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Study on the Aggregation and Deaggregation Behaviors of Phosphatidylcholines†

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Phosphatidylcholines and their analogs, the functional building block of the membrane, are recently found to mediate multiple physiological processes and exhibit a broad range of desirable pharmacological effects, which involve hydrophobic lipophilic interactions (HLI) between the phospholipid and the cell membrane. The HLI behavior of phosphatidylcholines (L*n*) and their analogues 1, 2-diacyl-*sn*-glycerol-3-phosphoric acid bromoethyl ester (P*n*), have been investigated in MeOH-H_2O binary systems of different volume fractions (designated as Φ) of the organic component, by employing α-nephthylethyl lauryl ether (Np-12) as fluorescent probe. A very interesting observation is that the L*n* possesses double character, *i.e.*, it behaves both as an aggregator and as a deaggregator. The effects of the structure and the environment on the coaggregation and deaggregation are also discussed.

Keywords phosphatidylcholine, hydrophobic lipophilic interactions (HLI), aggregation, deaggregation

Introduction

The growing interest in the activity-structure relationship of phospholipid is based on its diverse biological activities. More and more studies reveal that the phospholipid and their analogs exhibit a broad range of desirable pharmacological effects and represent a new class of membrane-directed antitumor,[1] antiviral[2] and antifungal[3] agents. Although the exact mechanism(s) for these activities is not well understood, these agents are membrane-interactive and may function by fluidization of cell membranes.[4] It is well known that phospholipid is the building block of cellular membranes in virtually all living organisms, and it is the amphiphilic structures of phospholipid that determine their biological roles and the function of membrane.[3,5] Therefore studies on the hydrophobic interactions of phospholipids and their molecular assembly behavior might provide some insights into the mechanism of their membrane-directing activities.

On the other hand, the phenomenon of aggregation is a prerequisite for the development of life and the ability of cell to function.[5-7] We have made intensive study on aggregation, coaggregation and self-coiling of organic molecules brought by hydrophobic lipophilic interactions (abbreviated as HLI),[7] especially for neutral molecules[7a] and molecules carrying a unit positive or negative charge.[8] It has been recently found that the inherent coaggregating tendencies of organic molecules, *e.g.*, cholesteryl esters and triglycerides, might be directly related to their behaviors in causing atherosclerosis.[7,9] For instances, coaggregating tendencies of cholesteryl oleate and linoleate have been measured to be much larger than that of the stereate,[9] which probably provides a reasonable answer to the question why cholesteryl oleate and linoleate are the major components of the arterial plaque.[10] Since the atherosclerotic plaque might be looked upon as a precipitated coaggregate, an extremely effective yet nontoxic deaggregaor (deAgr.), which tends to break up simple aggregates, might turn out to be useful in preventing or even curing atherosclerosis. Some effective neutral deaggregators, *e.g.*, *n*-dedocyl-α-*D*-glucopyranoside, 17,21-dioxa-19-heptatriacontanyl-β-maltoside, have recently been reported by our group.[11] What would be the aggregation behavior of phospholipid, one of the main components of plasma lipoproteins and traditional treating agents for the atherosclerosis?[12] It would be interesting to see if these amphiphilic molecules with zwitterions possess some novel or unexpected properties.

Hence we are intrigued to investigate the HLI of phosphatidylcholine, the most abundant phospholipid, and their analogs. In this paper, we report our study on the coaggregating tendencies and deaggregating abilities of the phosphatidylcholine (L*n*s, *n* stands the number of carbon atoms in the side chain) and their analogs 1,2-diacyl-*sn*-glycerol-3-phosphoric acid bromoethyl ester (P*n*s) in MeOH-H_2O binary systems of different Φ values, *i.e.*, the volume fraction of the organic component of the aquiorgano mixture, by employing nephthylethyl lauryl ether (Np-12) as the fluorescent probe.

