MATRIX EFFECT IN OLEATE MICELLES-VESICLES TRANSFORMATION

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Abstract. It is accepted by many authors that the formation of closed molecular structures is a key step in the evolution of life. Oleate vesicles represent a good model system in this framework due to the fact that they self-assemble spontaneously and that fatty acids are considered as possible prebiotic structures. In this contribution, we will focus the attention on the transition from oleate micelles to oleic acid/oleate vesicles induced by a pH change. This transformation is strongly influenced by the presence of pre-formed vesicles. We called this phenomenon the matrix effect. The influence of pre-added POPC liposomes (POPC = 1-palmitoyl-2-oleoyl-*sn*-glycerol–3-phosphocholine) and oleic acid/oleate vesicles on the process rate and on the final size distribution will be discussed elucidating the main differences between these two systems.

Keywords: dynamic light scattering, liposomes, matrix-effect, oleic acid, POPC, vesicles

1. Introduction

Since the work of Oparin (1924), compartmentalization has been recognized as a key step in the origin of life on earth. Many authors have then proposed the emergence of lipidic membranes and the formation of closed bilayer structures (vesicles) as a possible route for the formation of protocells in a prebiotic scenario (Goldrach, 1958; Deamer *et al.*, 1980; Morowitz *et al.*, 1988; Ourisson *et al.*, 1999). In this framework, we studied over the last years the aggregates formed by long-chain fatty acids such as caprylic (Bachmann *et al.*, 1992), methyl dodecanoic (Morigaki *et al.*, 1997), and oleic acid (Walde *et al.*, 1994), including giant vesicles obtained from oleic acid (Wick *et al.*, 1994). There are several reasons for this interest. First, long-chain fatty acids vesicles are examples of spontaneous vesiculation, i.e. they form by simple addition of the surfactant in water (Wick *et al.*, 1995), without external work except of mixing. In addition, the fast and rapid uptake of monomer oleate by oleate vesicles as well as by POPC liposomes (POPC = 1-palmitoyl-2-oleoyl-*sn*-glycerol–3-phosphocholine) make these systems excellent experimental models to study the mechanism of vesicles growth and fission (Blöchliger, 1998; Lonchin, 1999; Berclaz, 2001a, b). Furthermore, these surfactant molecules could have been synthesized in prebiotic environment (Hargreaves *et al.*, 1977; Orò *et al.*, 1978).

In this work the transformation of oleate micelles (OM) into oleic acid/oleate vesicles (OAV) will be studied focusing the attention on the influence that preexisting OAV vesicles and POPC liposomes (POL) can exhibit on this process.

Oleate micelles are well known to be stable in water solution at pH higher than 10.5 and to be able to spontaneously transform into vesicles if the pH decreases to a value between $9.0 \div 8.0$ (Small, 1986; Fukuda, 2001; Cistola, 1986, 1988). As reported in a previous work (Blöchliger, 1998), if an aqueous solution of oleate micelles is added to water buffered solution at pH 8.5, unilamellar oleate/oleic acid vesicles form spontaneously due to the pH change. The vesicles prepared in this way present a very broad size distribution. By contrast, the addition of the same OM solution volume to a preexisting OAV suspension with a very narrow size distribution results in a final vesicle suspension size distribution very close to the preexisting one. Moreover, in the presence of preformed OAV, the micelle-vesicle transformation results to be faster than in their absence, as shown by the time evolution of the optical density. Analogous results can be also observed by adding oleate micelles to a POPC liposomes suspension. We called this phenomenon the 'matrix effect', since the preexisting aggregates seem to act as a template effect for the formation of the new ones. The aim of this work is to elucidate the main differences between AOV vesicles and POL liposomes matrix effect.

2. Materials and Methods

2.1. CHEMICALS

Sodium oleate (>99%), oleic acid (puriss, standard for gas chromatography), and bicine (>99.5%) were from Fluka, Buchs, Switzerland and used as received.

2.2. VESICLES AND LIPOSOMES SUSPENSIONS

Oleic acid/oleate vesicles were prepared by dispersing oleic acid in 0.2 M bicine buffer (pH 8.5) under magnetic stirring at room temperature overnight. Liposomes were prepared by dissolving POPC in chloroform (5 ml) in a 50 ml round-bottom flask and removing the solvent using a rotary evaporator ($p = 400$ mbar, T $= 25\,°C$). The obtained lipidic film was dried under vacuum overnight and then hydrated with a defined volume of bicine buffer.

