Biochimica et Biophysica Acta, 646 (1981) 185–187
Elsevier/North-Holland Biomedical Press

BBA Report

BBA 71527

EXCLUSION OF FLUID LIPID DURING COMPRESSION OF MONOLAYERS OF MIXTURES OF DIPALMITOYLPHOSPHATIDYLCHOLINE WITH SOME OTHER PHOSPHATIDYLCHOLINES

MICHAEL W. HAWCO, KAY P. COOLBEAR, PHILIP J. DAVIS and KEVIN M.W. KEOUGH *

Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, A1B 3X9 (Canada)

(Received January 20th, 1981)

Key words: Lipid monolayer; Surface tension; Phosphatidylcholine; Compression; Lipid exclusion

Monolayers composed of dipalmitoylphosphatidylcholine and one of four fluid phosphatidylcholines have been studied for their ability to attain low minimum surface tension during compression at two different speeds. The minimum surface tension depended on the compression rate and the proportions of the fluid and rigid lipid in the monolayer. The type of fluid lipid used in the monolayer also affected the minimum surface tension.

The pulmonary surfactant of mammalian lungs contains a large number of lipids in addition to the principal phospholipid, DPPC. Since these other lipids will increase the fluidity of the final mixture it has been suggested that a monolayer of surfactant must undergo some process of refinement to reduce the content of the more fluid lipids, leaving a monolayer even more enriched in DPPC which can achieve the very low surface tension needed in the contracted lung [1]. Recent results with monolayers made with mixtures of DPPC with egg lecithin and cholesterol [2,3] or with 'egg' phosphatidyl glycerol [4] are consistent with the possibility that the fluid lipid could be excluded during the compression of the monolayer. Using monolayers composed of DPPC and a single unsaturated mixed-acid phosphatidylcholine, SOPC, it has been found that the extent of exclusion of fluid lipid depended on both the compression rate and the proportions of SOPC and DPPC [5]. It seemed possible that the amount of molecular motion in the fluid lipid might also influence the extent of exclusion and thus the maximum surface pressure attained by a film during high compression. Here we describe experiments to test this possibility using monolayers composed of DPPC and four different fluid phosphatidylcholines. In keeping with a body of literature on pulmonary surfactant we will report the minimum surface tension (γ_{min}) achieved during compression of the film (γ = (surface tension of clean water) – (surface pressure of the film); γ_{min} = the minimum value of γ attained during compression of a monolayer. This value is usually reached at or after what is considered the point of monolayer collapse [5]).

DLPC and DPPC were obtained from Sigma Chemical Co., St. Louis, MO. SOPC was synthesized by a small modification of the procedure of Gupta et al. [6], and POPC and SLPC were made by a different procedure [7,8]. The lipids were pure as judged by this-layer chromatography [9] except for the presence of trace amounts of 1,3-isomers. DPPC was purified by thin-layer chromatography and stored in $CHCl_3/CH_3OH$ (1 : 1, v/v). while DLPC was stored dry at −20°C and used as received. The SOPC, POPC and SLPC, which were stored in $CHCl_3/CH_3OH$ (1 : 1,

* To whom correspondence should be addressed.

Abbreviations: DLPC, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; SLPC, 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; SOPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; γ_{min}, minimum surface tension.

v/v) at −20°C under N_2, contained equimolar amounts of appropriate fatty acids, but contained between 6 and 10% of the reversed positional isomers of the 1,2-phosphatidylcholines as determined by enzymatic degradation and gas-liquid chromatography. The SLPC was analyzed by uv spectroscopy prior to use and found to be essentially free from conjugated dienes or hydroperoxides.

