydro and Dynarowicz-Łątka, 2006b). The negative deviations from linearity observed for the majority of the studied monolayers prove the existence of strong intermolecular forces in the mixed films.

The negative values of the ΔG^{Exc} suggest stronger attractions (or weaker repulsions) between a phospholipid and nystatin in mixed monolayers as compared to nystatin/nystatin and phospholipid/phospholipid interactions in their respective pure films. Moreover, the minimum in the ΔG^{Exc} versus X_{nystatin} plots corresponds to the mixture of the highest thermodynamic stability. For the mixed monolayers containing nystatin and DPPE, DSPE or DSPC, respectively, the equimolar mixtures show the highest stability, while in DMPE/nystatin and DPPC/nystatin mixed films the most negative values of the ΔG^{Exc} and the same the highest stability for 1:2 mixtures ($X_{\text{nystatin}} = 0.7$) are observed. The strongest interactions between molecules exist in the mixed films containing the unsaturated phospholipid (DOPC) and in these mixtures (DOPC/nystatin) the lowest values of the ΔG^{Exc} appear for 2:1 monolayer.

4. Discussion

The analysis of the interactions between nystatin and PC/PE phospholipids allow us to examine the influence of the polar head structure on the intermolecular forces between the components of the mixed monolayers. The results obtained for the phospholipids differing in the chain length and their degree of saturation on the other hand, are a source of information on the effect of the hydrophobic part of the phospholipid on its interactions with nystatin.

