

Oxidative stress on liposomes with chlorophyll a monitored by spectral studies

M. BARBINTA-PATRASCU*, L. TUGULEA, A. MEGHEA^a, A. POPESCU

Faculty of Physics - University of Bucharest, Romania

^a*Faculty of Chemistry- University "POLITEHNICA" of Bucharest, Romania*

Chlorophyll a (Chla) incorporated into lipid bilayers is a suitable spectral marker for the liposomes characterization in oxidative stress studies. Liposomes with Chla have been obtained by the thin-film hydration method; small unilamellar liposomes (SUVs) were obtained from multilamellar vesicles. Different types of SUVs were obtained with and without vitamin E, using the lipids: β -stearoyl- γ -oleoyl L- α -phosphatidylcholine (SOPC), dimyristoyl phosphatidylcholine (DMPC), soybean lecithin, dissolved in chloroform together with Chla. Spectral criteria were developed in order to monitor the oxidative stress on liposomes with Chla.

(Received January 28, 2008; accepted February 7, 2008)

Keywords: Small unilamellar liposomes, Chlorophyll a (Chla), Vitamin E, Oxidative stress, Spectral criteria

1. Introduction

Liposomes are spherical self-closed vesicles that are made up of one or more curved lipid bilayers which enclose an aqueous volume. The value of liposomes, as membrane model systems, derives from the presence of the same bilayer structure of the lipid component as in the natural cell membranes [1].

The liposomes with Chla are useful cell membrane models for the oxidative stress studies and constitute an important tool to investigate the antioxidant capacity of different molecules [2]. Due to the spectral features (strong visible absorption and fluorescence), the Chla incorporated into the liposomal lipid bilayer was used as a sensor to monitor the oxidative effects on liposomes.

The present study was designed to estimate spectral criteria in order to monitor the oxidative stress on liposomes with Chla. Vitamin E (as α -tocopherol acetate) was used as antioxidant, to protect the lipid vesicles against oxidative damage.

2. Experimental

Reagents:

The lipids: β -stearoyl- γ -oleoyl L- α -phosphatidylcholine (SOPC), dimyristoyl phosphatidylcholine (DMPC) and soybean lecithin were purchased from Sigma Aldrich (Germany). α -tocopherol acetate (α -toc acetate), KH_2PO_4 , Na_2HPO_4 and the organic solvents of analytical purity (chloroform, ethanol, petroleum ether, methanol, n-propanol) were supplied from Merck (Germany). All solutions were prepared with bidistilled water.

Methods and applied procedures

Chla was obtained from fresh spinach leaves, using a chromatographic method on powdered sugar column and different organic solvents (petroleum ether, n-propanol and ethanol) as mobile phase, according to the procedure of Strain and Svec [3]. The purity of Chla samples was monitored by using spectral criteria in VIS range.

Liposomes preparation. The lipid model membranes were prepared using the thin-film hydration method [1]. The lipids were dissolved in chloroform together with Chla (Chla/lipid molar ratio = 1/100) and the solvent was evaporated using a rotary evaporator (BIOBLOCK SCIENTIFIC – Heildolph 94200, 60–90 rpm). The obtained lipid thin film was hydrated with a phosphate buffer solution (NaH_2PO_4 - K_2HPO_4), pH = 7.2. The resulted multilamellar vesicles were mechanically dispersed, using a VIBRAX stirrer (200 rpm) and then sonicated (BRANSON 1210), above the critical temperature (T_c) of phase transition of lipids, in order to obtain small unilamellar vesicles (SUVs). To improve the liposome stability, the α -toc acetate was incorporated into liposomal membrane during the lipid film preparation. Different types of lipid vesicles were obtained in the presence or the absence of this antioxidant.

All the absorption spectra were recorded on a PERKIN-ELMER LAMBDA 2S spectrophotometer (PECSS software).

3. Results and discussion

Both lipids and Chla are susceptible to oxidative degradation by different physical or chemical factors. The absorption spectra of liposomes containing Chla were recorded, in UV-VIS range, in order to monitor the Chla and lipid oxidation.

