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ORIGINAL CONTRIBUTION

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Inhibitory effects of fibrinogen on the dynamic tension-lowering activity of dipalmitoyl phosphatidylcholine dispersions in the presence of tyloxapol

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Abstract Inhibitory effects of fibrinogen on the dynamic surface tension-lowering ability of dipalmitoyl phosphatidylcholine (DPPC)/tyloxapol dispersions at pulsating air/liquid interfaces were investigated. When 10 ppm tyloxapol was added into a 1000 ppm DPPC dispersion prepared by sonication alone, the dynamic surface activity of the mixture was controlled by DPPC and was significantly inhibited by the presence of 1000 ppm fibrinogen. If the added tyloxapol concentration was as high as 100 ppm, tyloxapol then dominated the dynamic adsorption behavior of the dispersion, and the addition of 1000 ppm fibrinogen only showed minor influence on the dynamic surface tension behavior. When a 1000 ppm DPPC dispersion was prepared by sonication with 10 ppm tyloxapol, improved dynamic adsorption behavior resulted and thus the fibrinogen inhibition on the dynamic surface tension-lowering activity of the dispersion was slightly lessened. If the concentration of tyloxapol present during the sonication of a 1000 ppm DPPC dispersion was increased to 100 ppm, less interference by tyloxapol and/or fibrinogen with the dynamic adsorption of DPPC was found, which appears related to the tyloxapol-enhanced DPPC dispersion status with a reduced particle size.

Keywords Air/liquid interface · Dynamic adsorption behavior · Dynamic surface tension · Mixed surfactants · Surfactant inactivation

Introduction

One of the major functions of lung surfactants, which are synthesized in alveolar type II cells, is to reduce the dynamic surface tension at the air/liquid interface of the alveolar lining layer during breathing [1]. Dipalmitoyl phosphatidylcholine (DPPC) as the main component of lung surfactants is responsible for the surface tension-lowering ability of lung surfactants [2]. However, it has been generally accepted that plasma proteins, especially fibrinogen, have the ability of inhibiting the dynamic surface activity of lung surfactants [3].

The inhibitory effects of bovine serum albumin on equilibrium surface tension-lowering properties of two surfactant replacement formulations, Survanta and Exosurf, have been investigated by Bummer et al. [4]. It has been found that the equilibrium surface tension of Survanta was significantly elevated due to the existence of albumin. By comparison, only minor inhibitory influence of albumin on the equilibrium surface tension of Exosurf was observed even with an albumin concentration ten times higher than that applied in the case of Survanta. The unexpected resistance of Exosurf surface activity to the inhibitory effect of albumin was attributable to the presence of the nonionic surfactant tyloxapol in the Exosurf formulation. In a previous study, it has also been reported that tyloxapol seemed to improve the resistance of DPPC dynamic surface activity to albumin inhibition [5].

However, it has been suggested that tyloxapol dominated the dynamic adsorption behavior of Exosurf and was responsible for the comparatively higher surface tension of Exosurf [6, 7]. A detailed analysis on the dynamic surface tension behavior of DPPC/tyloxapol dispersions at pulsating air/liquid interfaces has been performed, and it has been concluded that the presence of tyloxapol may either improve or inhibit DPPC dynamic adsorption, depending on the tyloxapol concentration and preparation method for the dispersions [8]. By considering the potential applications of tyloxapol in enhancing the resistance of lung surfactant formulations to the inhibitory effects of plasma proteins, it appears important to further examine the influence of tyloxapol on the inactivation of DPPC dynamic surface activity by a plasma protein.

Experimental

L-α-Dipalmitoyl phosphatidylcholine (DPPC) (>99% pure), tyloxapol (SigmaUltra grade), and fibrinogen (from bovine plasma, fraction 1, type IV) were supplied by Sigma Chemical Company, USA and were used without further purification. Tyloxapol, as a nonionic surfactant, is a liquid polymer of the alkyl aryl polyether alcohol type [9]. The water used in all experiments was purified by means of a Milli-Q plus water purification system (Millipore, USA) with an initial resistivity of 18.2 MΩ-cm. A 0.1 M buffer solution of NaH_2PO_4/Na_2HPO_4 with a pH value of 7.0 was used for preparing all dispersions or solutions.

