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Biophysical Chemistry 80 (1999) 31–40

Biophysical Chemistry

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Interaction of type-I collagen with phospholipid monolayer

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Received 9 December 1998; received in revised form 4 March 1999; accepted 16 April 1999

Abstract

The effects of type-I collagen on dipalmitoyl phosphatidylcholine (DPPC) and dimyristoyl phosphatidylcholine (DMPC) monolayer films with different compositions were studied using monolayer technique. The addition of collagen in the subphase of different monolayer films induced a considerable shift towards larger area/molecule in the compression–isotherm curves. This is either referred to the insertion of collagen into the monolayer by its hydrophobic residues or to an adsorption process causing a protein layer to be located parallel to the lipid monolayer [1]. The variation of collagen interaction with different lipid compositions was also verified through the penetration-kinetics experiment. Comparing our results to the results of Pajean et al. [2] and Pajean and Herbage [3] on the effect of collagen on the stability of lipid vesicles implies that the collagen induced stability could be explained on the basis of collagen–lipid monolayer interaction.

Keywords: Type-I collagen; Monolayer; Collagen–lipid interaction; DPPC; DMPC

1. Introduction

Collagen is a well known protein with a great variety of industrial and medical applications [4]. It is a potentially useful biomaterial since it is a major structural component of many tissues such as skin, bone, cartilage, tendons, and basement membranes. There are numerous observations on associations between collagen fibres and lipids in both normal and pathological tissues [5–9]. Cohen and Barenholz found a strong interaction between collagen and sphingomyelin liposomes in vitro [10]. Lipids and collagen have been used to prepare a gel matrix which was reported as an effective drug delivery system [11]. Martinez del Pozo et al. described interaction of type-I collagen with

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0301-4622/99/$ - see front matter
PII: S0301-4622(99)00058-7

phospholipid vesicles [12,13]. Pajean et al. found that collagen increases the stability of liposome vesicles [2,3]. Fonseca et al. found that coating of liposomes with collagen involves both a higher stability in vitro and a selective and almost immediate accumulation of the vesicles in liver Kupffer cells, therefore, it might be effective in the treatment of some infectious diseases located within macrophages [14]. Monolayers of phospholipids at the air/water interface provide a suitable experimental system to investigate molecular interactions occurring in an oriented molecular array. This model system is used in the present work to investigate the interaction of type-I collagen with membrane model of different compositions of DPPC and DMPC mixed with different stabilising lipids such as cholesterol, α-tocopherol (vitamin E) and PEG-PE.

2. Materials and methods

2.1. Materials

L-α-Dipalmitoyl phosphatidylcholine (DPPC) specified 99% pure, L-α-dimyristoyl phosphatidylcholine (DMPC) specified 99% pure, α-tocopherol (vitamin E), cholesterol (CHOL) type 99% pure and type-I collagen from Bovine were purchased from Sigma (St Louis, MO, USA). Distearoylphosphatidylethanolamine derivatized at the amino position with 2000 molecular weight segment of poly(-ethylene glycol), PEG-PE, was obtained from Calbiochem (La Jolla, CA). Organic solvents (chloroform and ethanol) were of analytical grade and obtained from Merck. Water used was double distilled in glass apparatus (final distillation over alkaline $KMnO_4$).

2.2. Monolayer experiments

Films of pure DPPC, DPPC/CHOL (100:20), DPPC/α-tocopherol (100:20) and DPPC/CHOL/PEG-PE (100:20:4) mole ratio dissolved in chloroform/ethanol (5:1, v/v) were spread on a subphase of double distilled water in a Teflon trough. The same sample compositions were also prepared using DMPC. Compression isotherms were measured as previously described [15] [using an electromicrobalance (Sartorius A-120-S) based on the Wilhelmy method and coupled to a chart recorder to give a continuous reading of the force on the dipping plate (Langmuir system was made by Barry Wright corp. UK). The dimensions of the trough were $28.5 \times 16.2 \times 2.5$ cm]. Surface pressure was measured with accuracy, 0.1 mN/m. Compression started at least 35 min after film spreading to ensure complete evaporation of solvent and the compression rate was 5 $Å^2$/molecule per min. Measurements were repeated at least three times and reproduced after expansion and recompression. The temperature was kept at 25°C. For each experiment, collagen (dissolved in 0.5 M acetic acid) was injected in the subphase using a Hamilton microsyringe. The final collagen concentration in the subphase was 9 nM. Penetration kinetics were performed by spreading the phospholipid film at an initial pressure of 5 mN/m, and the pressure increases $\Delta\pi$ (produced after injection of collagen into the subphase) were recorded for 35 min.