Mixtures of the lipids were formed in $CHCl_3/CH_3OH$ (1 : 1, v/v) which was removed under N_2 and they were redissolved in hexane/methanol (98 : 2, v/v). An aliquot containing 7.4 ± 0.1 nmol of the lipid was applied to the subphase to form the monolayer. Solvent was allowed to evaporate for 10 min before starting the compressions. Monolayer studies were carried out on a Kimray-Greenfield surfactometer (KMA Associates, Oklahoma City, OK) which consists of a teflon trough (5.0 × 11.5 cm) and barrier, and which has a total available area of 53.4 cm^2 [5]. The apparatus was housed in a homemade environment chamber which was used to maintain the temperature at 36 ± 1°C. Monolayers were formed on 0.15 M NaCl made from doubly distilled water (second distillation from $KMnO_4$ solution). The pH of these solutions was monitored periodically and found it to be between 5 and 6. The balance was modified so that compression rates up to 3.6 $cm^2 \cdot s^{-1}$ could be employed. Surface tension was monitored with a platinum dipping plate which was rubbed with fine carborundum paper and flamed prior to use.

The minimum surface tension (γ_{min}) attained during compression (full compression reduces the pool area to 15% of the original size) was monitored for four different kinds of monolayers composed of DPPC mixed with one of either DLPC, POPC, SLPC or SOPC. Each kind of monolayer was also studied at four different proportions of the two constituent lipids and at two compression rates (1.02 and 3.6 $cm^2 \cdot s^{-1}$). The results are summarized in Fig. 1 where three properties of these monolayers are evident. First the γ_{min} attained by any one kind of monolayer depends upon the proportion of fluid lipid to DPPC in the mixture. Second, for any given proportion the γ_{min} depends upon the compression rate. Third, the γ_{min} attained also depends upon which fluid lipid is in the mixture, that is, for any given lipid ratio and given compression rate the γ_{min} is different for each kind of monolayer. Monolayers of mixtures of SOPC

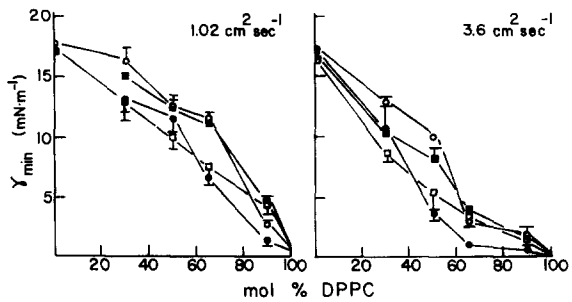

Fig. 1. The effect of compression rate and lipid composition on the γ_{min} attained during compression of monolayers made from DPPC and four fluid lipids. ○———○, POPC-DPPC; □———□, DLPC-DPPC; ■———■, SLPC-DPPC; ●———●, SOPC-DPPC. Values are mean ± range (n = 2) except for SOPC-DPPC at 1.02 $cm^2 \cdot s^{-1}$ where the values are mean ± S.D. (n = 4). Where the range is not given, it is within the size of the symbol. For clarity, the error bars are given on one side of each symbol.

and DPPC generally reach the lowest γ_{min}. Mixtures of POPC with DPPC were generally the least effective in reaching low γ_{min}. The other mixes showed intermediate behaviour. SLPC-DPPC mixes reached slightly lower values of γ_{min} than did POPC-DPPC when the DPPC content was low, and slightly higher values when the DPPC content was high. DLPC-DPPC mixes reached lower γ_{min} than did POPC-DPPC at low concentrations of DPPC, but the γ_{min} were close to that of POPC-DPPC at high concentrations of DPPC (90% at 1.02 $cm^2 \cdot s^{-1}$; and 65% and 90% at 3.6 $cm^2 \cdot s^{-1}$).

The results confirm and extend previous findings about lipid exclusion during compression of mixed phosphatidylcholine monolayers [2–5]. We have observed with SOPC-DPPC mixes [5] that the ultimate γ_{min} attained during the compression of monolayers depended upon the ratios of the components and the compression rate. These results confirm that both of these effects are demonstrable in other monolayers, and that they are not dependent in any special way on the presence of unsaturated phosphatidylcholines since DLPC is also excluded during compression. It would appear that the exclusion is dependent upon the fluidity or phase of the lipid. In our other studies [5] compressions could only be carried out at rates up to 1.02 $cm^2 \cdot s^{-1}$. The rate of change in an alveolus during exhalation is not known, but it seemed desirable to be assured that the previously observed effect of compression rate was not related only to the

slow rates employed. At compression rates which were 3.5 times the faster rate used before, the general pattern of a dependence of the γ_{min} on the proportions of fluid and rigid phosphatidylcholines is still observed.

