Studies the interactions of ascorbic acid isoforms with a simple model of DPPC monolayer as a biomimetic membrane by Langmuir-Blodgett technique

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Abstract. There is ongoing interest motivated by the desire regarding monolayer to understand the nature of interaction forces within oriented structures. Monolayer of phospholipids dipalmitoylphosphatidylcholine DPPC were examined mainly because they are accepted as membrane model system and can offer a stable frame to investigate the interactions of various biomolecules and biomaterial compounds with the lipid membrane. Identifying the monolayer behavior in the presence of ascorbic acid (AscA) isoforms and if this isoforms could alter packing and organizing the thin film. By Langmuir-Blodgett technique, the DPPC monolayer were studied in absence and presence of (Conc.: 10⁻³M, 10⁻²M and 10⁻¹M) L- and D- AscA isoforms in water (subphase) at temperatures 25, 37 and 41°C and fixed pH=7. It's ascertained in this study that, the subphase with L- and D-AscA addition created a fixed monolayer at 25°C, while at high temperature 41°C causes alteration in DPPC monolayer to somewhat less densely pack especially L-AscA this is for instance disclosed by left-area shifted of the DPPC monolayer curve shape. The Langmuir monolayer studies revealed that AscA isoforms interrupt the DPPC monolayer during its formation, leads to make the variations in such monolayers properties. This work demonstrates that AscA addition in subphase has applied promising significant disturbing in monolayer play an essential role in biomimetic membrane.

1. Introduction

The study of monolayer is currently of particular interest, which is motivated by the desire to understand the nature of interacting forces within oriented structures. Formation of Monolayer by using Langmuir technology as a model system of a biological cell membrane, which surveyed by many researchers in biomedical sciences.[1,2] Lipids represent excellent models in a biomimetic membrane, and therefore the Langmuir technology is successfully utilized to studying the properties or behavior of biomembranes. This technology is useful to investigate the mechanism effect of several biomolecules such as ions, drugs, vitamins, enzymes, hormones, nanoparticles etc. on biomembrane. Lipids represent in biomimetic membrane is one of the excellent models for using this technique. The main composition of cell
membrane is phospholipid especially phosphatidylcholines (PCs) which have capture current attention in various fields. Recently, attention is increased towards the molecular films As a result of their prospective applications in biosensors, bioreactors, electrical and photo devices and materials science. The biochemical properties of PCs and the temperature-mediated effects of biomolecule interactions with monolayer at air-water interfaces still not well enough understood. In this work, the particular biomolecule is Ascorbic acid (AscA) which called vitamin C. It cannot be synthesized in the human body and therefore its presence is dependent on food. For this reason, the studies are focused mainly on AscA L- and D-isoforms. The L-AscA, found naturally in foods and synthetically in most supplements while D-AscA does not. This vitamin, is a precursor to vitamin E, possesses antioxidant properties, and has a beneficial effect on human health by avoiding cancers, positively affecting the immune system, and preventing eye diseases and coronary vascular diseases.

The goal of this research is to study the interaction of L- and D-AscA isoforms with DPPC monolayer as a cell membrane model using the Langmuir technique in different concentrations and temperatures. It particular emphasis on how these isoforms could alter packing and organizing a thin film. Furthermore, an attempt to understanding, if this interaction can influence mechanical properties (such as fluidity, strength, etc.) of the model cell membrane on the example of DPPC monolayer.

2. Materials and Method

2.1 Materials
Phospholipid used in this work was 1,2-dipalmitoyl-sn-glycero-3-phosphocholine DPPC (C16) getting from the Avanti Polar Lipids, Inc. (Lot #: 160PC-256). AscA isoforms L- & D- received from (RF and Khimreaktiv corp, Meligen corp., RF). The purity of all chemicals 99% and they were used as received. The spreading solvent was spectroscopic grade chloroform (Aldrich). The resistivity of water was 18.2 MΩ×cm using a Millipore Milli-Q system.

![Figure 1](image1.png)

Figure 1. Molecular structures of (a) DPPC (C16), (b) L-AscA and (c) D-Asc.