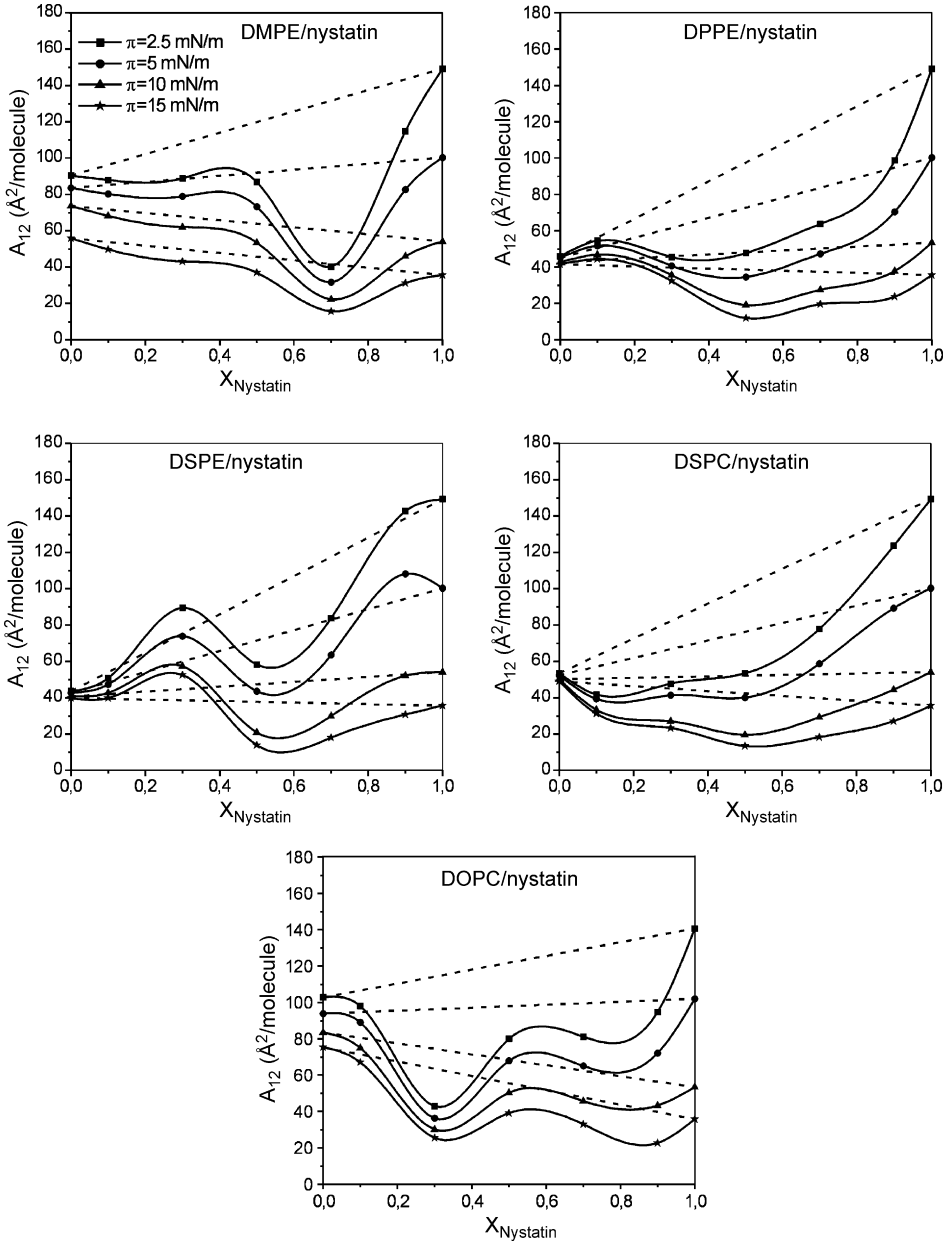


Fig. 3. Mean molecular area (A_{12}) vs. film composition (X_{nystatin}) plots for mixtures of nystatin and phospholipids at different constant surface pressures.

4.1. The influence of the polar head-group structure

The results of our investigations prove that mixed monolayers containing phosphatidylcholines or phosphatidylethanolamines show negative deviations from ideality in the wide range of the monolayer composition. This indicates that the interactions between the phospholipids and nystatin are more attractive than the interactions between molecules in the respective pure films.

To verify the influence of the head group of the phospholipids on their miscibility with nystatin the values of the ΔG^{Exc} for the mixed systems containing the antibiotic and the phospholipids of the same length of the hydrocarbon chain and different structure of the polar head were compared. Analyzing nystatin/DSPC and nystatin/DSPE mixed monolayers, more negative values of the ΔG^{Exc} for PC—as compared to PE-containing monolayers were found (see Figs. 4 and 5). To explain this effect, the phospholipid/phospholipid

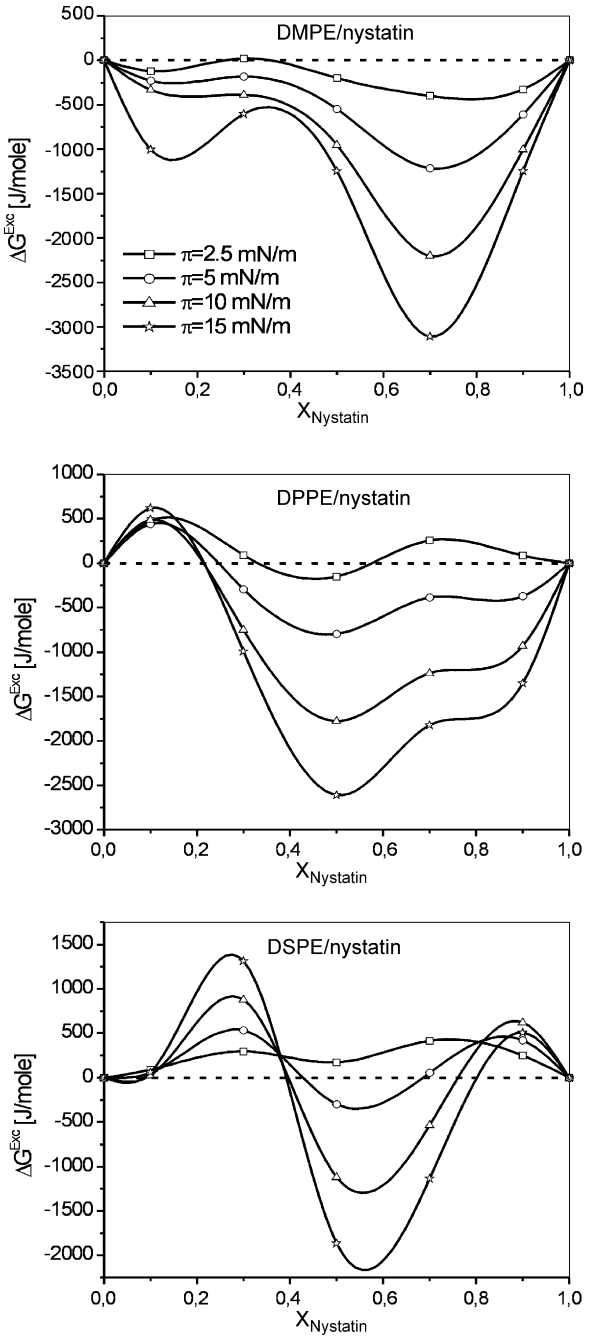


Fig. 4. Excess free energy of mixing (ΔG^{Exc}) vs. composition plots for mixtures of nystatin and phosphatidylethanolamines (DMPE, DPPE and DSPE) at different constant surface pressures.

