J. Pharm. Pharmacol. 1997, 49: 587–591
Received August 16, 1996
Accepted November 31, 1996

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An Interfacial Tension Model of the Interaction of Water-soluble Polymers with Phospholipid Composite Monolayers

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Abstract

The axi-symmetric drop-shape analysis-pendant drop technique has been used to measure interfacial tension at the chlorobenzene–water interface in the presence of adsorbed films of dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), DMPC–cholesterol, DPPC–cholesterol, DMPC–cholesterol–dicetyl phosphate (DCP) and DPPC–cholesterol–DCP.

A surface-pressure function, $\pi^* = \pi_{lipid-polymer} - \pi_{lipid}$ (where π_{lipid} is the surface pressure of the mono-layer without polymer and $\pi_{lipid-polymer}$ is the surface pressure of the lipid mono-layer and adsorbed polymer at equilibrium at the chlorobenzene–water interface) was used to characterize the interaction of eight water-soluble polymers with the lipid films. The equation, $\Delta\pi^* = \pi_{II}^* - \pi_I^*$ (where the subscripts II and I denote the higher and lower lipid composites, respectively) was used to determine the differential effect of cholesterol and DCP on mono-layer characteristics in the presence of 1% w/v polymer. Cholesterol or polymer individually condensed DMPC films and expanded DPPC films. However, composite films of DMPC–cholesterol–DCP and carboxymethylchitin (CM-chitin), poly(acrylic acid) (PAA) or poly(vinyl alcohol) (PVA) were more expanded than DMPC films whereas composite films of DPPC were neither more condensed nor expanded than DPPC films. A polymer impact ratio, $P^* = \pi_{lipid-polymer}/\pi_{lpolymer}$ was calculated and the polymers were ranked in order of their impact on the lipid film. PVA and polysaccharides gave low and high P* values, respectively, corresponding to high and low levels of film interaction, whereas PAA and hydrophobized polysaccharides gave intermediate values, indicating their affinity for and penetration of interfacial films with little disruption of the mono-layer.

The results show that measurement of interfacial pressures at the chlorobenzene–water interface might be advantageous for evaluating the action of polymers on biological membranes.

Air–water and oil–water interfaces have been used to study the characteristics of biological membranes in the presence of many water-soluble additives, including macromolecules. Although the air–water interface has been most widely investigated, because of its experimental simplicity, it is generally conceded that the oil–water interface would represent a superior physical model of a bilayer by enabling examination of the behaviour of one-half of a bi-layer, i.e. a monolayer in this environment (Ohki & Ohki 1976). At the liquid–hydrocarbon interface films of biological molecules, such as phospholipids, are presumed to be in an expanded state owing to assimilation of their hydrocarbon chains by the bulk liquid hydrocarbon. However, this might depend on the nature of the oil used and the composition of the lipids in the interfacial film. In many situations phospholipid monolayers become exposed to polymers, such as proteins, polysaccharides, or synthetic derivatives. Hence, the nature of the interaction of water-soluble polymers with phospholipid films at the oil–water interface would be of interest. It was expected that measurements of interfacial pressures of polymers combined with lipids that often comprise a model biological membrane could help to elucidate these types of interaction and that the relative behaviour of the polymers could be assessed.

This study employed a rapid and convenient method for measuring interfacial tension previously described for measurements at the oil–water interface in which solutes in both the oil and aqueous phases co-adsorb to produce mixed films (Li et al 1995). Polymers under consideration for the formulation of polymer-coated liposomes as an improved liposomal drug delivery system have, in particular, been selected.

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Materials and Methods

Materials

Dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) were obtained from Princeton Lipids (Princeton, NJ). Dicetyl phosphate (DCP), cholesterol, pullulan (nominal MW 54 000), dextran (nominal MW 73 500), and poly(vinyl alcohol) (PVA, nominal MW 40 000) were obtained from Sigma (St Louis, MO) and used as received. Carboxymethyl chitin (CM-chitin) was a gift from Nanyo Kasei, Japan. Poly(acrylic acid) (PAA, nominal MW 250 000), chlorobenzene (HPLC grade), benzyl benzoate and chloroform were obtained from Aldrich (Toronto, ON). Cholesterol-derivatized pullulan and cholesterol-derivatized dextran were synthesized and purified by previously published methods (Hammerling & Westphal 1967; Sato & Sunamoto 1992). The purity of solvents was determined by surface-tension measurement and comparison with literature values. All water used in the preparation of solutions was double-distilled in glass apparatus.