A 2000 ppm DPPC dispersion was prepared by sonication with series of short bursts (5-s duration and 2-s interval) for 5 min by using an ultrasonic processor (model VCX600, Sonics & Materials, Inc., USA) with an actual energy output of 35–40 W. During the sonication, the sample was surrounded with a jacket circulated with water at room temperature to avoid the rise in temperature. The dispersion was then mixed with an equal volume of buffer solution or 2000 ppm fibrinogen solution to obtain a 1000 ppm DPPC or 1000 ppm/1000 ppm DPPC/fibrinogen dispersion. The volume mean particle size of the dispersions was 32 μm as measured by a laser diffraction technique [8]. A fibrinogen concentration of 1000 ppm was selected due to its significant inhibition on 1000 ppm DPPC surface activity.

Mixed DPPC/tyloxapol dispersions were prepared by two methods, and the compositions were chosen to be the same as that used in a previous study [8]. For the method I, a 2000 ppm DPPC dispersion was prepared alone by sonication, and then was mixed with an equal volume of 20 or 200 ppm tyloxapol solution to give a desired DPPC/tyloxapol dispersion with a composition of 1000 ppm/10 ppm or 1000 ppm/100 ppm. Under the circumstances, a DPPC particle size of 32 μm was expected [8]. For the method II, a dispersion containing 2000 ppm DPPC was sonicated in the presence of 20 or 200 ppm tyloxapol and then was mixed with an equal volume of buffer solution to obtain a dispersion with the same composition as prepared by the method I. For a 1000 ppm/100 ppm DPPC/tyloxapol dispersion prepared by the method II, a smaller volume mean particle size of 26 μm was found in comparison with that obtained for a dispersion prepared by the method I [8]. For the addition of 1000 ppm fibrinogen in a DPPC/tyloxapol dispersion, the 20 or 200 ppm tyloxapol solution used in the method I was replaced by a 20 ppm/2000 ppm or 200 ppm/2000 ppm tyloxapol/fibrinogen solution, and the buffer solution used in the method II was replaced with a 2000 ppm fibrinogen solution.

A commercial pulsating bubble surfactometer (PBS) (Electronetics Corporation, USA) was used to measure dynamic surface tensions of surfactant samples under pulsating-area conditions [10, 11]. Measurements were done at a temperature of 37 ± 1 °C, which was controlled by the internal heater of the PBS. A bubble with a radius of 0.40 mm was formed first in a surfactant sample contained in a sample chamber, and the surfactant molecules started adsorbing onto the bubble surface to reduce the surface tension at the air/liquid interface. The pressure difference across the air/liquid interface was monitored by a pressure transducer, and the surface tension was calculated from the pressure difference and bubble radius by using the Young-Laplace equation. After a steady tension value was reached, the volume displacement of the sample was controlled by a pulsating rod, and the bubble radius then oscillated between 0.40 and 0.55 mm. A pulsation rate of ~20 cycles/min was used in all experiments. During the pulsation, the pressure differences across the air/liquid interface were measured and the corresponding dynamic surface tensions were calculated. The steady tension value was confirmed by following the surface tension variation with time after the pulsation was finished, and then a repeated experiment was performed.

Results and discussion

The inhibitory effect of 1000 ppm fibrinogen on the dynamic surface tension-lowering activity of a 1000 ppm DPPC dispersion at a pulsating air/liquid interface is demonstrated in Fig. 1. It has been indicated that dynamic surface tension behavior of a dispersed surfactant system strongly depended on its preparation protocol and thus dispersion status [12, 13, 14, 15, 16]. For a 1000 ppm DPPC dispersion prepared by the sonication procedure described in the experimental section, a volume mean particle size of 32 μm measured by a laser diffraction technique has been reported and its dynamic surface tension behavior has been investigated [8]. As shown in Fig. 1a, the dynamic surface tension response of a DPPC dispersion at pulsating-area conditions was improved by the sonication process, if one considered the poor adsorption characteristic of DPPC [17]. However, an initial surface tension (γ_i) of ~49 mN/m was detected for the DPPC/fibrinogen mixture (Fig. 1c), which was about 20 mN/m higher than that measured in the absence of fibrinogen. This initial surface tension was almost the same as that observed for a pure fibrinogen solution (Fig. 1b), implying that fibrinogen has an advantage over DPPC when competing for the space at the interface.