2.2 Method
Technique of Langmuir-Blodgett KSV-Nima KN2002 (Biolin Scientific) supply with a paper Wilhelm plate controlled an automatically Langmuir film balance. It was set to gain a curve at
air/water interface of monolayer. For all tests, DPPC dissolved into chloroform at concentration $10^{-3}$ molarity. DPPC was sprayed firstly on the surface of water subphase (pure) and secondly on the surface of AscA subphase $L$- and $D$- isoforms at concentrations $10^{-3}$ M, $10^{-2}$M and $10^{-1}$M. An external water bath circulation was used to maintain the temperature at 25, 37 and 41°C. Teflon barriers were compacted by Air/water interface and expanded symmetrically at demand rate. The cleaning of trough was warranted by suck out the surface subphase before each test, DPPC sample was spread via a microsyringe (USA, Hamilton Co.) when the surface pressure has been changed to less than 0.1mN/m through compression on the subphase surface. The experiment was started after ten minutes when solvent evaporated and the monolayer equilibrated. At this time, the rate of monolayer compressed on air/water interface was 15cm²/min to get the curves. A sharp-collapse was exhibited of the curve shape followed by the abnormal alteration for surface pressure at additional compression. This is indicated to the point of the monolayer collapse at appointed experimental state [Error! Reference source not found.]. From the principle of Langmuir technology, the monolayer molecular organization data and interactions therein could be drawn the curve include the location, the course and shape. First, the area of molecular ($A$) is estimated in a highly compressed monolayer which corresponds to the surface area occupied by a single molecule. This was done by estimating the reduction of the linear part of the curve to the zero surface pressure. Second, pressure collapse ($\pi_{col}$), which is the pressure at breaks monolayer, was estimated at the point where curve stop increasing at the monolayer compression. Third, the variation in the surface characteristic of the monolayers as fluidity reflected through the variation in the value of compression modulus. Which is defined as [Error! Reference source not found.,Error! Reference source not found.]

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

Where the surface pressure is ($\pi$) is and the area per molecule is ($A$). $C_S^{-1}$ coefficient provides the phase of monolayer information in which is occurs. The monolayer states are classified at the basis of $C_S^{-1}$ at the maximal values in the $C_S^{-1}$ plots versus $\pi$ in the following manner: the liquid phase (L) is $C_{S,max}^{-1} = 12.5-50$ mN/m; the liquid-expand phase (LE) is $C_{S,max}^{-1} = 50-100$ mN/m; the liquid condense phase (LC) is $C_{S,max}^{-1} = 100-250$ mN/m and the solid phase (S) is $C_{S,max}^{-1} > 250$ mN/m. The minima in plots of $C_S^{-1}$ versus $\pi$ are phase transitions.

3. Discussion
3.1 DPPC monolayer in $L$- & $D$- AscA as a subphase at 25°C
DPPC monolayer curve on the surface of purified water subphase (0AscA) at 25°C, 37°C and 41°C are shown in figure 2a. In our system the transition phase from liquid expand LE to liquid condense LC state represented through the reduction in molecular area at compression that noted in the curve. The main variation between them is to the initial point and the phase transition range. The decrease in the molecular region and the increase in temperature are proportional to the starting point of the phase transition. The transition of LE-LC phase is much better approaching through the compression curve figure 2b. By the assistance of the compression curve could be easily determined the transition phase range from point to point. Table 1 shows significant increase in surface pressure due to long hydrocarbon chains of DPPC. This fact showed at normal temperatures 25°C. While at high temperatures 37 and 41°C monolayer curve showed the lift-off molecular area $A_0$ and different collapse surface pressures that grows significantly with the temperature.