Interfacial-tension measurements

Interfacial-tension measurements were performed using the axi-symmetric drop-shape analysis-pendant drop technique

(Rotenberg et al 1983; Cheng et al 1990). Drops of oil phase were formed from a cleaned, 26-gauge Teflon cannula immersed in water or aqueous solution in a 20-mL quartz glass cuvette at ambient temperature (24 ± 1°C). Drop-profile images were collected and digitized over 15 min and shape analysis was performed by use of a Sun Sparc 10 work station which generates spline curve fits to the drop-profile coordinates. The accuracy of the technique is of the order of ±0·1 mN m^{-1} (Li et al 1995). The averages of at least six measurements of each drop were determined. Equilibrium at the interface was obtained after 10 min as determined by observation of the kinetics of interfacial tension as a function of time.

When polymers were used, 1% w/v aqueous solutions were equilibrated with pendant-drop solutions of lipids in chlorobenzene. The polymer concentration, although somewhat arbitrary, was selected with reference to the use of polymers to stabilize liposomes and to maintain a relatively low solution viscosity.

Density measurements

Densities of solutions, required for interfacial tension calculations, were measured at ambient temperature (24 ± 1°C) by means of a Paar DMA 55 densitometer.

Results and Discussion

Fig. 1 illustrates the variation of interfacial tension at three oil–water interfaces as a function of DMPC concentration on a log scale. In each case the interfacial tension was independent of DMPC concentration up to approximately 10^{-3} to 10^{-2} mM then the interfacial tension fell linearly up to about 10^{-1} mM after which it remained fairly constant, indicative of the formation of a packed mono-layer at the interface. Chlorobenzene was selected for further studies because the adsorption equilibrium of the phospholipid was attained faster than with chloroform, and interpretation of interfacial tension data was expected to be simpler than at the chloroform–benzyl benzoate–water interface. Also, as seen in Fig. 1, the concentration of DMPC at which the linear decrease in the interfacial tension occurred was lower at the chlorobenzene–water interface than at the chloroform–water interface. Experimentally, an organic liquid of greater density than water was convenient to use (the density of chlorobenzene at 20°C, $d_{20} = 1{\cdot}10$ g mL^{-1}).

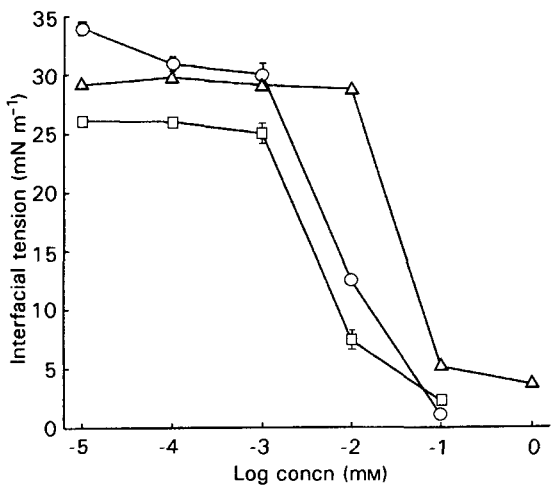

FIG. 1. Interfacial tension at various oil–water interfaces as a function of DMPC concentration at 24 ± 1°C. ○ Chlorobenzene–water, △ chloroform–water, □ chloroform–benzyl benzoate (1:3 v/v)–water.

The interfacial tensions of DMPC or DPPC monolayers containing cholesterol and DCP, formed at the oil–water interface from chlorobenzene solutions over four orders of magnitude of concentration are graphically represented in Fig. 2. The shapes of all the curves are approximately the same; however, in general, it can be seen that mixtures of DMPC–cholesterol (3:1 mole ratio) or DMPC–cholesterol–DCP (3:1:0·5 mole ratio) resulted in higher interfacial tensions than DMPC alone whereas comparable mixtures with DPPC yielded lower interfacial tensions than DPPC alone.