The minimum and maximum surface tensions, γ_{min} and γ_{max}, achieved by the mixture during the surface pulsation were much higher than that obtained for a pure DPPC dispersion, showing strong fibrinogen inhibition on the dynamic surface tension-lowering ability of DPPC (Fig. 1c). Furthermore, the dynamic tension curves in Fig. 1b and c almost overlapped with each other, indicating that the dynamic surface tension response of the mixture was dominated by fibrinogen and the DPPC ability of adsorbing onto the interface was greatly depressed. It has been proposed that

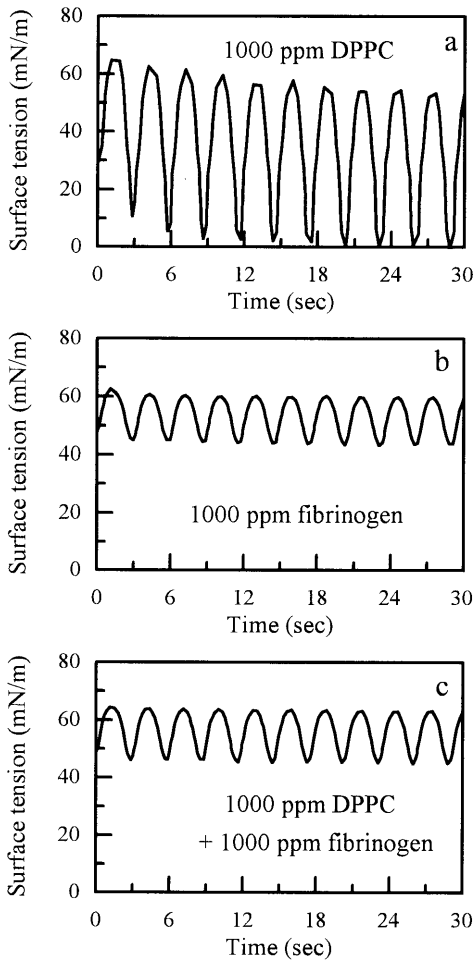

Fig. 1a–c. Inhibitory effect of 1000 ppm fibrinogen on the dynamic surface tension-lowering activity of a 1000 ppm DPPC dispersion under pulsating-area conditions

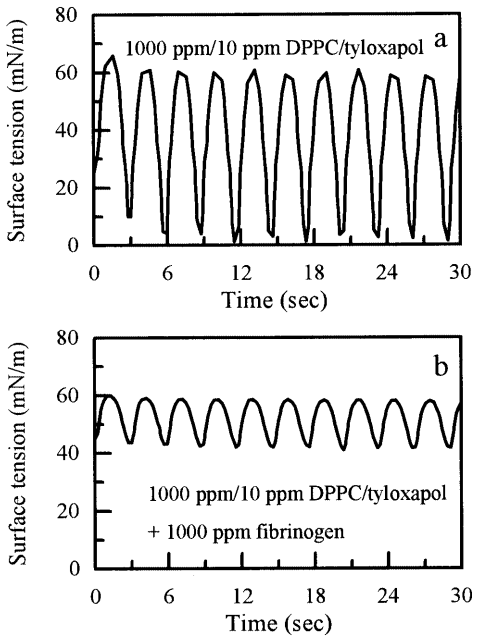

Fig. 2a, b. Influence of added 1000 ppm fibrinogen on the dynamic surface tension behavior of a 1000 ppm/10 ppm DPPC/tyloxapol dispersion prepared by the method I

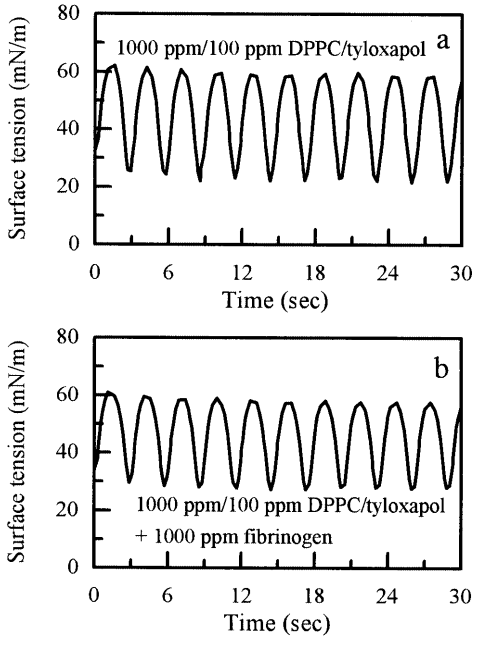

Fig. 3a, b. Influence of added 1000 ppm fibrinogen on the dynamic surface tension behavior of a 1000 ppm/100 ppm DPPC/tyloxapol dispersion prepared by the method I

plasma proteins may form surface inactive complexes with DPPC and thus inhibit the DPPC surface activity [18, 19]. However, since the fibrinogen-dominated dynamic surface tension response was clearly observed in Fig. 1c, it is unlikely that the DPPC inactivation resulted from the formation of surface inactive complexes with fibrinogen. A reasonable explanation for the inactivation of DPPC dynamic surface activity by fibrinogen may be similar to that proposed for the DPPC/bovine serum albumin system [20]. That is, fibrinogen occupied most of the interface before the pulsation probably due to its preferential adsorption, and consequently only a negligible surface concentration of DPPC resulted through dynamic adsorption during the surface pulsation.