3. Results and discussion

The variation of area/molecule with collagen concentration is shown in Fig. 1. At 9 nM collagen concentration, there is a maximum increase in the area/molecule for different surface pressures (so this concentration is chosen to study the interaction between collagen and lipid monolayer). Fig. 2 shows the compression isotherm for type I-collagen alone (without lipid) spread directly at the interface as an insoluble monolayer. Compression isotherms were carried out after 30 min of collagen injection to ensure its complete spreading [16]. It is clear that the collapse pressure for collagen is 25 mN/m. This result suggests that collagen is a good surface-active agent and demonstrates its ability to spontaneously penetrate a lipid-free interface. Compression isotherms of DPPC films spread on collagen solution are shown in Fig. 3a–d. Collagen is added in the subphase in the region of the liquid expanded state (surface pressure 5:10 mN/m) at which the monolayer is sensitive to any interaction [17–19].

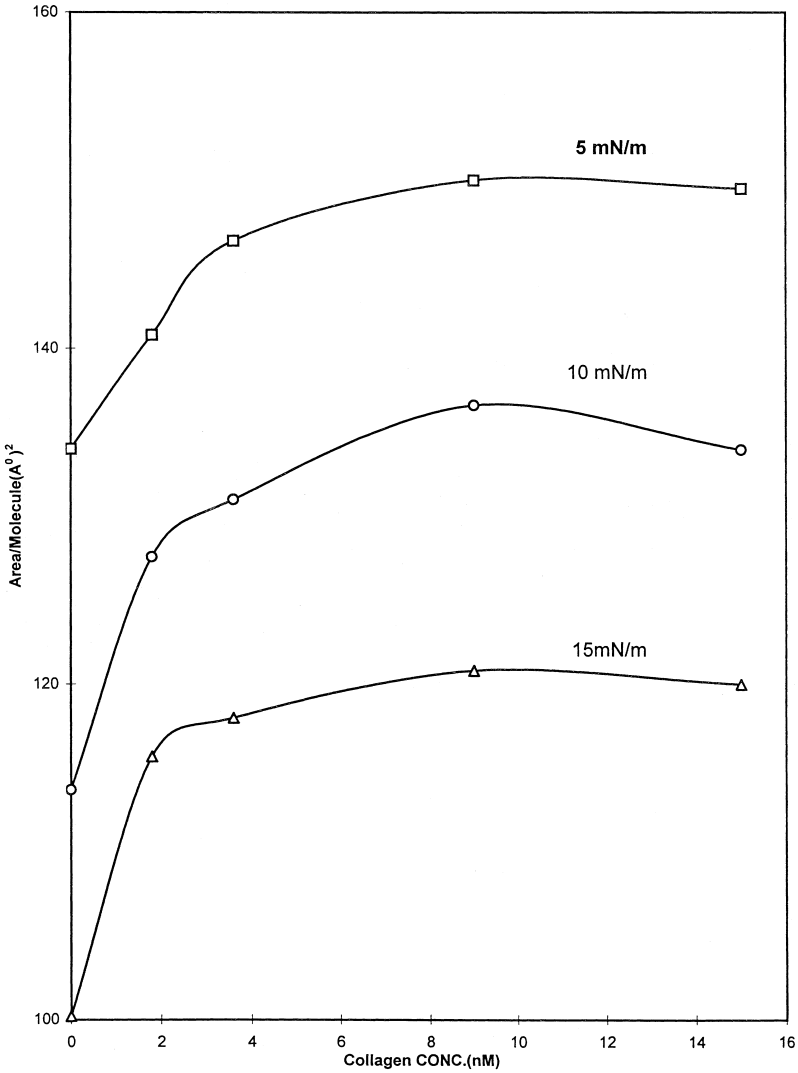

Fig. 1. Variation of area/molecule (Å^2) of pure DPPC monolayer with collagen concentration (nM) at constant values of surface pressure.