To form 100 and 50 nm unilamellar sized aggregate suspensions, both vesicles and liposomes underwent a 5-times freeze-thaw cycle (freezing in liquid nitrogen and thawing at room temperature) to reduce the lamellarity (Mayer *et al.*, 1986), followed by a 10-times passage through polycarbonate membranes of decreasing pore diameters.

2.3. OD MEASUREMENTS

Optical density was measured with a Cary 1E UV/vis multi-cell spectrophotometer from Varian, Australian using a quartz cells with a path length of 1.0 cm at $\lambda = 400$ nm.

2.4. PHOTON CORRELATION SPECTROSCOPY

Dynamic light scattering analysis were performed with a ZetaSizer 5000 ($\lambda = 633$ nm, scattering angle 90° degrees) from Malvern, United Kingdom.

3. Results

The general experimental procedure used in this work was the injection by a Hamilton syringe 200 μL of a 22.0 mM oleate aqueous solution (pH = 10.5) into 2.0 mL of a bicine buffer solution at a pH = 8.5, to obtain a final surfactant concentration equal to 2.0 mM. The injection is done directly into a spectroscopy cell that is gently shaken by hand before measuring. Since oleate molecules at high alkaline pH at a concentration above the CMC (critical concentration for micelle formation) spontaneously self-assemble into micelles, the process of vesicle formation at lower pH corresponds to the spontaneous transformation of micelles into vesicles. Herein, this experiment will be addressed as the *control experiment*, to distinguish it from the injection of the same micelles solution volume to 2.0 ml of a buffer solution with pre-added aggregates: oleic acid/oleate vesicles or POPC liposomes. In both cases, the overall surfactant concentration is 2.2 mM and it will result doubled after the micelles addition. In Figure 1 the time course of vesicle formation is followed by monitoring the optical density increase due to light scattering. Notice that the process is faster and the final plateau value is lower than those observed in the control experiment. This behavior can be observed also for POPC pre-added liposomes (data not shown) and it suggests a strong influence of the pre-added aggregates on the micelles-vesicles transformation: the matrix effect. To better elucidate this phenomenon the time evolution of the average hydrodynamic radius and the size distribution of vesicles before and after the micelles injection have been determined using photon correlation spectroscopy. It is worthwhile to mention that this technique is sensitive to large aggregates much more than small ones (Schurtemberger *et al.*, 1993).

In Figure 2 the case of pre-added OAVs is reported along with the control experiment. In the control experiment the average hydrodynamic radius increases up to large values and the size distribution results very broad (see Figure 3). Conversely while, when pre-added vesicles are present in solution, they grow in size by taking up the new surfactant molecules. The measured size distribution remains close to the initial one and no large aggregates are formed as in the control experiment (see for instance Figure 3). This can be observed also in the case of pre-added POPC
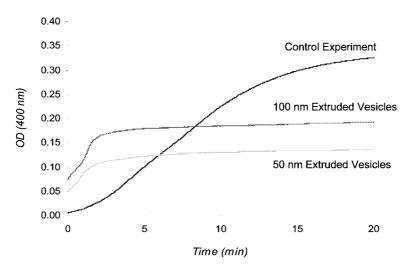
Figure 1. Effect of oleate/oleic acid vesicles on the transformation of oleate micelles: OD measured as a function of time at $25.0° \pm 0.1$: micelle added to buffer *(curve a)*, 100 nm (c*urve b)* and 50 nm *(curve c)* extruded oleic acid/oleate vesicles.

liposomes, but in this case the average hydrodynamic radius undergoes a small decrease, see Figure 4. Table I summarizes the initial and the final average radius of both the OAV and POL cases at different initial sizes.