The light scattering of liposomes, having dimensions (diameters of tens to hundred of nm) close to the wavelengths in UV-VIS range, influence the absorption of liposome suspension in this range (being more important in the range of short wavelengths). The over-estimated absorbance values were corrected for the contribution of light scattering on the basis of the optical density (OD) measured at 325 nm, where the scattering is the sole contributor to the OD. The corrected values for the optical density (OD) at a given wavelength λ (in nm) were calculated using the expression [4]:

$$(OD_{\lambda})_{\text{corrected}} = (OD_{\lambda})_{\text{measured}} - (OD_{325})_{\text{measured}} \cdot (325/\lambda)^4 \quad (1)$$

Fig. 1 shows an example of the effect of correction, using this formula, upon the VIS absorption spectrum of liposomes containing Chla and vitamin E.

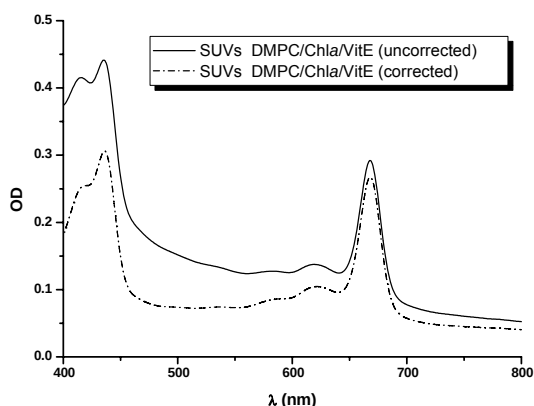


Fig. 1. The effect of correction against the contribution of light scattering.

The modifications in the lipid bilayers induced by factors like oxidation, ageing, were monitored by exploiting the spectral properties (strong absorption in VIS) of the Chla, used as a sensor. From our previous experience [5, 6], it is known the existence of a connection between lipid oxidation (i.e. liposome oxidative degradation) and Chla oxidation. The oxidation of the Chla results in a Soret band modification and a decrease of the red absorption. Fig. 2 illustrates the change of Chla spectrum in liposomes during the liposome ageing, along an interval of 22 days. During this long interval, the liposome suspension was kept at 4 °C and prior recording the spectrum the liposome suspension was sonicated for 20 minutes.

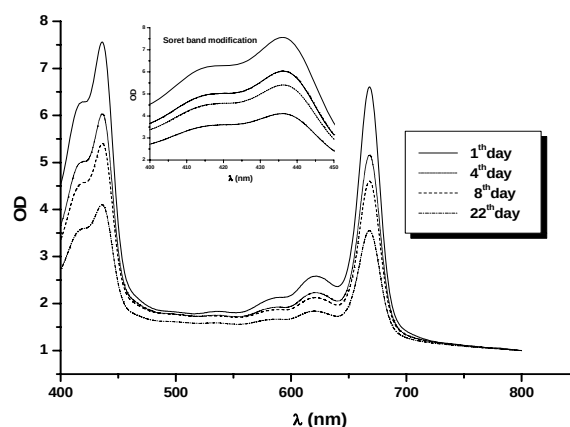


Fig. 2. Time course of VIS absorption spectra of DMPC SUVs (0.25 mM) containing vitamin E (1.014 mol % of phospholipids) and Chla (Chla/lipid molar ratio = 1/100) Inset: the Soret band modification (all spectra were corrected and normalized at 800 nm).

In order to monitor the oxidative stress on liposomes with Chla, by using the spectroscopic properties of Chla incorporated into lipid bilayer, the following parameters have been evaluated:

- *bleaching* %: $[(OD_0 - OD_i) / OD_0] \cdot 100$, where OD_0 is the absorbance at the main

red peak for the unsonicated liposomes and OD_i is the absorbance at the main red peak for the sonicated liposomes at different times.