The incorporation of collagen within the lipid monolayer was monitored by the increase of the surface area of the lipid monolayer at constant surface pressure [20]. Fig. 3a shows the effect of collagen on a monolayer composed of pure DPPC. It is clear that the molecular area of DPPC in the protein–lipid monolayer is very much expanded compared with that of pure DPPC monolayer at the same surface pressure which is a consequence of the collagen–lipid interaction. This finding is evident, for example, at a surface pressure of 10 mN/m. At this pressure there is a shift of 10.37% towards a larger area/molecule. These results are in good agreement with those described elsewhere [16,20]. The protein–lipid interaction is either referred to as the insertion of protein into the monolayer by its hydrophobic residues or to an adsorption process causing a protein layer to be located parallel to the lipid monolayer [1]. Cohen and Barenholz demonstrated an interac-

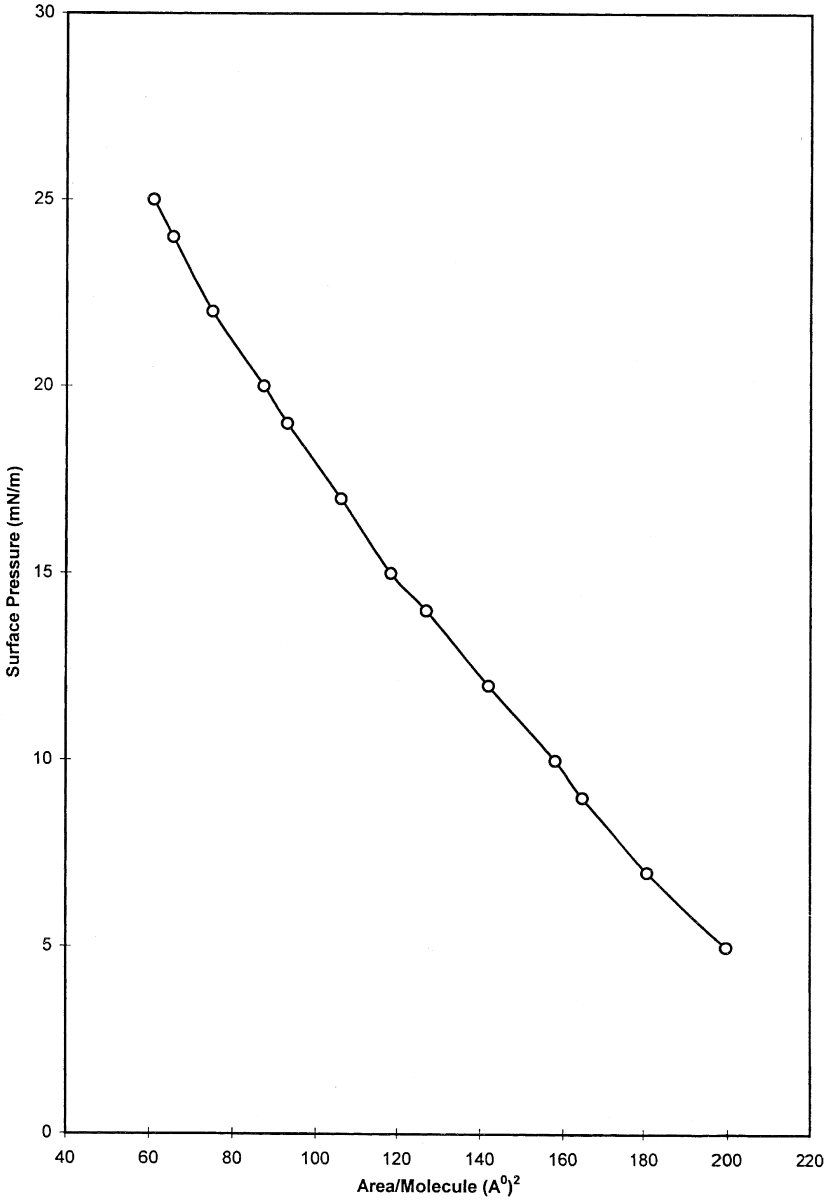

Fig. 2. Compression isotherm for type- I collagen (without lipid monolayer) of concentration 9 nM.

