

THE POLYMERIZATION OF α -ELEOSTEARIC ACID IN THE DIPALMITOYLPHOSPHATIDYLCHOLINE- α -ELEOSTEARIC ACID LIPOSOME*

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ABSTRACT

The polymerization of α -eleostearic acid (α -ESA) in the dipalmitoylphosphatidylcholine (DPPC) - α -ESA liposome was studied. The polymerization was carried out by UV irradiation and was kinetic first order. The rate constant depends on the ratio of DPPC to α -ESA in the liposome. A transition point of reaction rate could be observed in the course of the polymerization, and it appeared earlier with elevated temperature. Before the transition point, the higher the DPPC to α -ESA ratio the higher was the reaction rate; while after the transition point, the higher the DPPC to α -ESA ratio the lower was the reaction rate.

EXPERIMENTAL

Materials and methods

Dipalmitoylphosphatidylcholine (DPPC) was from Sigma Chemical Co., α -Eleostearic acid (α -ESA) was prepared from Chinese Tung oil. Distilled water was deionized. All reagents used were reagent grade.

Preparation of liposome: Liposome was prepared by rapid injection method developed by Batzri^[6]. Typically, a mixture of 70 μ l 50 mM. DPPC in ethanol and 10 μ l 50 mM. α -ESA in ethanol was rapidly injected into two 2 ml deoxygenized H₂O at 50°C under vigorously whirling. After injection, the dispersion was whirled for 3 minutes. Liposome formed had a composition of DPPC: α -ESA = 7:1 (M/M). Liposomes with other compositions were prepared by the same procedure.

Electron micrographs were taken on a JEOM JEM-200CX electron microscope. Liposomes were negatively stained by an equal volume of 1% ammonium molybdate just before the observation.

Polymerization: Polymerizations were carried out by UV irradiation. The UV source was a 25 W low pressure mercury lamp having a peak intensity at 254 nm. Liposomes were irradiated in N₂ atmosphere by UV through a quartz window on the polymerization cell. Irradiated liposomes were dissolved in ethanol for spectrum determinations. The spectra were taken on a Specord UV VIS spectrophotometer.

Received January 9, 1986

* Project supported by the Science Fund of the Chinese Academy of Sciences.

RESULTS AND DISCUSSION

1. *DPPC- α -ESA liposome*

Fig. 1 showed the electron micrograph of DPPC: α -ESA=1:1 (*M/M*) liposome prepared by injection method. The sample was negatively stained by an equal volume of 1% ammonium molybdate. Electron micrographs showed that the prepared DPPC- α -ESA liposomes were unilamellar vesicles.

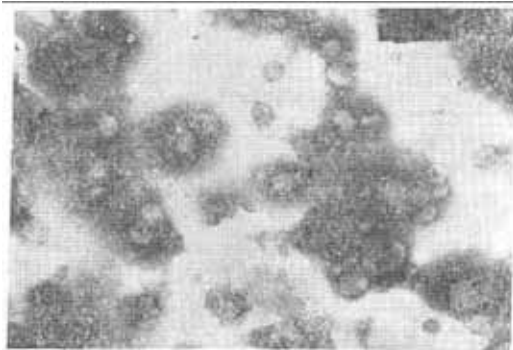


Fig. 1 Electron micrograph of DPPC- α -ESA liposome DPPC: α -ESA=1:1 (*M/M*). Sample was negatively stained with an equal volume of 1% ammonium molybdate (20,000 $\times$).

2. *The change of the spectra of DPPC- α -ESA liposomes after UV irradiation*

Fig. 2Aa is the spectrum of DPPC: α -ESA=7:1 (*M/M*) liposome before UV irradiation. It is almost the same as the spectrum of the ethanol solution of α -ESA. The existence of DPPC had no effect on the spectrum of α -ESA because DPPC has much smaller extinction coefficient in this region. In order to avoid the effect of the light scattering caused by the

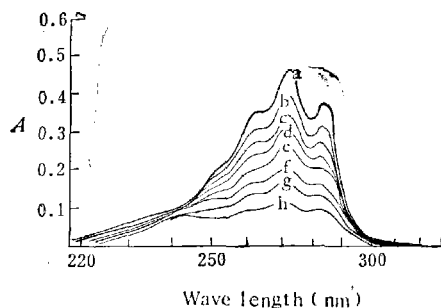


Fig. 2A Spectra of DPPC- α -ESA liposomes dissolved in ethanol after different UV irradiation time DPPC: α -ESA=7:1 (*M/M*). Liposome was prepared by injection. UV irradiation time (in min): a) 0; b) 2; c) 5; d) 8; e) 10; f) 15; g) 20 and h) 30. Polymerization temperature: 14°C.