- *the Soret band absorbance ratio*: $R = OD^{437nm} / OD^{419nm}$;
- *the relative variation of the absorbance ratio* % : $(\Delta R / R_0) \cdot 100$, where $R = OD^{437nm} / OD^{419nm}$, R_0 = the absorbance ratio in the first day; R_i = the absorbance ratio at different moments; $\Delta R = R_0 - R_i$

The degradation by oxidation of Chla, incorporated in the lipid bilayer, is conducting to the increase of the *bleaching* value and to the decrease of the *Soret band absorbance ratio*.

The time course of the *bleaching* parameter for two different lipids used for liposomes preparation is presented in Fig. 3. The values of the *bleaching* parameter are lower in the case of Chla-vitamin E-SUVs as compared with the Chla - SUVs. This finding suggests that vitamin E - containing bilayers are more stable in time.

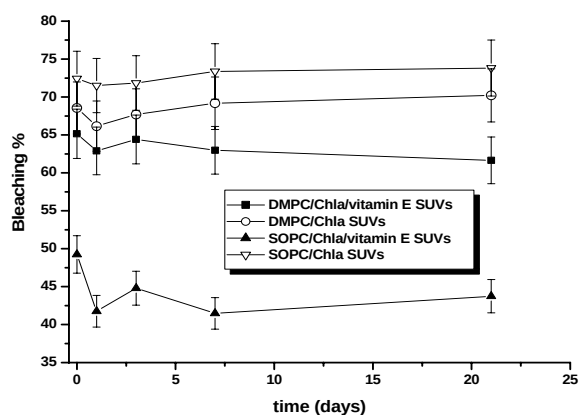


Fig. 3. The bleaching values of the DMPC (0.25 mM) and SOPC (0.25 mM) Chla- liposomes with and without vitamin E (1.014 mol % of phospholipids final concentration).

The oxidative damage of Chla-liposomes is related to the Chla oxidation which can be monitored also by the spectral modification in the Soret band, namely the decrease of the absorbance ratio $OD^{438\text{ nm}}/OD^{418\text{ nm}}$.

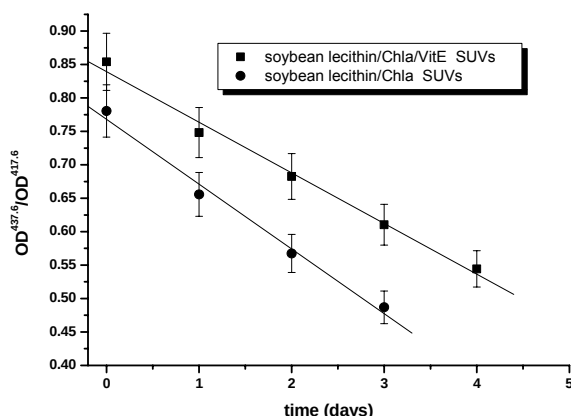


Fig. 4. The Soret band absorbance ratio of the soybean lecithin (0.5 mM)/ Chla SUVs (correlation coefficient $r = 0.99438$) and the soybean lecithin (0.5 mM)/Chla/vitamin E (0.7 mol % of phospholipid final concentration) SUVs (correlation coefficient $r = 0.99529$).

As it is seen in the Fig. 4, the values of the absorbance ratio $OD^{437.6\text{ nm}}/OD^{417.6\text{ nm}}$ for the vitamin E - bilayers are higher than in the case of Chla-liposomes without vitamin E, this fact suggesting the protective effect of vitamin E (as α -toc – acetate) incorporation in the liposomal bilayer.

The increase of the parameter: *relative variation of the absorbance ratio* indicates also a degradation of the Chla incorporated into liposomes. The values of this parameter are higher in the case of Chla-SUVs as compared with the Chla-vitamin E-SUVs, irrespective of the type of lipid (see Fig. 5 and 6).