tion involving hydrogen bonding between the hydroxyproline and/or hydroxylysine residues of collagen-like tail acetylcholinesterase and the interface region of the sphingolipid molecule [10]. Martinez del Pozo et al. observed an interaction (simple surface binding) between the triple helix of type-I collagen and phosphatidylcholine liposomes resulting in relative immobilisation of the phospholipid molecules around the protein and consequently a decrease in their fluidity [12]. This shift to a larger area/molecule is smaller for other mixed monolayers giving 7.1% for DPPC/CHOL (100:20), 5.9% for DPPC/α- tocopherol (100:20) and 1.55% for DPPC/CHOL/PEG-PE (100:20:4) as shown in Fig. 3b–d. Similar to DPPC, collagen produced a considerable shift towards a

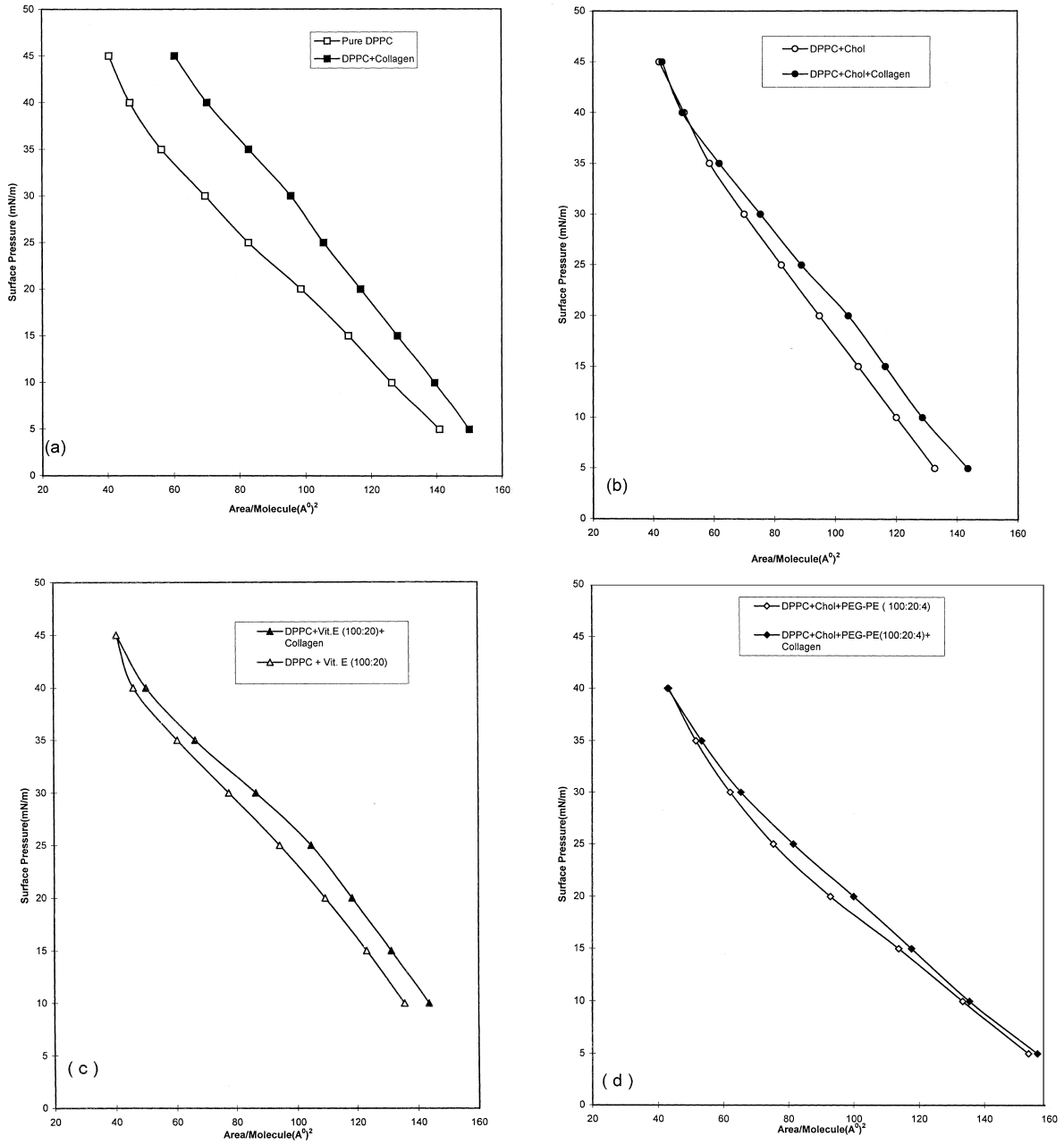

Fig. 3. Compression isotherms of DPPC monolayers spread on type-I collagen: (a) pure DPPC; (b) DPPC/CHOL (100:20); (c) DPPC/ α-tocopherol (100:20); (d) DPPC/CHOL/PEG-PE (100:20:4) in molar ratios, the collagen concentration in the subphase is 9 nM.