Table 1 lists the interfacial tensions (γ_{12}) and corresponding surface pressures (π) of lipid composites and polymers at the chlorobenzene–water interface where:

$$\pi = \gamma_0 - \gamma_{12} \quad (1)$$

and γ_0 is the interfacial tension at the clean chlorobenzene–water interface. It is apparent that both phospholipids are highly surface active at this interface but DMPC resulted in a higher value of π than DPPC. The addition of cholesterol to DMPC resulted in a lower value of π, in contrast with the addition of cholesterol to DPPC which resulted in a higher value. Increasing the cholesterol content from 25 to 50 mol%

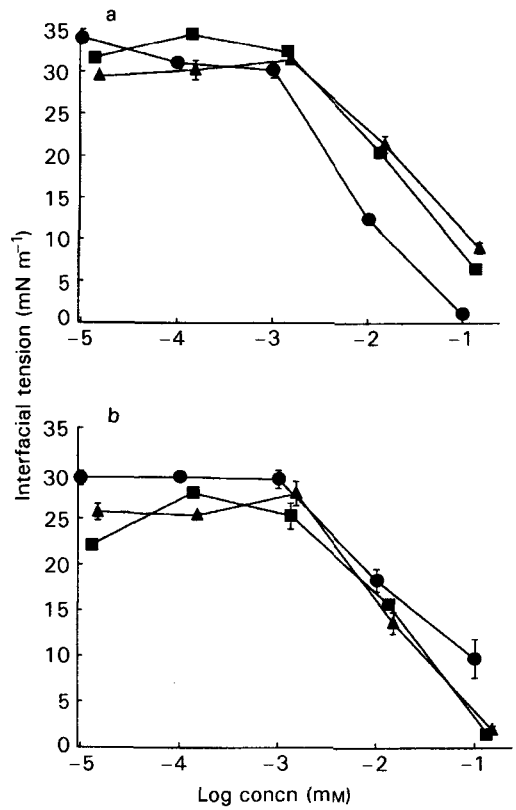

FIG. 2. Interfacial tension of phospholipid composites at the chlorobenzene–water interface as a function of total lipid concentration. ● PC, ■ PC–cholesterol (3:1, mol ratio), ▲ PC-cholesterol–DCP (3:1:0·5, mol ratio). a. PC = DMPC. b. PC = DPPC.

Table 1. Interfacial tensions (γ_{12}) at the chlorobenzene–water† interface and surface pressures (π) of adsorbed lipid films.

Lipid composition†† (mole ratio)	Interfacial tension (mN m^{-1})	Surface pressure (mN m^{-1})
Dimyristoylphosphatidylcholine	0·68 ± 0·35	35·50
Dimyristoylphosphatidylcholine–cholesterol (3:1)	6·23 ± 0·34	29·95
Dimyristoylphosphatidylcholine–cholesterol (1:1)	4·25 ± 0·75	31·93
Dimyristoylphosphatidylcholine–cholesterol–dicetyl phosphate (3:1:0·5)	7·99 ± 0·61	28·19
Dipalmitoylphosphatidylcholine	6·59 ± 2·11	29·59
Dipalmitoylphosphatidylcholine–cholesterol (3:1)	0·80 + 0·48	35·38
Dipalmitoylphosphatidylcholine–cholesterol (1:1)	4·10 ± 0·77	32·08
Dipalmitoylphosphatidylcholine–cholesterol–dicetyl phosphate (3:1:0·5)	1·35 ± 0·45	34·83
Cholesterol-derivatized pullulan	22·12 ± 2·07	14·06
Pullulan	28·82 ± 0·42	7·36
Cholesterol-derivatized dextran	22·69 ± 0·93	13·49
Dextran	29·38 ± 2·66	6·80
Carboxymethylchitin	21·21 ± 0·48	14·97
Poly(acrylic acid) (pH 6)	21·90 ± 0·28	14·28
Poly(acrylic acid) (pH 1·8)	20·05 ± 1·04	16·13
Poly(vinyl acetate)	4·16 ± 0·98	32·02