Concerning now the meaning of the radius changes upon addition of fresh surfactant, the first question is whether they can be interpreted simply in terms of a growth size model or also in an increase of the aggregates concentration. In order to clarify this point, in the approximation of spherical aggregates and neglecting the bilayer thickness, the following formula can be derived:

$$[S] = \int_0^\infty [N]\frac{8\pi R^2}{a}P(R)dR = \frac{8\pi}{a}[N]\overline{R^2}. \tag{1}$$

This equation links the overall surfactant concentration $[S]$ to the overall aggregate concentration $[N]$, times the aggregate average squared radius $\overline{R^2} = (\overline{R}^2 + \sigma^2)$, being $P(R)$ the size distribution and a the average surface area of a surfactant molecule respectively. Therefore, a change in $[S]$ can produce an increase of the aggregate concentration and/or variations of the size distribution. Two indexes can be derived (see the Appendix) in terms of the squared average radius $\overline{R}^2$ and the variance σ^2, before and after the micelles addition:

$$f_R = \frac{1}{c}\left(\frac{\overline{R}^2 + \sigma^2}{\overline{R_0}^2 + \sigma_0^2} - 1\right) \qquad f_N = \frac{\overline{R_0}^2 + \sigma_0^2}{\overline{R}^2 + \sigma^2}(1 - f_R), \tag{2}$$

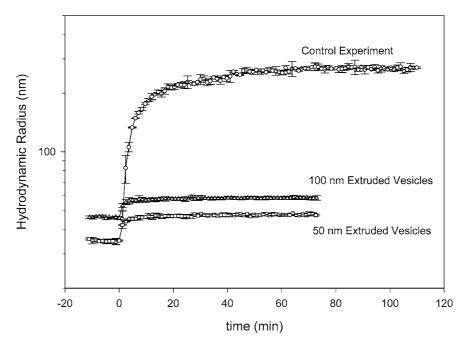where subscript 0 indicates values before the surfactant addition and $c = \Delta[S]/[S_0]$.

Figure 2. Influence of oleic acid/oleate vesicles on the transformation of oleate micelles: average hydrodynamic radius (logarithmic scale) as a function of time, determined by photon correlation spectroscopy cumulant analysis at 25.0° ± 0.1 C.
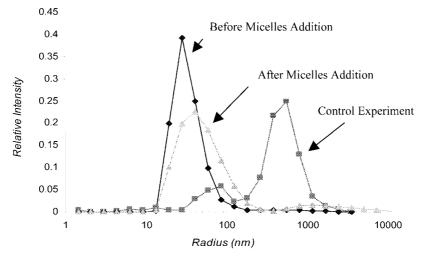

Figure 3. Influence of 50 extruded oleate/oleic acid vesicles on the transformation of oleate micelles: vesicle size distribution determined by photon correlation spectroscopy CONTIN method at 25.0° ± 0.1 C.

TABLE I

Average hydrodynamic radius before and after micelle addition and indexes of growth. Each radius value has been determined as the mean of at least 4 different measurements by Photon Correlation Spectroscopy: cumulant analysis. Indexes of growth are calculated by Equation (2)

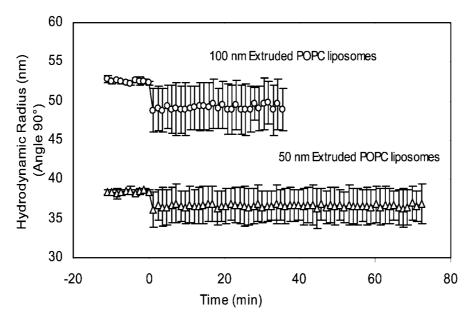| Solution	Filter pore Ø (nm)	Before micelle addition		1.5 h after micelle addition		f_N	f_R
		$\overline{R}$ (nm)	σ (nm)	$\overline{R}$ (nm)	σ (nm)		
Bicine Buffer	–	–	–	268 ± 9	239 ± 9	–	–
Oleic acid/oleate vesicles	50	35.0 ± 0.8	12.5 ± 2.0	47.7 ± 0.8	20.0 ± 4.0	0.03	0.94
	100	46.3 ± 0.8	15.0 ± 2.3	58.1 ± 1.0	22.3 ± 2.1	0.22	0.64
POPC liposomes	50	38.4 ± 0.2	6.6 ± 0.7	36.6 ± 2.0	10.2 ± 5.1	1.15	−0.10
	100	52.5 ± 0.2	10.9 ± 0.9	48.7 ± 3.0	13.7 ± 1.9	1.37	−0.22

Figure 4. Influence of POPC liposomes on the transformation of oleate micelles: average hydrodynamic radius as a function of time, determined by photon correlation spectroscopy cumulant analysis at $25.0° \pm 0.1$ C.