It could explain that the curves of DPPC are controlled by the hydrophobicity of alkyl chains. As a result, the thermal motion of alkyl chains is increased and the surface pressure is increased by increasing temperature. The DPPC curves corresponding to the condensed state of DPPC monolayers are the same at 25 and 37°C hence their $C_S^{-1}$ values will be similar this is agreement with literature [19, 20].
Table 1. DPPC monolayer $C_S^{-1}$, $A$, and $\pi_{\text{coll}}$ in water subphase at 25, 37 and 41°C.

| Subphase | Temperature, °C | $A$, Å² | $\pi_{\text{coll}}$, mN/m | $C_S^{-1}$, mN/m |
|----------|----------------|----------|---------------------------|-----------------|
| Water    | 25             | 110      | 45.2                      | >250            |
|          | 37             | 117      | 43.46                     | >250            |
|          | 41             | 120      | 31.2                      | 164.6           |

Figure 2. DPPC monolayer curves (a) and comparison of compression modulus $C_S^{-1}$ (b) in purified water at 25, 37 and 41°C.

Table 2 showed that the addition of $L$- and $D$-AscA isoforms to the subphase changed the DPPC monolayer properties. AscA made DPPC monolayer lift off at $A_0$ value higher than the respective pure subphase (0AscA) in the same concentration $10^{-3}$, $10^{-2}$ and $10^{-1}$ M at 25°C figure 3 a, b. The values of $A_0$ and the slopes of the curves corresponding to the condensed state of the monolayers at the concentrations of $L$- and $D$-AscA isoforms $10^{-3}$ and $10^{-2}$ M are practically similar; thus they will have the same $C_S^{-1}$ values figure 3c, d. The plateaus shape of the LE-LC in the DPPC monolayer curve became smoother in $L$ (natural)-AscA curve rather than in $D$ (synthetic)-AscA.

Table 2. DPPC monolayer $A$, $\pi_{\text{coll}}$ and $C_S^{-1}$ in $L$- and $D$-AscA as a subphase concentration $10^{-3}$, $10^{-2}$ and $10^{-1}$ M in 37°C.

| Subphase | $C$, M | $A$, Å² | $\pi_{\text{coll}}$, mN/m | $C_S^{-1}$, mN/m |
|----------|--------|---------|---------------------------|-----------------|
| $D$-AscA | $10^{-3}$ | 110     | 63                        | >250            |
|          | $10^{-2}$ | 114     | 63                        | >250            |
|          | $10^{-1}$ | 140     | 66                        | >250            |
|          | $10^{-3}$ | 113     | 66                        | >250            |
| $L$-AscA | $10^{-2}$ | 114     | 65                        | >250            |
|          | $10^{-1}$ | 150     | 67                        | >250            |
Figure 3. DPPC monolayer curves and comparison of compression modulus $C^{-1}_S$ in $D$-AscA (a), (c) and $L$-AscA (b), (d) subphases concentration $10^{-3}, 10^{-2}$ and $10^{-1}$ M and purified water at 25°C.

This being more significant in high $L$-AscA concentration $10^{-1}$M, which is due to the activity and sensitivity of natural biomolecule ($L$-AscA isoform) properties. The expansion of AscA monolayer was attributed to the addition of AscA to the subphase and this is due to the hydrophobic interactions and hydrogen bonding. The importance of these expansions are proving these interactions, which having the key role in the cell membrane disturbing by AscA activity. The addition of AscA curves into DPPC monolayer in different concentrations causes increasing in the maximum value of $C^{-1}_S$ (table 2). The higher $C^{-1}_S$ propose formation of monolayer with more solidity in subphase containing ($L$- and $D$-AscA) if compared with monolayer formed in pure water subphase ($0$AscA).

3.2 DPPC monolayer plots in L- and D- AscA isoforms as a subphase at 37 and 41°C
Tables 3 and 4 observed significant effect of high temperatures on area, compression modulus $C_S^{-1}$ and curve of DPPC monolayer. Addition of different AscA concentrations causes alteration in DPPC monolayer property of its fluidity, which reflected by the variations in compression modulus value and this due to the synergistic effect of high concentration and high temperature. As suggested from the LE-LC plateaus shape of the curves figure 4 a, b and figure 5 a, b that became smoother, the subphase with AscA addition showed alteration in DPPC monolayer property to less densely especially in high $L$- and $D$-AscA concentration at high temperature 37 and 41°C. This is more observed at the higher concentration of $L$-AscA at 41°C. In the presence of $L$- and $D$-AscA and absence (0AscA) in subphase, the LC grew with increasing temperature (41°C), indicating that the expanded phase continued for a longer compression period. More importantly, pressure of LC DPPC monolayer on AscA is higher, proving that the monolayers were fluidized as a result of $L$- and $D$-AscA action (figure 4 c, d) and (figure 5 c, d) and this effect be more significant in $L$-AscA isoform. However, the magnitude of this fluidization (significant decrease in $C_S^{-1}$), as measured by pressure of LC due to the pure subphase (0AscA) in the same conditions, was incrementally lower with increasing temperature at 37 and 41°C. These results are in an agreement with calorimetric data on phase transition analysis of DPPC liposomes [Error! Reference source not found.].