The interference of tyloxapol with the inhibitory effect of fibrinogen on the DPPC dynamic adsorption is shown in Figs. 2 and 3. With the addition of 10 ppm tyloxapol, the dynamic surface activity of 1000 ppm DPPC seemed unaffected, except that a slightly higher γ_{max} value was detected (Fig. 2a). This result indicates that the presence of 10 ppm tyloxapol did not cause significant influence on the DPPC adsorption,

apparently because of the much smaller concentration of tyloxapol. When 1000 ppm fibrinogen was added into the 1000 ppm/10 ppm DPPC/tyloxapol dispersion, an initial surface tension of 45 mN/m was observed (Fig. 2b), which is much higher than that obtained without fibrinogen. It is evident that fibrinogen played an important role in the initial adsorption of the mixture. In addition, the pronounced rise in γ_{min} during the pulsation shows the extraordinary inhibition on the dynamic surface tension-lowering ability of a 1000 ppm/10 ppm DPPC/tyloxapol dispersion by 1000 ppm fibrinogen (Fig. 2b). In comparison with the data reported in Fig. 1c, it appears that 10 ppm tyloxapol caused insignificant influence on the fibrinogen inhibition.

When the tyloxapol concentration in a DPPC/tyloxapol mixture prepared by the method I was increased to 100 ppm, a less adsorption effect of DPPC was detected during the pulsation, as judged from the much higher γ_{min} values (Fig. 3a). This is obviously due to the comparatively fast adsorption of tyloxapol corresponding with a higher concentration [8]. When 1000 ppm fibrinogen was present in the 1000 ppm/100 ppm DPPC/tyloxapol dispersion, only a slight rise in γ_{min} during the pulsation was found (Fig. 3b). Apparently, the existence of 100 ppm tyloxapol resulted in the minor influence of 1000 ppm fibrinogen on the dynamic adsorption behavior of the mixture. As compared with the results demonstrated in Fig. 1c, the role of fibrinogen in the dynamic adsorption seemed insignificant. However, the dominant adsorption effect of 100 ppm tyloxapol was detrimental to the dynamic surface tension-lowering ability of DPPC.

In a recent study, Wen and Franses have reported that by breaking large DPPC liposomes into smaller vesicles by sonication, one could significantly improve the adsorption rate and tension-reduction ability of DPPC dispersions [16]. Also, it has been suggested that by sonication with the nonionic surfactant tyloxapol, the particle size of a DPPC dispersion was reduced and thus its dynamic adsorption was enhanced [8]. When the method II was applied to prepare a 1000 ppm/10 ppm DPPC/tyloxapol dispersion, the dynamic surface tension response of the dispersion under pulsating-area conditions showed similar γ_i and γ_{min} values to that indicated in Fig. 2a, but with much lower γ_{max} values (Fig. 4a). The lower γ_{max} values imply the improved dynamic surface tension-lowering ability of the dispersion as compared with that of a DPPC/tyloxapol dispersion prepared by the method I, which appears to correlate with the enhanced dispersion status or reduced particle size of DPPC by sonication with tyloxapol [8].

If 1000 ppm fibrinogen was added into a 1000 ppm/10 ppm DPPC/tyloxapol dispersion prepared by the method II, the inhibitory effect of fibrinogen on the dynamic surface tension response of the dispersion was clearly observed (Fig. 4b). As judged from the change in γ_i values of Fig. 4a and b, one can conclude that fibrinogen strongly affected the initial adsorption of the mixture. However, lower γ_{min} values were detected in Fig. 4b in comparison with that indicated in Fig. 2b. Apparently, the improved DPPC dynamic adsorption resulting from tyloxapol-enhanced DPPC dispersion status with a reduced particle size lessened, to a certain extent, the inhibitory effect of fibrinogen on the DPPC dynamic surface activity at the pulsating interface.