Two limit cases can be in principle defined: *the growth model* $f_N = 0$ and $f_R = 1$, i.e. no new aggregates are created and only the pre-added ones increase in size and/or in polydispersity, and *the template effect* $f_N = 1$ and $f_R = 0$, i.e. new aggregates are formed but the size distribution does not change. The previous formulae can be simply extended to the case of two different amphiphiles by defining $c = (a_2[S_2])/(a_1[S_1])$, where the subscripts indicate different molecules and a_i ($i = 1, 2$) is the surfactant head area. In Table I, both f_N and f_R are reported for all the studied systems. Note that, although the final surfactant concentration is doubled in both systems, OAVs and POLs behave in very different ways. In particular, the growth model can account for the 50 nm extruded vesicles results ($f_N = 0.03$ and $f_R = 0.94$), while, in the case 100 nm OAVs a size distribution change is not the only effect observed. In fact, here also a certain number of new vesicles are formed ($f_N = 0.22 > 0$). On the other hand, both 50 and 100 nm extruded POPC pre-added liposomes show a decrement in size parameter ($f_R < 0$) and the formation of new vesicles is the main result after the oleate micelles additions ($f_N > 1$).

The mechanism of the formation of new vesicles is not completely clear, but some important insights came out of the EM investigations with ferritin-labeled vesicles. It has been shown that mechanisms of vesicle fission are present when oleate is added to POPC liposomes (Berclaz *et al.*, 2001a, b) and this effect may

contribute to a shift of the average radius toward lower values-as observed in Table I.

4. Discussion

Although the mechanism of the process is not completely clear and it will be the subject of a forthcoming paper, it appears evident that the rapid uptake and growth model can account for the OAV system, specially in the case of smaller pre-added vesicles (50 nm). On the other hand, the decreased average radius observed in the case of POPC liposomes suggests that the rapid uptake of oleate molecules make the lipidic membrane more unstable. This can be due to the fact that the membrane, at the beginning globally neutral, becomes negatively charged by the adsorption of oleate molecules and this can stress the lipidic bilayer and then induce fission processes as already mentioned. Finally, we would like to underline that, the different behaviors may be due to different overall surfactant compositions. These data can also be seen as experimental implementations of the GARD model proposed by Lancet (Segré *et al.*, 1998).

Appendix

In this section the two indexes reported in Equation (2) will be derived. By differentiating Equation (1), it is possible to obtain:

$$d[S] = \frac{8\pi}{a}([N]d\overline{R^2} + \overline{R^2}d[N]).$$

This equation shows as a change in the surfactant concentration can determine a change in the aggregate concentration and at the same time in the size distribution. However, given a certain $\Delta[S]$, the maximum possible change in the size distribution $\Delta\overline{R^2}_{max}(d[N] = 0)$ and in the vesicles concentration $\Delta[N]_{max}$ $(d\overline{R^2} = 0)$ can be calculated, keeping in mind Equation (1):

$$\Delta\overline{R^2}_{max} = \frac{a}{8\pi}\frac{\Delta[S]}{[N_0]} = \frac{\Delta[S]}{[S_0]}\overline{R_0^2} = c\overline{R_0^2} \qquad \Delta[N]_{max} = \frac{a}{8\pi}\frac{\Delta[S]}{\overline{R_0^2}},$$

where 0 indicates initial values and $c = \Delta[S]/[S_0]$. Now defining the two searched indexes as the ratio of the observed variation divided by the maximum possible value:

$$f_R = \frac{\overline{R^2} - \overline{R_0^2}}{\Delta\overline{R^2}_{max}} \quad f_N = \frac{[N] - [N_0]}{\Delta[N]_{max}} = \frac{a}{8\pi}\left(\frac{[S_0] + \Delta[S]}{\overline{R^2}} - \frac{[S_0]}{\overline{R_0^2}}\right)\frac{1}{\Delta[N]_{max}}$$

formulae in Equation (2) can be easily derived remembering the relationship: $\overline{R^2} = \overline{R}^2 + \sigma^2$.

Finally, it is worthwhile to stress as an implicit assumption in the previous treatment is to neglect the amount of surfactant present in solution as monomers.

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