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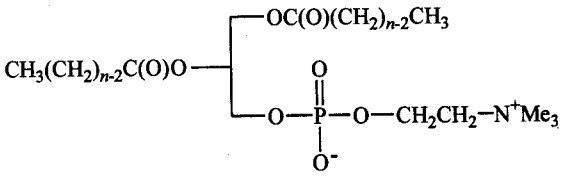
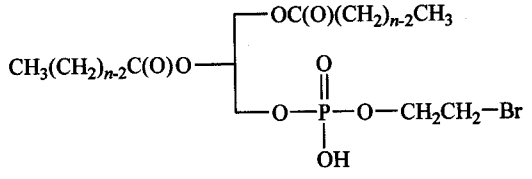


Ln (n = 8, 12, 16)



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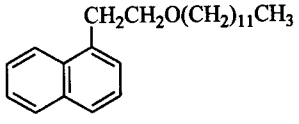


Np-12

Results and discussion

Formation of aggregate in aqueous or aquiorgano solvents from organic molecules with a long hydrocarbon chain is almost solely driven by HLI; thus aggregates may serve as one of the simplest models for such studies.

Aggregation can affect the chemical reactivity or the spectroscopic behavior of the studied molecules (*e.g.*, kinetic or fluorescent probes).[13,14] Critical aggregate concentration (CAgC), *i.e.*, the molar concentration (a narrow region) of an organic species (a substrate or probe) at onset of aggregation, is one of the most useful and powerful concepts for studying and understanding intermolecular aggregation. It is probably the most reliable quantitative measure for evaluating the aggregating tendencies of the aggregators (Agr).[7] The methodology for evaluating CAgC values from log k (rate constants of hydrolysis) or the I_e/I_m (the ratio of the excimer and monomer fluorescence emission intensity) vs. initial probe concentration plots has been well established and thoroughly tested in recent years.[9,15,16] The results of fluorescence experiments are very consistent with the kinetic measurements.

The solvent aggregating power (SAgP) is an important factor which affects the aggregating tendency of the organic molecules, and it has been well proved that the Φ values are the direct measure of SAgP.[7a] Two solvent systems with different SAgP are selected in our present research, *i.e.*, MeOH-H_2O with Φ = 0.45 and Φ = 0.50 at 25 ℃.

Fluorescent studies on coaggregation

Fluorescence spectroscopy is a useful tool for evaluating the coaggregating tendencies of organic molecules which possess fluorophores. In an aggregating media, aggregation of long chain fluorescent probe molecules may bring the chromophores close enough to form excimers.[14] In this work, the naphthylethyl lauryl ether (Np-12) was chosen as the fluorescent probe. The aggregation behavior of Np-12 in MeOH-H_2O system was first investigated in this work. Fig. 1 shows the concentration effect of Np-12 on its fluorescence spectra in the Φ = 0.45 MeOH-H_2O system at 25 ℃ and a plot of I_e/I_m vs. [Np-12] is given in Fig. 2, where I_m and I_e represent the fluorescence emission intensity of the Np-12 monomer and excimer respectively. The CAgC of Np-12 is obtained from the breaking point of the plot of I_e/I_m vs. [Np-12], *i.e.*, 2.0×10^{-6}mol/L under this condition. It is generally accepted that below CAgC, the Agr exists in its monomeric form. In this region, its fluorescent behavior is independent of its initial concentration. But in the presence of a target molecules, *e.g.*, L*n*s or P*n*s, which possess a long hydrocarbon chain, the fluorescent behavior of the probe will be changed rather abruptly just at the coaggregation occurring as the concentration of target molecules species becomes large enough. The critical coaggregate

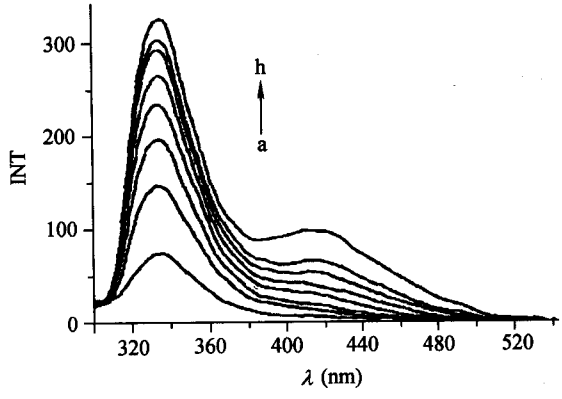


Fig. 1 Fluorescence spectra of Np-12 at different concentrations in the Φ = 0.45 MeOH-H_2O system at 25 ℃, λ_{ex} = 280 nm, [Np-12] (10^{-6} mol/L): a, 0.5; b, 1.0; c, 1.5; d, 2.0; e, 2.5; f, 3.0; g, 3.5; h, 4.5.