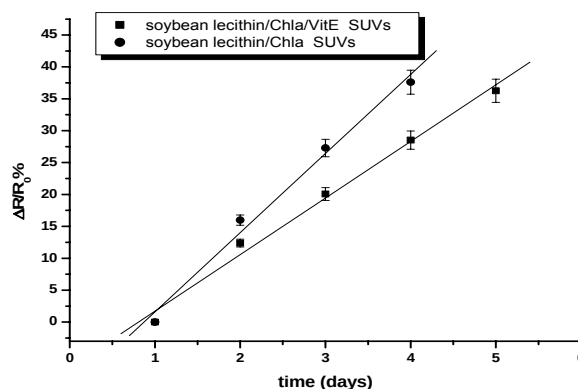


Fig. 5. The time course of the the relative variation of the absorbance ratio % for the Chla- soybean lecithin (0.5 mM) liposomes (correlation coefficient $r = 0.99438$) and Chla- vitamin E (0.7 mol % of phospholipids) - soybean lecithin (0.5 mM) liposomes (correlation coefficient $r = 0.99529$).

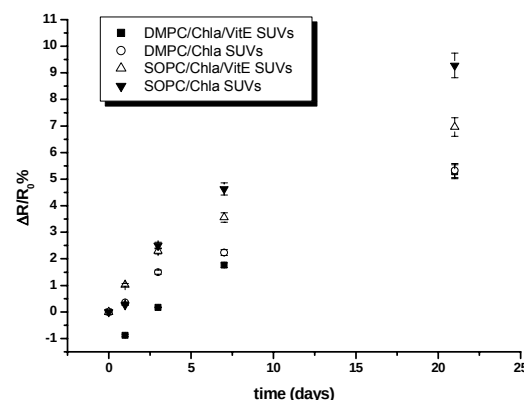


Fig. 6. The time course of the the relative variation of the absorbance ratio % for the DMPC (0.25 mM) and SOPC (0.25 mM) Chla- liposomes with and without vitamin E (1.014 mol % of phospholipids final concentration).

The time course of the *relative variation of the absorbance ratio* suggests that the vitamin E (as α -tocopherol) acetate – containing bilayers are more stable in time. The antioxidant effect of vitamin E is more evident for SOPC liposomes, as compared with DMPC liposomes (Fig. 6). The values of this spectral parameter are higher for SOPC liposomes than in the case of DMPC liposomes and the effect of vitamin E presence results in significant differences for aged liposomes (22 days after preparation). The SOPC system contains unsaturated fatty acids which are more susceptible to oxidation than the DMPC system, which contains only saturated compounds. This difference in fatty acid composition could be a plausible explanation for the obtained experimental results.

4. Conclusions

The spectral properties of Chla incorporated into liposomal lipid bilayer were used to monitor the oxidative

effects on liposomes. Several spectral criteria were developed to monitor the oxidative degradation of these membrane models.

The experiments revealed that Chla is an adequate sensor for liposomes systems characterization and for oxidative stress studies.

All of the evaluated parameters indicate a higher oxidative damage during a time interval of the order of days, in the case of liposomes without vitamin E.

References

- [1] R. R. C. New, *Liposomes: A practical approach*. New, R. R. C. (ed.), IRL press, Oxford University, 1990.
- [2] M. E. Barbinta-Patrascu, M. Voinea, L. Tugulea, *Bucharest University Annals, Physics, LII*, 35-42 (2003).
- [3] H. H. Strain, W. A. Svec: *The Chlorophylls* (Vernon L. P. and Seely G. R., eds.) pp. 21-26, Academic Press, New York, USA, 1966.
- [4] O. Bittner, S. Gal, I. Pinchuk, D. Danino, H. Shinar, D. Lichtenberg, *Chemistry and Physics of Lipids*, **114**, 81-98 (2002).
- [5] L. Tugulea, S. Pascanu, *Romanian J. Biophys.* **6**(1-2), 1-10 (1996).
- [6] L. Tugulea, I. M. Iacovache, W. Mejbaum-Katzenellenbogen's *Molecular Biology Seminars Liposomes, From Models to Applications*, Wrocław/Piechowice by Szklarska Poręba, Poland, May 26-29, 2002, *Proceedings in Cell. Mol. Biol. Lett.* **7** (2): 302 (2002).

*Corresponding author: p_mareli@yahoo.com