larger area/molecule in the compression isotherm of different DMPC monolayers (Fig. 4a–d) at a surface pressure of 10 mN/m. Recorded shifts were 10.9% for pure DMPC, 7.75% for DMPC/CHOL (100:20), 6.95% for DMPC/α-tocopherol (100:20) and 3.7% for DMPC/CHOL/PEG-PE (100:20:4). In Fig. 5 the increase in surface pressure (at constant area) of

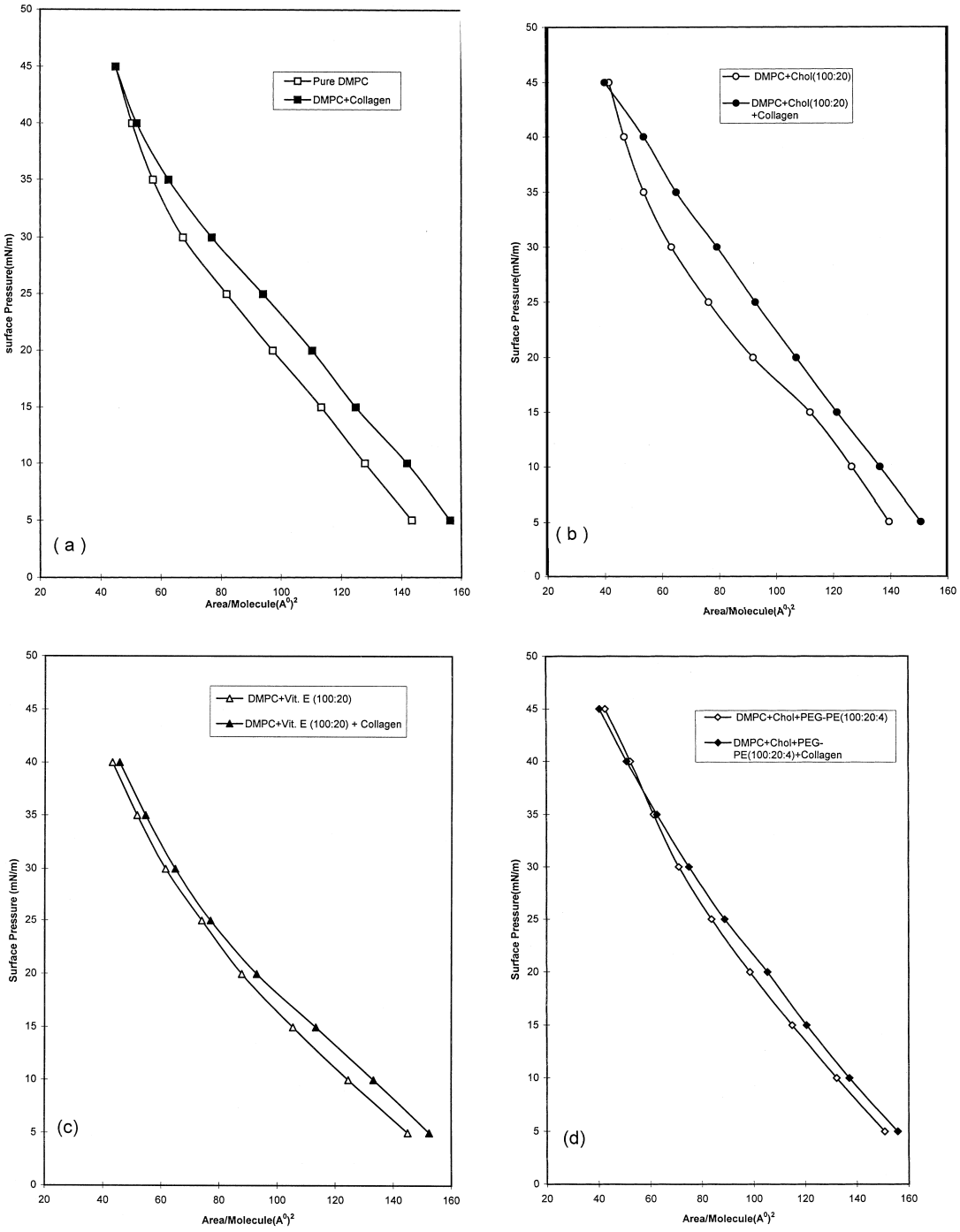

Fig. 4. Compression isotherms of DMPC monolayers spread on type-I collagen: (a) pure DMPC; (b) DMPC/CHOL (100:20); (c) DMPC/ α-tocopherol (100:20); (d) DMPC/CHOL/PEG-PE (100:20:4) in molar ratios, the collagen concentration in the subphase is 9 nM.