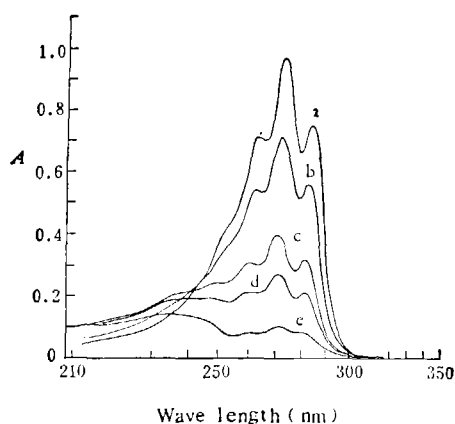


Fig. 2B Spectra of DPPC- α -ESA liposomes dissolved in ethanol after UV irradiations
 DPPC: α -ESA=1:3 (*M/M*).
 Liposome was prepared by injection.
 UV irradiation time (in min.):
 a) 0; b) 8; c) 20; d) 25 and e) 40.
 Polymerization temperature: 14°C.

liposome particles and prevent the additional polymerization of α -ESA caused by room light before the spectrum determination, liposome samples were injected into ethanol immediately so that the lipid aggregates were destroyed and solutions were formed. All spectra given below were the spectra of the ethanol solutions of the liposomes.

Fig. 2A showed the spectra of DPPC: α -ESA = 7:1 (*M/M*) liposomes irradiated for various times. The decrease of the intensity of the spectrum with the irradiation time indicated that the α -ESA polymerized easily in liposome during the UV irradiation period. When the intensities of the characteristic absorptions of the three conjugated double bonds at 262 nm, 272 nm and 282 nm reduced, the characteristic absorption of two conjugated double bonds at 233 nm appeared. This process could be seen more clearly from the DPPC: α -ESA = 1:3 (*M/M*) liposome in Fig. 2B.

3. The rates of the polymerizations

The relation between the absorbance (*A*) and the irradiation time (*T*) of the UV polymerization of α -ESA in DPPC: α -ESA = 3:1 (*M/M*) liposome was showed in Fig. 3A. The log *A*-*T* plotting of this relation was a straight line (Fig. 3B). This indicated that the polymerization was a first order reaction. The rate constant calculated from the log *A*-*T* line was $3.2 \times 10^{-4} \text{ s}^{-1}$. For the liposome with higher α -ESA concentration, for example DPPC: α -ESA = 5:7 (*M/M*) liposome, a rate transition could be seen during the polymerization (Fig. 4A). The log *A*-*T* plotting for this system gave a broken line (Fig. 4B). It means that the polymerization process involved two stages, and both reactions were first order. The reaction rate was higher in the second stage than in the first one. For the liposomes with lower α -ESA concentrations, for example the DPPC: α -ESA = 7:1 (*M/M*) liposome, a rate transition was also found (Fig. 5A and 5B). The difference between the DPPC: α -ESA =

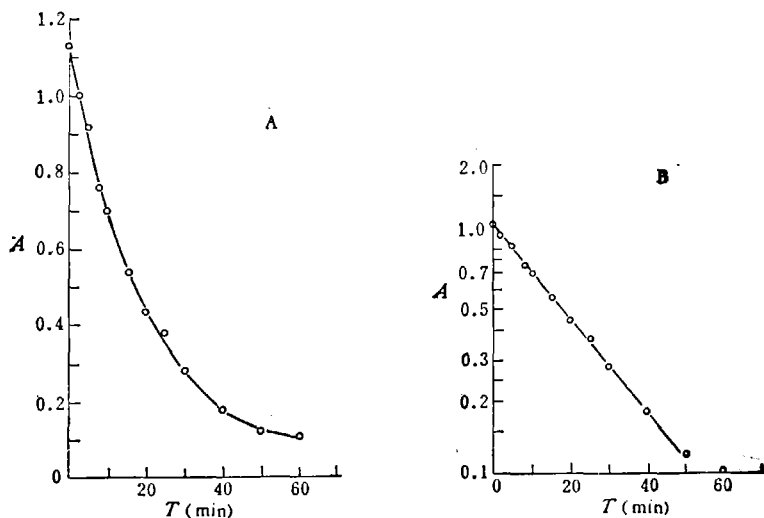


Fig. 3 Relation between absorbances of α -ESA in liposome and UV irradiation times for DPPC: α -ESA=3:1 (M/M) liposome
Polymerization temperature: 14°C.
A) A - T relation; B) Log A - T relation.