† $\gamma_{12} = 3618$ mN m^{-1}. †† Concentrations of DMPC and DPPC were 0·1 mM; concentration of PC–cholesterol was 0·133 mM; concentration of PC–cholesterol–DCP was 0·15 mM; concentration of the polymer was 1% w/v. Interfacial tension is given as mean ± s.d.

modestly increased and decreased, respectively, the π value of DMPC and DPPC monolayers. In typical plots of π against A (area molecule^{-1}) at a given concentration of adsorbate, it is known that curves with higher values of π represent films having more expanded characteristics (Demel & Joos 1968; Cadenhead 1969; Ohki et al 1976). Conversely, curves having lower values of π represent films in a more condensed state. Thus, the addition of cholesterol resulted in a more condensed DMPC film but a more expanded DPPC film, a finding that is consistent with the effect of cholesterol on the fluidity of phospholipid bilayers above and below the phase-transition temperature (T_m) of the phospholipid (Browning 1981) but not in agreement with mixed films of cholesterol and phospholipid at the CCl_4–water and heptane–water interfaces, at which cholesterol and phospholipid behaved ideally (Demel & Joos 1968). This is in contrast with the air–water interface at which cholesterol condenses spread films of phospholipid (Albrecht et al 1986). The addition of DCP further condensed both the DMPC–cholesterol and DPPC–cholesterol films (i.e. DCP slightly reduced the degree of expansion of the DPPC due to cholesterol). In comparison, the π value of adsorbed polymers at the chlorobenzene–water interface was low, except for PVA which has a substantial surface activity as seen by its γ_{12} value in Table 1.

The relative surface pressure (π^*), defined as:

$$\pi^* = \pi_{\text{lipid–polymer}} - \pi_{\text{lipid}} \quad (2)$$

(where π_{lipid} is the surface pressure of the mono-layer without polymer and $\pi_{\text{lipid–polymer}}$ is the surface pressure of the lipid mono-layer and adsorbed polymer at equilibrium at the chlorobenzene–water interface) was studied to investigate the role of mono-layer composition on polymer interaction at an oil–water interface. A positive π^* value is indicative of a polymer that was adsorbed at the interface and contributed to an increased surface pressure (film expansion) owing to a combination of its affinity for the interface and its effect on the molecules of the lipid monolayer. A negative π^* value is indicative of a polymer that by virtue of its surface action with the molecules of the lipid monolayer increased the packing of lipid molecules (condensation) at the oil–water interface. Table 2 compares the π^* values for each of eight water-soluble polymers adsorbed at the interface in conjunction with either DMPC or DPPC. It can be seen that all of the polymers yielded positive values of π^* at the DPPC-covered interface and negative values of π^* at the DMPC-covered interface. Hence, the film of DMPC underwent condensation as a result of polymer adsorption whereas the film of DPPC became expanded as a result of polymer interaction at the interface.

The modulation of polymer interaction at the phospholipid interface by commonly incorporated lipids such as cholesterol and DCP can be described by a difference expression of π^* ($\Delta\pi^*$) given by:

$$\Delta\pi^* = \pi_{II}^* - \pi_{I}^* \quad (3)$$

where the subscripts II and I refer to the higher and lower lipid composites, respectively. Table 3 presents $\Delta\pi^*$ values for each polymer. The addition of 25 mol% cholesterol to DMPC films in the presence of 1% w/v polymer clearly resulted in its expansion because $\Delta\pi^*$ is positive and the degree of polymer

Table 2. π^* (mN m^{-1}) ± s.d. values of polymers adsorbed at the chlorobenzene–water interface in the presence of phospholipid.