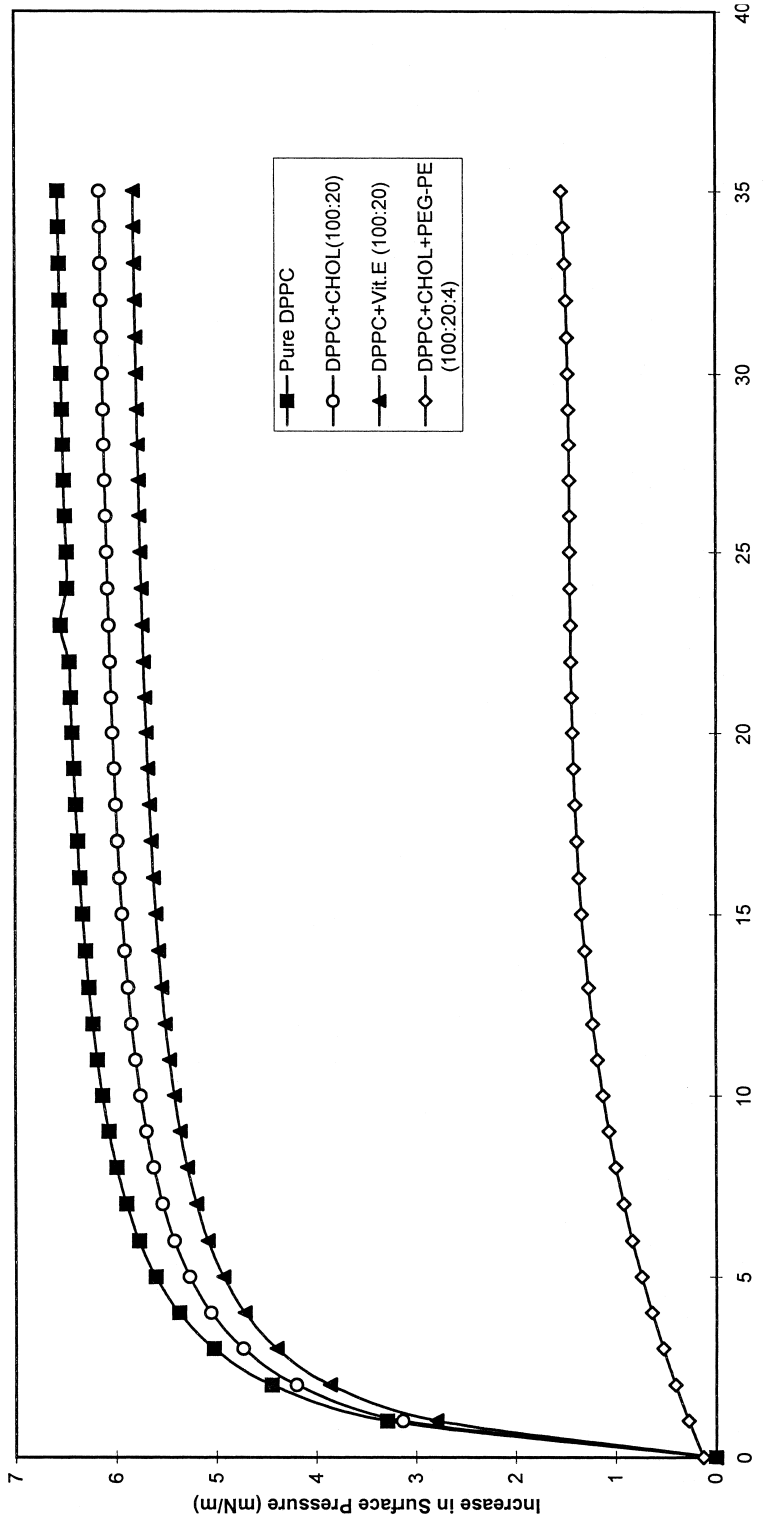

Fig. 5. Surface pressure increases induced by type-I collagen injected under different DPPC monolayers (35 min. after injection) as a function of time, at initial surface pressure of 5 mN/m, the collagen concentration in the subphase is 9 nM.