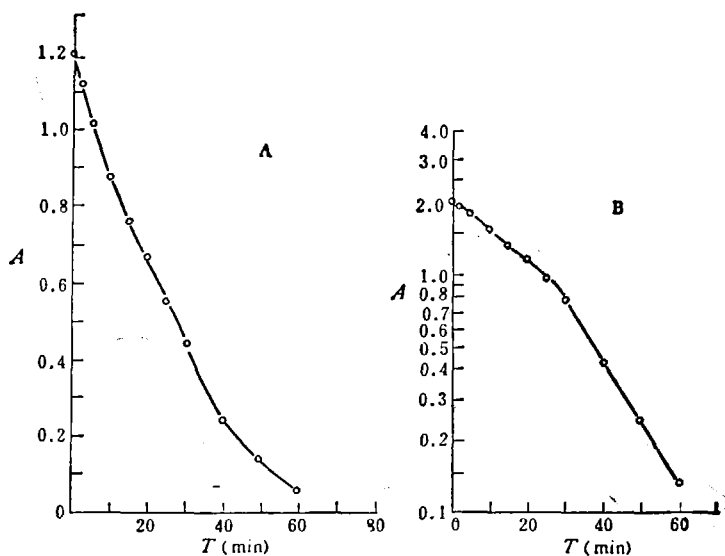


Fig. 4 Relation between absorbance of α -ESA in liposome and UV irradiation times for DPPC: α -ESA=5:7 (M/M) liposome
Polymerization temperature: 14°C.
A) A - T relation; B) Log A - T relation.

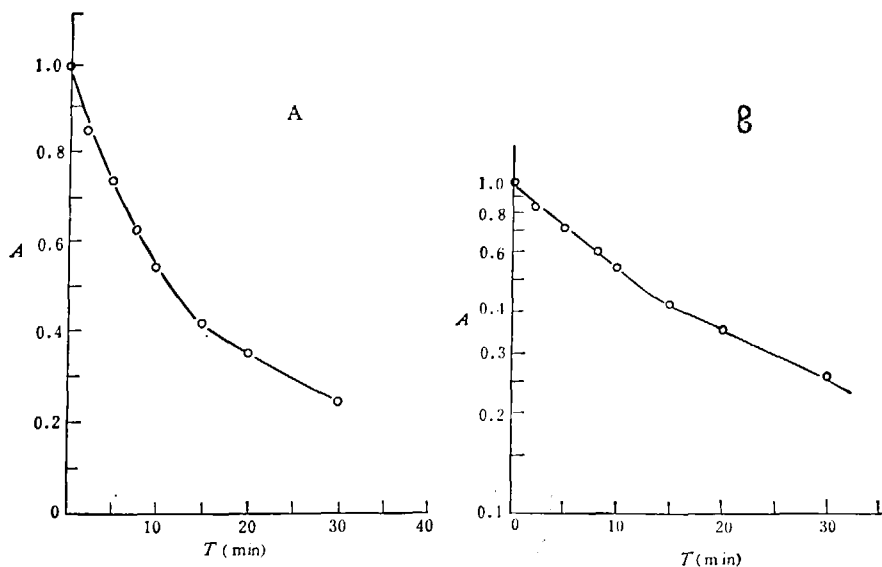


Fig. 5 Relation between absorbance of α -ESA in liposome and UV irradiation times for DPPC: α -ESA=7:1 (M/M) liposome

Polymerization temperature: 14°C.

A) A-T relation; B) Log A-T relation.