	Lipid composition*	
	Dimyristoylphosphatidylcholine	Dipalmitoylphosphatidylcholine
Cholesterol-derivatized pullulan	− 9·34 ± 0·10	3·18 ± 0·33
Pullulan	− 9·39 ± 0·24	3·05 ± 0·50
Cholesterol-derivatized dextran	− 9·70 ± 0·28	3·43 ± 0·56
Dextran	− 8·07 ± 0·05	3·49 ± 0·51
Carboxymethyl-chitin	− 6·66 ± 0·23	4·99 ± 0·23
Poly(acrylic acid) (pH 6)	− 4·08 ± 0·67	6·15 ± 0·17
Poly(acrylic acid) (pH 1·8)	− 10·09 ± 0·08	5·74 ± 0·31
Poly(vinyl acetate)	− 2·36 ± 0·58	4·47 ± 0·60

*PC concentration was 0·1 mM. Means ± s.d.

Table 3. Differential effects of cholesterol and dicetyl phosphate on π^* ($\Delta\pi^*$, mN m^{-1})† of lipid films.

Lipid composition	Cholesterol-derivatized pullulan	Pullulan	Cholesterol-derivatized dextran	Dextran	Carboxy-methylchitin	Poly(acrylic acid)		Poly(vinyl acetate)
						pH 6	pH 1·8	
Dimyristoylphosphatidylcholine-cholesterol (3:1)	4·70	3·22	4·77	8·05	3·65	5·02	10·21	6·50
Dimyristoylphosphatidylcholine-cholesterol (1:1)	6·00	6·43	3·63	− 0·08	3·22	0·65	2·76	− 0·46
Dipalmitoylphosphatidylcholine-cholesterol (3:1)	− 4·86	− 4·54	− 4·77	− 6·04	− 6·28	− 6·35	− 8·16	− 6·58
Dipalmitoylphosphatidylcholine-cholesterol (1:1)	3·51	2·76	2·57	3·25	1·29	2·06	4·63	2·96
Dimyristoylphosphatidylcholine-cholesterol–dicetyl phosphate (3:1:0·5)	3·88	4·62	4·79	0·02	5·93	6·26	3·85	3·22
Dipalmitoylphosphatidylcholine-cholesterol–dicetyl phosphate (3:1:0·5)	− 0·02	− 0·20	0·66	0·79	0·53	0·68	1·60	1·84

† $\Delta\pi^* = \pi_{II}^* - \pi_I^*$ where II and I refer to the higher and lower lipid composites, respectively.

Table 4. Polymer impact ratios (P*).

Lipid composition†	Cholesterol derivatized pullulan	Pullulan	Cholesterol-derivatized dextran	Dextran	Carboxymethyl chitin	Poly(acrylic acid)		Poly(vinyl acetate)
						pH 6	pH 1·8	
Dimyristoylphosphatidylcholine	1·86	3·55	1·91	4·03	1·93	2·20	1·58	1·04
Dimyristoylphosphatidylcholine-cholesterol (3:1)	1·80	3·23	1·86	4·40	1·80	2·16	1·86	1·06
Dimyristoylphosphatidylcholine-cholesterol (1:1)	2·37	4·37	2·27	4·68	2·15	2·35	2·16	1·11
Dimyristoylphosphatidylcholine-cholesterol-dicetyl phosphate (3:1:0·5)	1·95	3·62	2·08	4·15	2·08	2·48	1·99	1·11
Dipalmitoylphosphatidylcholine	2·33	4·43	2·45	4·87	2·31	2·50	2·19	1·06
Dipalmitoylphosphatidylcholine-cholesterol (3:1)	2·40	4·60	2·52	4·83	2·28	2·46	2·04	1·04
Dipalmitoylphosphatidylcholine-cholesterol (1:1)	2·41	4·53	2·47	4·82	2·14	2·38	2·12	1·03
Dipalmitoylphosphatidylcholine-cholesterol-dicetyl phosphate (3:1:0·5)	2·36	4·50	2·53	4·86	2·28	2·47	2·11	1·08

† Concentrations of DMPC and DPPC were 0·1 mM; concentration of PC–cholesterol was 0·133 mM; concentration of PC–cholesterol-DCP was 0·15 mM; concentration of the polymer was 1% w/v.