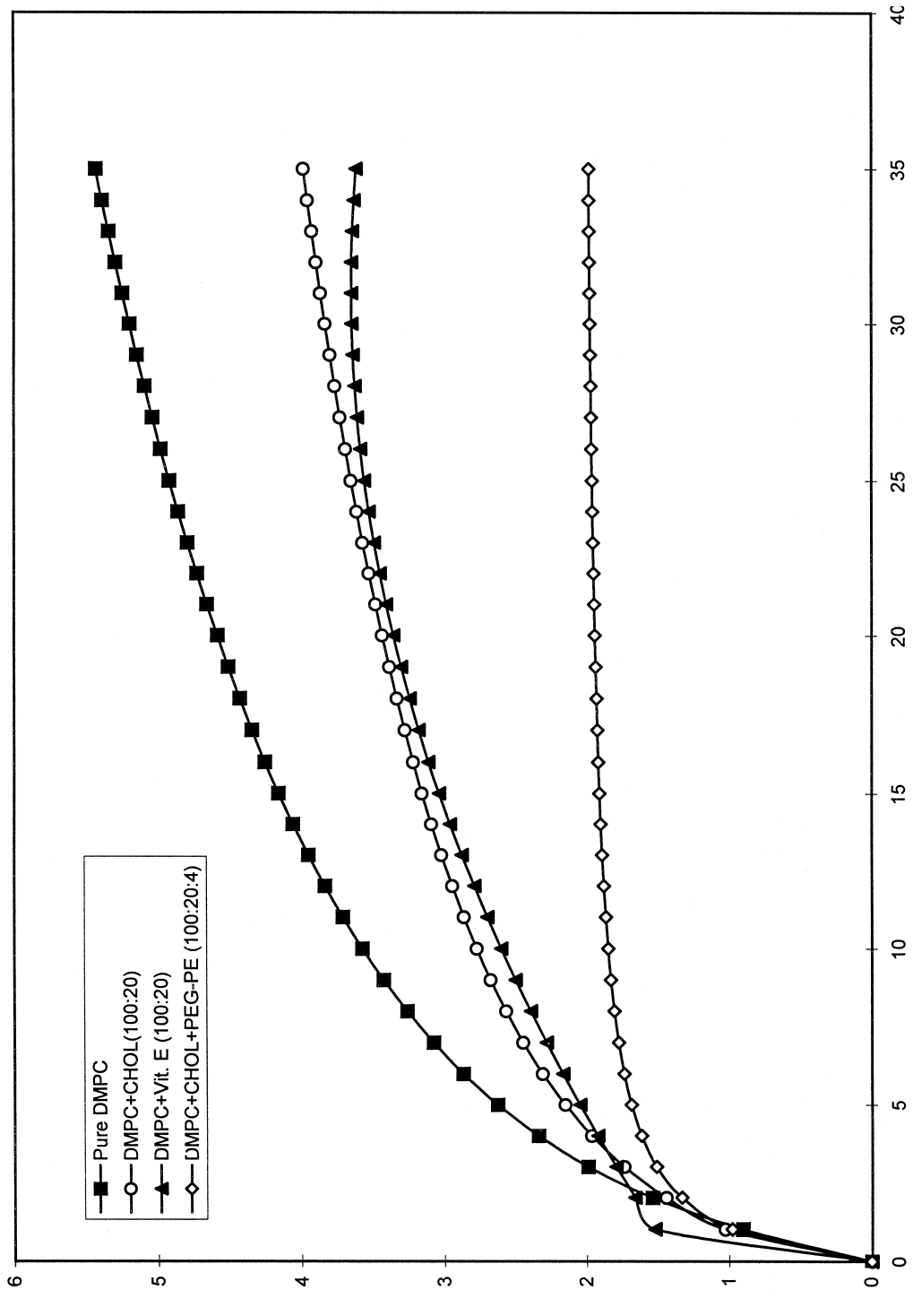

Fig. 6. Surface pressure increases induced by type-I collagen injected under different DMPC monolayers (35 min after injection) as a function of time, at initial surface pressure of 5 mN/m, the collagen concentration is 9 nM.