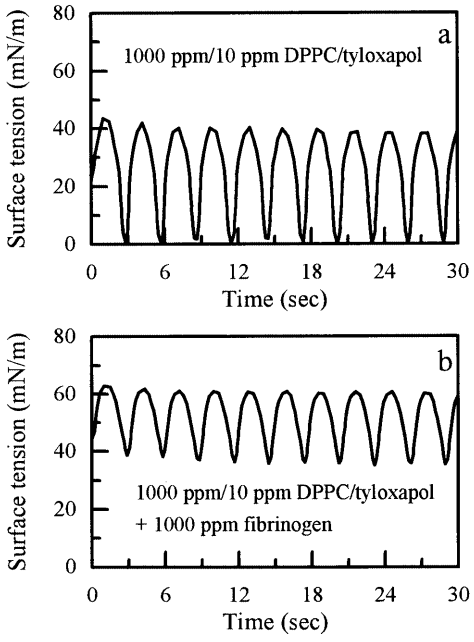

Fig. 4a, b. Influence of added 1000 ppm fibrinogen on the dynamic surface tension behavior of a 1000 ppm/10 ppm DPPC/tyloxapol dispersion prepared by the method II

When a 1000 ppm/100 ppm DPPC/tyloxapol dispersion was prepared by the method II, much better dynamic tension-lowering ability of the dispersion as compared with that reported in Fig. 3a has been found in a previous study (Fig. 5 a) [8]. The improved dynamic adsorption of the mixture was evidently caused by the enhanced DPPC dispersion status with a reduced particle size of 26 μm by 100 ppm tyloxapol during the sonication preparation. With the addition of 1000 ppm fibrinogen, γ_{min} values of the DPPC/tyloxapol dispersion under pulsating-area conditions became higher (Fig. 5b). However, by comparison with that shown in Fig. 3b, improved dynamic surface tension-lowering activity of the 1000 ppm/100 ppm DPPC/tyloxapol dispersion was detected. This appears to correlate with the improved DPPC dynamic adsorption through the enhanced dispersion status by the application of the preparation method II.

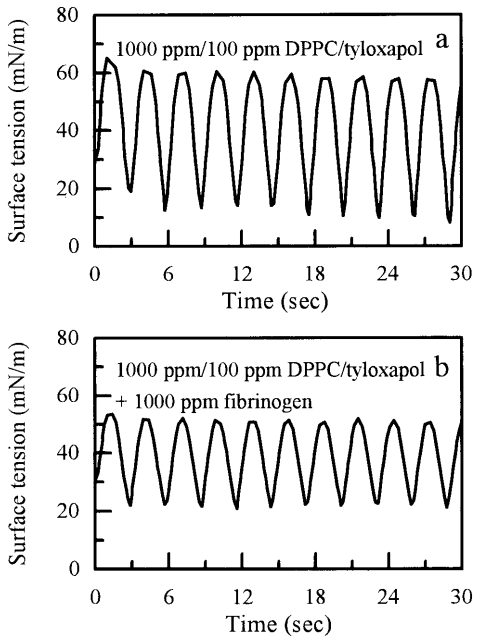

Fig. 5a, b. Influence of added 1000 ppm fibrinogen on the dynamic surface tension behavior of a 1000 ppm/100 ppm DPPC/tyloxapol dispersion prepared by the method II

Conclusions

The roles of tyloxapol in fibrinogen inhibition on DPPC surface activity at pulsating air/liquid interfaces were examined by dynamic surface tension measurements. The results demonstrate that the dynamic surface activity of 1000 ppm DPPC was markedly inhibited by 1000 ppm fibrinogen. When tyloxapol was added into a previously sonicated 1000 ppm DPPC dispersion, tyloxapol concentration-dependent interference with DPPC dynamic adsorption was observed. At a higher tyloxapol concentration of 100 ppm, tyloxapol dominated the dynamic adsorption behavior of the DPPC/tyloxapol dispersion apparently due to the fast adsorption characteristic of tyloxapol, and the added 1000 ppm fibrinogen only caused a minor effect on the dynamic tension response of the dispersion.

When a 1000 ppm DPPC dispersion was prepared by sonication in the presence of tyloxapol, the dynamic adsorption of DPPC was evidently enhanced with an extent depending on the tyloxapol concentration due to the improved DPPC dispersion status by tyloxapol. For a 1000 ppm/100 ppm DPPC/tyloxapol dispersion, the interference of 100 ppm tyloxapol and/or added 1000 ppm fibrinogen with the dynamic surface activity of 1000 ppm DPPC became less significant. The results suggest that the deleterious effect of fibrinogen on DPPC dynamic surface activity may be reduced if a suitable amount of tyloxapol is incorporated into a DPPC dispersion by an appropriate preparation method.

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