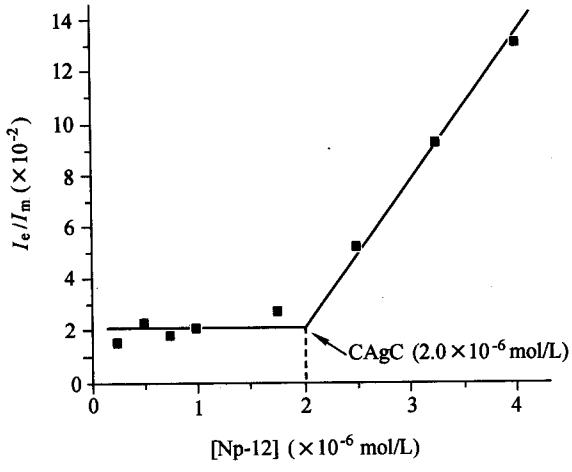


Fig. 2 Plot of I_e/I_m vs. [Np-12] in the Φ = 0.45 MeOH-H_2O system at 25 ℃. Values of I_e/I_m are given in Fig. 1.

concentration (CoCAgC) values of the target molecule with the probe can be obtained by using the plots abovementioned, and it is used to evaluate the coaggregating tendency of the target molecules in the present work.

In the study of coaggregation of target molecules with Np-12, the concentration of Np-12 was kept lower than its CAgC value so that the Np-12 molecules were in the monomeric state. The coaggregating abilities of L*n*s and P*n*s with the monomeric Np-12 were studied in the Φ = 0.45 MeOH-H_2O system at 25 ℃ and shown in Figs. 3 and 4 for L16 and P16, respectively. When Np-12 molecules exist in the monomeric form just by itself, it gives only monomeric emission at 337 nm, but when the Np-12 concentration increases or when L*n*s or P*n*s are added untill aggregation or coaggregation occurs, the intensity of the monomeric emission decreases while the excimer emission at 420 nm appears and increases (Figs. 3 and 4). From the plots of I_e/I_m vs. [L*n*s] or [P*n*s], the coaggregating ability of target molecules could be evaluated in terms of CoCAgC values. Since plotting CoCAgC values vs. [Np-12] gave a good straight line, the CAgC values of L*n*s or P*n*s themselves could be obtained by means of extrapolation to [Np-12] = 0.[15] The results are summarized in Table 1.

As shown in Table 1, both L*n*s and P*n*s can form coaggregates with monomeric Np-12. The orders of decreasing coaggregating tendencies are: L12 > L16 > L8, P12 ~ P16 > P8, P12 > L12, P16 > L16.

The results of fluorescence experiments can be understood in terms of the following effects, *i.e.*, chain-length effect[16] and chain-foldability effect.[7b,9] In general, the organic molecule with longer hydrocarbon chain possesses larger coaggregating tendency, however, the hydrocarbon chain with more than 10—12 CH_2 moieties tends to fold on itself so that its HLI could be partially self-satisfied, thus L16 has less coaggregating tendency than that of the shorter L12. Furthermore, the probe molecule Np-12 possesses a 12-carbon chain, and if there was a "chain-length matching effect", it might possibly lead to preferential coaggregation with the same chain-length molecules, *e.g.*, L12 or P12. The difference of coaggregating tendencies between L*n*s and P*n*s with the same chain length clearly indicates that the zwitterionic charges of L*n*s reduce the coaggregating tendency.

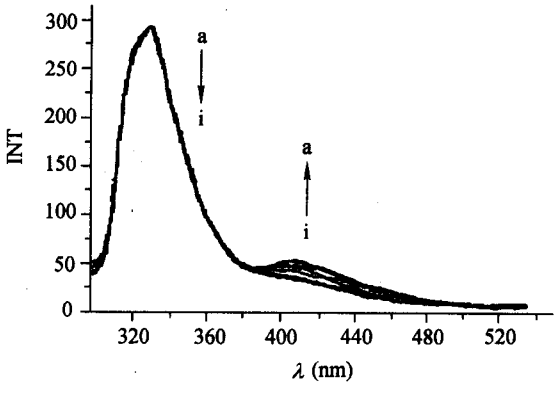


Fig. 3 Effect of L16 concentration on the fluorescence spectra of monomeric Np-12 in the Φ = 0.45 MeOH-H_2O system at 25 ℃, λ_{ex} = 280 nm, [Np-12] = 1.2×10^{-6} mol/L, [L16] (10^{-6} mol/L): a, 0; b, 0.6; c, 1.2; d, 1.8; e, 3.0; f, 4.2; g, 5.4; h, 7.2; i, 9.6.