DPPC monolayers produced by collagen (9 nM) are plotted as a function of time at initial surface pressure of 5 mN/m. Surface pressure ($\Delta\pi$) of pure DPPC increased gradually until 6.5 mN/m where it presented a plateau over a period of 35 min. The increase in surface pressure reached 6.1, 5.8 and 1.45 mN/m for other DPPC compositions [DPPC/CHOL (100:20), DPPC/α-tocopherol (100:20) and DPPC/CHOL/PEG-PE (100:20:4), respectively] as shown in Fig. 5. The increase in surface pressure ($\Delta\pi$) with time (at constant area) for DMPC monolayers after injection of collagen (9 nM) into the subphase, at initial surface pressure of 5 mN/m is shown in Fig. 6. Also like DPPC, the increase in the surface pressure presented a plateau over a period of 35 min giving values of 5.4, 4, 3.6 and 2 mN/m for different DMPC monolayers [pure DMPC, DMPC/CHOL (100:20), DMPC/α-tocopherol (100:20) and DMPC/CHOL/PEG-PE (100:20:4), respectively] as shown in Fig. 6. It is clear from Figs. 5 and 6 that the curves became closer to each other and the change in surface pressure was reduced to a greater extent after the addition of CHOL and PEG-PE to the lipid monolayer. The effect of cholesterol, α-tocopherol, and PEG-PE on the stiffness of different lipid monolayers is evident in the compression isotherms and the penetration kinetics curves. When we add cholesterol to the pure lipid monolayer, there is a shift in the compression isotherm to a smaller area/molecule than that of pure lipid monolayer. Cholesterol condenses the monolayer of DMPC (above the phase transition temperature) due to an interaction between cholesterol and the acyl chains of the phospholipid molecules [21–24]. The limited freedom of acyl chains causes the membrane to condense, with a reduction in area, closer packing and decreased fluidity [25,26]. For DPPC monolayer (below the phase transition temperature) cholesterol increases the motion of the polar head group of DPPC due to its liquefying effect [27]. The shift (caused by collagen) to a higher area/molecule in the case of the lipid monolayer containing α-tocopherol is lower than that containing CHOL of the same molar ratio as shown in Figs. 3 and 4. These results are in good agreement with those described else where [3,28]. It was assumed that the stabilising effect of α-tocopherol is mediated via its binding with lipid (such as DPPC) by the formation of a hydrogen bond with either of the four oxygens of the phosphate group or with the oxygen atoms in the glycerol moiety. Thus, the interaction between α-tocopherol and lipids involves both hydrophobic and hydrogen bonding type interactions [29], which restricts the molecular mobility of lipid chains. The shift to a higher area/molecule is minimum in the case of adding CHOL 20% and PEG-PE 4% to the lipid monolayer, which agrees with Gaber et al. [15,30]. This minimised shift is partially referred to the addition of PEG-PE by 4% mole ratio to the pure DPPC or DMPC which decreases the motion of the choline methyl group, predominantly through coulombic and hydrophobic interaction forces [31].

4. Conclusion

Collagen is proved to interact with all investigated lipid compositions. This interaction could be a marker of expected improvement in the stability of lipid vesicles composed of any of these compositions. Comparing these results to the results of Pajean et al. [2,3] on the effect of collagen on the stability of lipid vesicles implies that the collagen induced stability could be explained on the basis of collagen-lipid monolayer interaction. These results indicate that a lipid–collagen complex could be used either in Cosmetology or Pharmacology as an effective drug delivery system.

Acknowledgements

We are indebted to our colleagues Said A. Ali and Wael M. Elshemey in the Biophysics Department for all their help and valuable suggestions.

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