The data show that the γ_{min} reaches is dependent upon the type of lipid which is mixed with the DPPC. While all the fluid lipids are excluded, some appear to be more so than others. The greatest differences are seen between mixtures of SOPC-DPPC and POPC-DPPC. While it has not been demonstrated unequivocably in any study, it seems reasonable to assume that exclusion or squeeze-out of fluid lipid during compression of these monolayers leaves a more rigid monolayer capable of attaining very low γ_{min}. That being the case, there can be at least two explanations for low γ_{min}. First, for any given ratio of fluid lipid to DPPC the total amount squeezed out may be the same, and the ultimate fluidity (and consequently γ_{min}) in the resulting monolayer may thus be determined by the fluidity of the low-melting component. The bulk phase transition temperature of the fluid components are in the order SLPC < POPC $\simeq$ DLPC < SOPC [10–12] and thus the extent of molecular motion in the chains at 36°C will be in the reverse order. The second explanation is that different amounts of fluid lipid may be excluded, depending upon the proportion of and type of fluid lipid. Mixing of the various phosphatidylcholines in the monolayer may not be equivalent for each combination. Patches enriched in fluid lipid may be more readily excluded from one monolayer than another. It can be seen that mixing is not equivalent for a number of these phosphatidylcholine combinations in bilayers [11,12] although extrapolation from the bilayers to the monolayer must be approached with caution (e.g. Ref. 13). It is likely that both effects contribute to the different γ_{min}.

The results confirm, with four simple mixtures, that the minimum surface tension achieved during compression of a phosphatidylcholine monolayer depends on the rate of compression and on the proportions of fluid and rigid lipid in the monolayer before compression. The results also suggest that the structure and phase of the fluid lipid may play a role in the way exclusion occurs and thus in the γ_{min} achieved. These results may have significance to the observation that the lipids of the surfactant phosphatidylcholines of newborn infants with respiratory distress syndrome have a different fatty acid composition that the phosphatidylcholines from surfactant of infants without respiratory distress [14].

This work was supported by the Canaduan Lung Association, the Medical Research Council of Canada and the Newfoundland Tuberculosis and Respiratory Disease Association. A major portion of the work described will constitute a portion of a thesis to be submitted to Memorial University of Newfoundland by M.W. Hawco. We thank Ms. J. Thomey for her technical assistance.

References

1 Clements, J.A. (1977) Am. Rev. Respir. Dis. 115, 67–71
2 Hildebran, J.N., Goerke, J. and Clements, J.A. (1979) J. Appl. Physiol. Respir. Environ. Excercise Physiol. 47, 604–611
3 Wildeboer-Venema, F. (1978) Respir. Physiol. 32, 225–237
4 Bangham, A.D., Morley, C.J. and Phillips, M.C. (1979) Biochim. Biophys. Acta 573, 552–556
5 Hawco, M.W., Davis, P.J. and Keough, K.M.W. (1981) J. Appl. Physiol. Respir. Environ. Exercise Physiol., in the press
6 Gupta, G.M., Radhakrishnan, R. and Khorana, H.G. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 4315–4319
7 Cubero Robles, E. and Van den Berg, D. (1969) Biochim. Biophys. Acta 187, 520–526
8 Roseman, M.A., Lentz, B.R., Sears, B., Gibbes, D. and Thompson, T.E. (1978) Chem. Phys. Lipids 21, 205–222
9 Keough, K.M.W. and Davis, P.J. (1979) Biochemistry 18, 1453–1459
10 Davis, P.J., Coolbear, K.P. and Keough, K.M.W. (1980) Can. J. Biochem. 58, 851–858
11 Coolbear, K.P. and Keough, K.M.W. (1980) Proc. Can. Fed. Biol. Soc. 23, 142
12 Van Dijck, P.W.M., Kaper, A.J., Oonk, H.A.J. and De Gier, J. (1977) Biochim. Biophys. Acta 470, 58–69
13 Nagle, J.F. (1980) Annu. Rev. Phys. Chem. 31, 157–195
14 Shelley, S.A., Kovacevic, M., Paciga, J.E. and Balis, J.U. (1979) N. Engl. J. Med. 300, 112–116