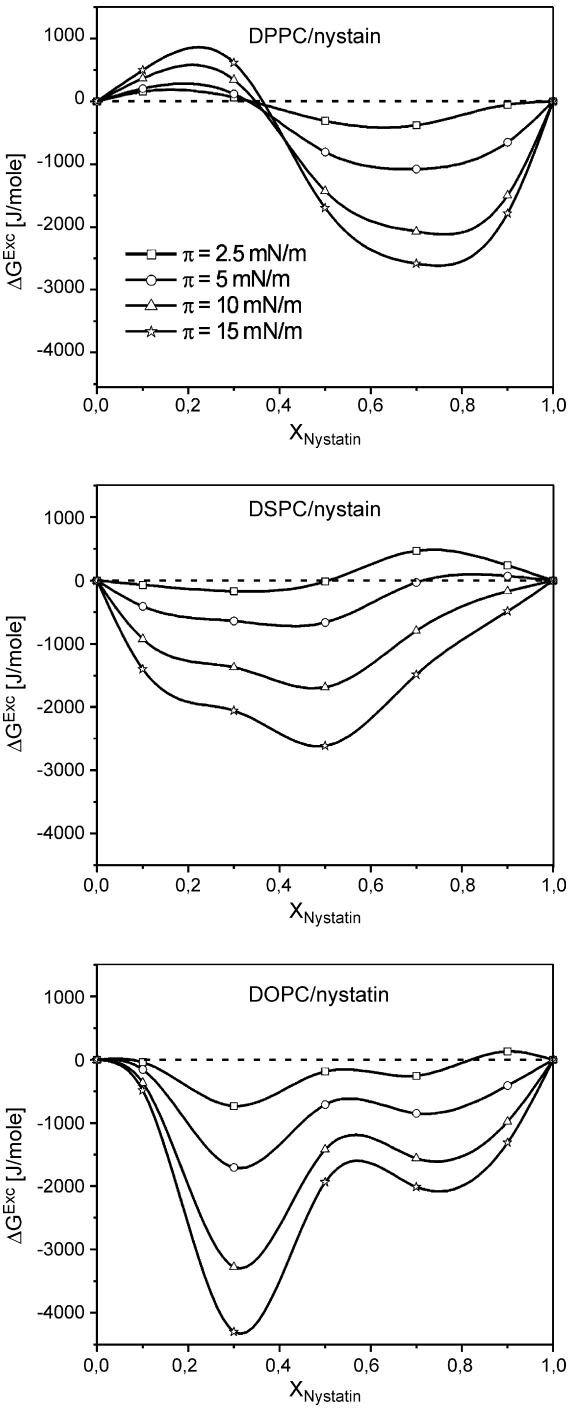


Fig. 5. Excess free energy of mixing (ΔG^{Exc}) vs. composition plots for mixtures of nystatin and phosphatidylcholines (DPPC, DSPC and DOPC) at different constant surface pressures.