Table 3. DPPC monolayer $C_S^{-1}$, $A$, and $\pi_{coll}$ in $L$- and $D$-AscA as a subphase concentration $10^{-3}$, $10^{-2}$ and $10^{-1}$ M at 37°C.

| Subphase | $C$, M | $A$, Å² | $\pi_{coll}$, mN/m | $C_S^{-1}$, mN/m |
|----------|--------|---------|-------------------|------------------|
| $D$-AscA | $10^{-3}$ | 117     | 54                | $>250$           |
|          | $10^{-2}$ | 120     | 56                | $>250$           |
|          | $10^{-1}$ | 165     | 57                | $>250$           |
| $L$-AscA | $10^{-3}$ | 120     | 56                | 200              |
|          | $10^{-2}$ | 125     | 55                | 167              |
|          | $10^{-1}$ | 200     | 45                | 95               |

Table 4. DPPC monolayer $C_S^{-1}$, $A$, and $\pi_{coll}$ in $L$- and $D$-AscA as a subphase concentration $10^{-3}$, $10^{-2}$ and $10^{-1}$ M at 41°C.

| Subphase | $C$, M | $A$, Å² | $\pi_{coll}$, mN/m | $C_S^{-1}$, mN/m |
|----------|--------|---------|-------------------|------------------|
| $D$-AscA | $10^{-3}$ | 120     | 50                | 165              |
|          | $10^{-2}$ | 125     | 48                | 147              |
|          | $10^{-1}$ | 165     | 46                | 149              |
| $L$-AscA | $10^{-3}$ | 120     | 40                | 121              |
|          | $10^{-2}$ | 136     | 40                | 129              |
|          | $10^{-1}$ | 200     | 38                | 115              |
Figure 4. DPPC monolayer curves and comparison of compression modulus $C_S$ in $D$-AscA (a),(c) and $L$-AscA (b), (d) subphases concentration $10^{-3}, 10^{-2}$ and $10^{-1}$ M and purified water at 37°C.
4. Conclusion

To study the influence of synthetic and natural AscA isoforms the DPPC monolayer has been successfully utilized as a simple model of biomimetic cell membrane. The Langmuir monolayer studies revealed that biological molecules interrupt the DPPC monolayer during its formation, leads to make the variations in such important the phospholipids layers properties as its fluidity. Phase transition from the liquid expanded to liquid condensed phase for DPPC curve properties are also affected due to the incorporation of AscA plots in the subphase and this effect enhance with the synergistic effect of temperature.

5. References

[1] Hac-Wydro, K, Dynarowicz-Łatka P 2008 Annales Universitatis Mariae Curie-Sklodowska Luilin - Polonia. 4 47 doi:10.2478/v10063-008-0027-2

[2] Oliveira Jr.O.N. 1992 Brazilian Journal of Physics 22 60.