Table 1 Rate constants of polymerizations of α -ESA in liposomes by UV irradiation

Liposome compositions DPPC: α -ESA (M/M)	k_1 (s ⁻¹)	k_2 (s ⁻¹)
7:1	3.9×10^{-4}	2.8×10^{-4}
3:1	3.2×10^{-4}	3.2×10^{-4}
5:7	2.2×10^{-4}	4.3×10^{-4}

Polymerization temperature: 14°C.

k_1 : rate constant before rate transition.

k_2 : rate constant after rate transition.

5:7 (M/M) liposome and the DPPC: α -ESA = 7:1 (M/M) liposome was that the latter had a lower polymerization rate after the rate transition. The rate constants for the different liposomes were shown in Table 1. The data listed demonstrated that the higher the ratio of DPPC to α -ESA the larger was the rate constant in the first stage of the polymerization, whereas in the second polymerization stage, the higher the ratio of DPPC to α -ESA the smaller was the rate constant.

4. The effects of temperature on the polymerization of α -ESA in liposome

Polymerization temperature showed little effect on the polymerization rate of α -ESA in the DPPC: α -ESA = 7:1 (M/M) liposome in the first stage of the polymerization (Fig. 6). In the case of DPPC: α -ESA = 5:7 (M/M) liposome, polymerization temperature had a small

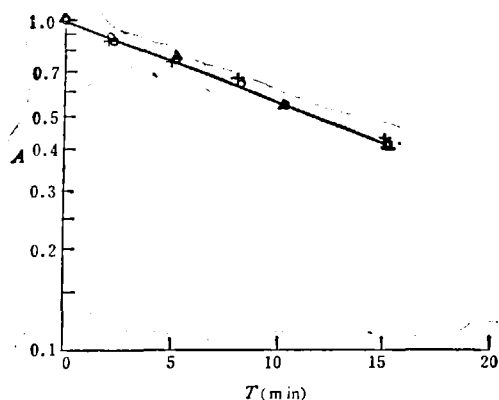


Fig. 6 Log A - T relations of α -ESA polymerizations in DPPC:
 α -ESA=7:1 (M/M) liposome at different temperatures
 + 14°C; Δ 25°C; o 35°C; • 45°C.

effect on the reaction rates. The increase of the temperature caused a little increase of α -ESA polymerization rate at the first stage, but a little decrease at the second stage (Fig. 7). Polymerization temperature had a significant effect on the transition point of the rate transition. The higher the polymerization temperature the earlier appeared the rate transition.

The presented results demonstrated that the UV irradiation polymerizations of α -ESA in liposomes had the behaviour of the normal photopolymerizations. The polymerization was kinetic first order and the rate constant only slightly depended on the polymerization

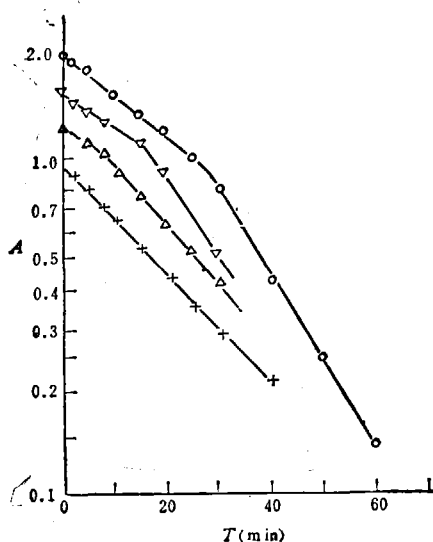


Fig. 7 Log A - T relations of α -ESA polymerizations in DPPC:
 α -ESA=5:7 (M/M) liposome at different temperatures
 o 14°C; ∇ 35°C; Δ 45°C; x 50°C.

temperature. On the other hand, the UV polymerizations in liposomes had their own characteristics. In these polymerizations, a rate transition could be observed, and the transition point had temperature dependence. The rate constant depended on the liposome composition.

Liposome is a liquid crystal system. Polymerizations carried out in such system should have special features. To understand the mechanism of this new kind of polymerizations more work are needed. From the results shown above, it is reasonable to suppose that the phase transition of the liposome may play an important role in this polymerization. The DSC study on DPPC liposome showed that the phase transition of this liposome, which appeared at 34.5°C and 41.6°C, were sensitive to the ab extra molecules. The polymerization of α -ESA may cause phase transition of DPPC- α -ESA liposome and phase transition may cause the change of the polymerization rate. The phase transition temperature varies with the polymerization extent, so that the transition point of the polymerization rate may vary with the polymerization temperature.

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