interactions in one-component monolayers should be considered. These interactions involve van der Waals forces between hydrocarbon chains and electrostatic interactions of polar heads. Both phosphatidylcholines and phosphatidylethanolamines at physiological pH are zwitterions. The negative charge is localized on the phosphate group of both kinds of phospholipids. In the phosphatidylcholine monolayers, the polar head group interactions involve the negative charge of the phosphate group and the positive charge on the nitrogen in the choline group. The interactions between phosphatidylethanolamines by contrast, involve the negatively charged phosphate group and a positive charge on the nitrogen atom of the amino group. However, the interactions between polar heads of PE are stronger than those between PCs, due to the hydrogen bonds formed between the amino and phosphate group of neighboring phosphatidylethanolamines (Saiz and Klein, 2002; Yeagle, 1985). The incorporation of nystatin into the phospholipid monolayers causes the separation of lipid molecules and decreases electrostatic interactions between them. This effect is thermodynamically more favorable in the mixed systems containing DSPC molecules, which interact in the monolayer in a weaker manner than DSPE. This is reflected in more negative values of the excess free energy of mixing calculated for the DSPC-containing system. Interestingly, the minimal values of the excess free energy of mixing for nystatin/DPPC and nystatin/DPPE systems are comparable (Figs. 4 and 5). However, these minima are attained at different monolayer compositions ($X_{\text{nystatin}} = 0.7$ for nystatin/DPPC mixtures and $X_{\text{nystatin}} = 0.5$ for nystatin/DPPC system). This is probably due to the fact that the monolayers formed by DPPC are packed more loosely as compared to DPPE films, which is why they are able to accommodate a larger amount of nystatin molecules.

4.2. The influence of the length and saturation degree of the phospholipid chains

The conclusions regarding the influence of the phospholipid acyl chain length on the phospholipid/nystatin interactions can be drawn from the ΔG^{Exc} versus X_{nystatin} plots (Figs. 4 and 5) obtained for the mixed monolayers. For the mixtures containing phosphatidylethanolamines (Fig. 4 and Table 1), the values of the excess free energy of mixing become progressively less negative with an increase in the length of the hydrocarbon chain. This is closely related to the phospholipid monolayer packing. Within the range of the investigated surface pressures, the highest packing of the molecules was observed in DSPE monolayer ($C_{\text{S max}}^{-1} \approx 180$ mN/m at $\pi = 15$ mN/m), whereas the loosest packing in DMPE film ($C_{\text{S max}}^{-1} \approx 40$ mN/m at $\pi = 15$ mN/m). As mentioned earlier, the interactions in one-component PE monolayers involve in addition to electrostatic forces and hydrogen bonds between polar groups also van der Waals forces between apolar tails, the latter ones known to grow stronger with the increasing length of the hydrocarbon tail. Moreover, the denser is the packing of molecules in the film, the stronger become van der Waals interactions. Also, the intermolecular distance affects the strength of the hydrogen bonds, which is the highest in the most condensed monolayer (DSPE). Thus, the incorporation of nystatin into DSPE versus, for example, DMPE monolayer is thermodynamically less favorable. This is reflected in less negative values of ΔG^{Exc}.

Table 1
The free energy of mixing values (ΔG^{Exc}) for mixed monolayers of the highest stability

Mixed system	ΔG^{Exc} (J/mol)	Monolayer composition/X_{nystatin}
DMPE/nystatin	−3100	1:2/0.7
DPPE/nystatin	−2600	1:1/0.5
DSPE/nystatin	−1900	1:1/0.5
DPPC/nystatin	−2600	1:2/0.7
DSPC/nystatin	−2600	1:1/0.5
DOPC/nystatin	−4300	2:1/0.3

For the mixtures containing phosphatidylcholines (Fig. 5, Table 1) the minimum values of ΔG^{Exc} are nearly identical for DSPC/nystatin and DPPC/nystatin. However, for DSPC-containing monolayers, at higher surface pressures, the values of the excess free energy of mixing are negative in the whole range of the monolayer composition, while for DPPC/nystatin mixed systems only for monolayers of $X_{\text{nystatin}} \geq 0.5$. Stronger interactions of nystatin with DSPC than DPPC result from stronger van der Waals attractions between hydrophobic parts of the antibiotic molecules and hydrocarbon chains of the phospholipid.

The results presented indicate that nystatin interacts more strongly with a PC containing unsaturated acyl chains (DOPC) than with that containing two saturated chains of the same length (DSPC) (see Fig. 5). The presence of *cis*-double bonds in DOPC molecule causes that its acyl chains are bent and therefore the monolayer molecules are packed more loosely as compared to DSPC film. This is why the incorporation of nystatin into DOPC monolayer requires lower energy. Moreover, the looser monolayer of DOPC enables more favorable packing of a bulky nystatin molecule than the condensed film of DSPC.