interaction with the mono-layer was similar in all instances (mean $\Delta\pi^* = 5{\cdot}77 \pm 2{\cdot}36$ mN m^{-1}). Increasing the cholesterol content to 50% resulted in further expansion of the films (except for dextran, PAA at pH 6, and PVA). In contrast, the addition of 25 mol% cholesterol to DPPC films in the presence of polymer resulted in condensation (negative $\Delta\pi^*$), but increasing the amount of cholesterol to 50% resulted in expansion of the film. For example, CHP condensed the DMPC film by 9·34 mN m^{-1} but including cholesterol resulted in expansion by 4·70 mN m^{-1} for a net π^* of − 4·64 mN m^{-1}, i.e. overall condensation of the DMPC film. In comparison, CHP expanded the DPPC film by 3.18 mN m^{-1} but including cholesterol resulted in a decrease of 4.86 mN m^{-1} for a net π^* of − 1·68 mN m^{-1}, i.e. overall condensation of the DPPC film also occurred. This compares with the condensation of DMPC film by 5 mN m^{-1} and the expansion of DPPC film by 6 mN m^{-1} with 25 mol% cholesterol in the absence of polymer (Table 1). The inclusion of 5 mol% DCP in DMPC–cholesterol films resulted in positive $\Delta\pi^*$ values for each of the polymers (except dextran) indicating expansion, and for CHP yielding a net π^* of − 0·8 mN m^{-1}. In other words, interfacial pressures of DMPC and DMPC–cholesterol–DCP lipid composite films to which CHP had become adsorbed were essentially the same at the chlorobenzene-water interface. This also held for pullulan, dextran, and cholesterol-derivatized dextran. There was, however, a net condensation of films containing CM-chitin of about 3 mN m^{-1} but a net expansion of films containing PAA or PVA from 4–7 mN m^{-1} The inclusion of 5 mol% DCP in DPPC–cholesterol films resulted in no significant change in interfacial pressure in the presence of each of the polymers, except when PAA at pH 1.8 or PVA were present, when slight expansion occurred (probably because of the large molecular weight of PAA and the high surface activity of PVA). In the DPPC composite films including cholesterol and DCP the net change in π was −1·8 mN m^{-1}.

The interaction of a polymer at a lipid interface can be based both on its surface activity and on the nature of its interaction

with the lipids. Using the π^* value of the polymer for a given composite lipid film and the surface pressure of the polymer at the clean interface (Table 1), a polymer impact ratio, (P*), was determined from:

$$P^* = \pi_{\text{lipid–polymer}}/\pi_{\text{polymer}} \quad (4)$$

where P* can be used to compare polymer performance at different lipid interfaces. A compilation of polymer impact ratios is presented in Table 4 for the eight different lipid monolayers. In general, low P* values indicate strong interaction with the lipid film and high values weak interaction. Intermediate values represent attachment but not extensive disruption of the film, which would be ideal if surface coatings are desired. Thus, the implication is that strong surface-active polymers represented by PVA might be detrimental to biological membranes whereas weakly interacting polymers such as pullulan or dextran would not be beneficial in the formulation of sterically-stabilized liposomes, for example. A $2{\cdot}0 < P^* < 2{\cdot}5$ obtained for cholesterol-derivatized pollulan, cholesterol-derivatized dextran, CM-chitin, and PAA at pH 6 would appear to be ideal because some of these poly-mers have also been shown to stabilize liposomes in simulated gastric and intestinal fluids (Dong & Rogers 1991; Sehgal & Rogers 1994).

One of the main findings was the opposite effects of mutually incorporated cholesterol–DCP and polymer on the state of packing of the monolayer, one counteracting the action of the other. This leads to the conclusion that cholesterol and polymer interact at different sites of the monolayer and do not necessarily interfere with each other. Hence, the water-soluble polymers caused changes in monolayer packing mainly in the polar head-group region whereas cholesterol altered van der Waals attractive forces between the hydrocarbon chains of the phospholipid even in an environment of chlorobenzene molecules, and the monolayer expansion tendencies of negatively charged DCP also predominated in the hydrophilic region. Thus, the state of condensation or expansion of model or biological membranes is highly dependent on the type of polymer and the composition of the membrane, the parameters of which can be readily evaluated by interfacial tension measurements at the oil–water interface.

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