[3] Stefania C, Brezesinski G and Möhwald H 2014 Adv. in Colloid and Interface Science. 208 197 doi.org/10.1016j.cis.2014.02.013

[4] Travkova O G, Brezesinski, G 2013 Chemistry and Physics of Lipids 167–168, 43–50. doi: 10.1016/j.chemphyslip.2013.01.010

[5] Ziblat R Leiserowitz L and Addadi L 2010 J. Am Chem Soc 132, 9920 doi:10.1021/ja103975g

[6] Girard-Egrot A P, Godoy S and Blum L J 2005 Adv Colloid Interface Sci 116 205 doi:10.1016j.cis.2005.04.006

[7] Brezesinski G , Möhwald H 2003 Adv Colloid Interface Sci 100 102, 563-584 doi:10.1016/S0001-8686(02)00071-4

[8] Boukherroub R, Morin S, Sharpe P. and Wayner D D M 2000 Langmuir 16 7429 doi:10.1021/la991678z
[9] Kim V P, Ermakov A V, Glukhovskoy E G, Rakhnyanskaya A A, Gulyaev Y V, Cherepenin V A, Taranov I V, Kormakova P A, Potapenkovic K V, Usmanov N N, Szelelsky A M, Koksharoy Y A and Khomutov G B 2014 Nanotechnol Russia 9 280
[10] Stefanici C, Violiotijevic I, Santer M, Silva D V, Brezesinski G and Seeberger P H 2012 Chem Int Ed 51 12874.
[11] Gopal A , Lee- K Y C 2001 J Phys Chem B 105 10348 doi:10.1021/jp012532n
[12] Matsuyama T J , Lanterna A E, Granados A M, Krause- R W M, Maggio B and Vico R V 2014 Langmuir 30 5888 doi:10.1021/la500903m
[13] Simons K , Toomre D 2000 Cell Biol 1 31
[14] Caseli L, Moraes M L, Zucolotto V, Ferreira M, Nobre T M, Zaniquelli M E, Rodrigues Filho U P and Oliveira Jr ON 2006 Langmuir 22 8501 doi:10.1021/la061799g
[15] Wan K, Chovelon J M and Jaffrezic-Renault N 2000 Talanta 52 663.
[16] Oliveira Jr O N, Dos Santos Jr D S, Balogh D T, Zucolotto V and Mendonca C R 2005 Colloid Interface Sci 116 179 doi:10.1016/j.cis.2005.05.008
[17] Oliveira O N, Zucolotto V, Balasubramanian S, Narwa H S, Kumar J and Tripathy S K 2002 Layer-by-layer polyelectrolyte-based thin films for electronic and photonic applications. In Handbook of Polyelectrolytes, American Scientific Publishers: Los Angeles, pp 1-37.
[18] Torrent-Burgués J 2018 Colloids Interfaces 2 2 doi:10.3390/colloids2020017
[19] Yun H, Choi Y W, Kim N J and Sohn D 2003 Bull Korean Chem Soc 24 377.
[20] Krajewska B, Kyziol A and Wydro P 2013 Physicochem Eng Aspects 434 359 doi:10.1016/j.colsurfa.2013.03.018
[21] Yaroslavov A A, Sybachin AV, Zaborova O V, Zezina A B, Talmon Y, Ballauff M and Menger F M 2015 Advances in Colloid and Interface Science 226 54.
[22] Li Y , Schellhorn H E 2007 J Nutr 137 2171 doi:10.1093/jn/137.10.217
[23] Carr A C and Frei B 1999 Am J Clin Nutr 69 1086 doi:10.1093/ajcn/69.6.1086
[24] May J M, Qu Z C and Whitesell R R 1995 Biochemistry 34 12721.
[25] Buettner G R 1993 Arch Biochem Biophys 300 535 doi:10.1006/abbi.1993.1074.
[26] Raić-Mlić S, Svedruzić D, Gazivoda T, Marunović A, Hergold-Brundić A and Nagl A 2000 J Med Chem 43 4806 doi:10.1021/jm0009540
[27] Yamamoto I, Tai A, Fujinami Y, Sasaki K and Okazaki S 2002 J Med Chem 45 462 doi:10.1021/jm010379f
[28] Nowotarska SW, Nowotarski K J, Friedman M and Situ C 2014 Molecules 19 7497 doi:10.3390/molecules19067497
[29] Morandat S , Bortolato M , Anker G , Doutheau A , Lagarde M , Chauvet J P and Roux B 2003 Biochimica et Biophysica Acta 1616 137, doi:10.1016/j.bbamem.2003.03.001