A higher affinity of nystatin towards unsaturated phospholipids as compared to those possessing saturated

acyl chains is in agreement with the results of the investigations of the role of fatty acids in various polyenes activity. It has been found that fatty acids, when interacting with the antibiotic, reduce its effective concentration and decrease its action on fungi. This effect, in the case of nystatin becomes more pronounced with increasing degree of unsaturation (IIannitelli and Ikawa, 1980).

4.3. The interactions with phospholipids and the activity and toxicity of polyenes

It is known that the site of action of polyene antibiotics is the cellular membrane and that the antibiotic/sterol interactions are crucial for polyene activity. Moreover, the similarity between fungi and mammalian membrane is responsible for the toxicity of these drugs. Although the presence of sterols in a membrane is believed to be required for polyenes activity, the interactions with other membrane components should be taken into consideration. In the present study the interactions of nystatin with various phospholipids in Langmuir monolayers were examined. Similar investigations were performed for the mixed systems containing mammalian and fungi sterol (cholesterol and ergosterol, respectively) (Hąc-Wydro and Dynarowicz-Łątka, 2006b). Comparing these results, it is evident that the affinity of nystatin towards phospholipids is significantly higher than towards sterols. Stronger nystatin/phospholipids interactions are reflected in the values of the excess free energy of mixing, which are about six times more negative for nystatin/phospholipids than for nystatin/sterol monolayers. Similar results, i.e. stronger interactions with phospholipids than with sterols were also found for amphotericin B (AmB) (Hąc-Wydro et al., 2005). So strong an affinity of polyenes towards membrane phospholipids plays undoubtedly an important role in the activity and toxicity of these antibiotics. We suggest that the antibiotic/phospholipid interactions compete with the antibiotic/sterol ones, thereby leading to a decrease in the effective antibiotic concentration in the membrane. Our hypothesis explains differences between activity and toxicity of amphotericin B versus nystatin. Namely, the results of biological studies proved higher activity of AmB as compared to nystatin (Johnson et al., 1998; Carrillo-Munoz et al., 1999; Kotler-Brajtburg et al., 1979), which is reflected in a lower dose of amphotericin B required to obtain the same therapeutic effect as nystatin. On the other hand, nystatin interacts stronger with phospholipids than AmB, and therefore, the amount of unbound nystatin molecules, capable of interacting with ergosterol, is lowered. Amphotericin B was reported to be more toxic than nystatin (Kotler-Brajtburg et al., 1979). In our opinion, this is due to a stronger affinity of nystatin versus AmB towards phospholipids ($\Delta G^{Exc} \approx -700$ and -2600 J/mole for DPPC/AmB and DPPC/nystatin mixed monolayers, respectively), resulting in a decrease in "free" antibiotic molecules which can interact with cholesterol. Stronger interactions of nystatin/DPPC versus AmB/DPPC result from the differences in the structure of the molecules of both antibiotics. Namely, amphotericin B is a heptaene with seven conjugated double bonds in the hydrophobic part, whereas nystatin is a tetraene (Hamilton-Miller, 1973). These differences lead to the existence of stronger hydrophobic interactions between nystatin and phospholipids. Moreover, the structure of the apolar part of nystatin makes its molecule more flexible, which causes that nystatin may incorporate into a phospholipid monolayer easier as compared to AmB (Volpon and Lancelin, 2002). It should be emphasized that the toxicity of both antibiotics is "naturally" decreased by the interactions between phospholipid and cholesterol (Dynarowicz-Łątka and Hąc-Wydro, 2004). These interactions occurring in the cellular membrane prevent cholesterol from its interactions with the antibiotic, and increase the selectivity of both AmB and nystatin. Since the interactions between phospholipids and ergosterol in fungi cellular membrane are significantly weaker than phospholipid/cholesterol forces in the mammalian membrane (Dynarowicz-Łątka et al., 2002), they do not affect polyenes antifungal activity.

5. Summary

The aim of this study was to examine how phospholipids of different structures interact with nystatin, a polyene antibiotic. The most important conclusions are: (i) nystatin exhibits strong affinity towards phospholipids; (ii) nystatin interacts more strongly with phosphatidylcholines than with phosphatidylethanolamines; (iii) the strongest interactions occur between nystatin and a phospholipid possessing unsaturated hydrocarbon chains (DOPC); (iv) nystatin/phospholipid interactions may compete in the natural membrane with nystatin/sterol interactions, thereby affecting polyene activity and toxicity towards mammalian cell.

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