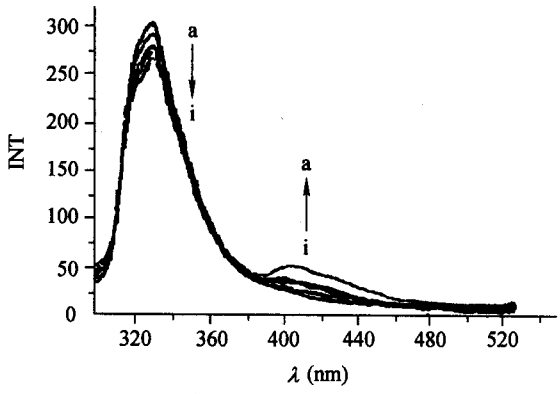


Fig. 4 Effect of P16 concentration on the fluorescence spectra of monomeric Np-12 in the Φ = 0.45 MeOH-H_2O system at 25 ℃, λ_{ex} = 280 nm, [Np-12] = 1.0×10^{-6} mol/L, [P16] (10^{-6} mol/L): a, 0; b, 0.4; c, 0.8; d, 1.2; e, 1.6; f, 2.5; g, 4.0; h, 8.0; i, 15.0.

Table 1 CoCAgC and CAgC values of L*n*s and P*n*s at constant concentration of Np-1[a]

[Np-12] (10^{-6} mol/L)	CoCAgC (10^{-6} mol/L)[c]					
	L8	L12	L16	P8	P12	P16
0.0[b]	11.1	2.95	8.05	41.0	1.13	1.36
0.4	8.40	2.35	6.45			
0.5					1.0	1.1
0.8	6.90	1.70	4.50		1.1	0.75
1.0					1.1	0.75
1.2	3.60	1.66	2.55			
1.4				13.0		
1.5					0.68	0.50
1.6	1.80	0.57	1.30	9.0		
1.8				5.0		
1.9					0.5	0.3

[a] In Φ = 0.45 MeOH-H_2O system at 25 ℃. [b] CAgC values of L*n*s or P*n*s are obtained by extrapolation to [Np-12] = 0.13. [c] Experimental uncertainty ±5%.

In order to investigate the effect of SAgP on the aggregating tendencies of L*n*s, we have studied the coaggregating between Np-12 and L*n*s in a system with weaker SAgP, *i.e.*, MeOH-H_2O system with Φ = 0.50 at 25 ℃. The concentration of L8, L12 and L16 were kept at constant, *i.e.*, 9.0×10^{-6} mol/L, and we measured the CoCAgC values of Np-12 with L*n*s of different chain length by plotting I_e/I_m vs. [Np-12]. As shown in Table 2, in the MeOH-H_2O system with Φ = 0.50 at 25 ℃, the CoCAgC values of Np-12 with L*n*s of fixed concentration are all larger than the CAgC value of Np-12, showing that the L*n*s actually retards the aggregation of the Np-12 mole-

Table 2 CAgC and CoCAgC values of Np-12 at constant concentration of L*n*s[a]

[L*n*] (10^{-6} mol/L)	I_e/I_m [Np-12] (10^{-6} mol/L)												CoAgC of Np-12 (10^{-6} mol/L)[c]
	1.0	2.0	3.0	3.5	5.0	7.0	9.0	11.0	12.0	13.0	14.0	15.0	
[L8] = 9.0	0.035	0.351		0.041	0.044	0.078	0.140	0.195			0.282		5.48
[L12] = 9.0	0.034	0.029		0.033	0.044	0.078	0.135		0.225			0.304	5.55
[L16] = 9.0	0.055	0.045		0.041	0.058	0.090	0.150		0.236			0.315	5.70
[L*n*] = 0	0.06		0.05		0.07	0.12	0.17	0.23		0.28		0.330	5.00[b]

[a] In $\Phi = 0.50$ MeOH-H_2O system at 25 ℃. [b] CAgC values of Np-12. [c] Experimental uncertainty ±4%.

cules. In other words, the L*n*s exhibited the character of the deaggregator

Previously we have proposed and demonstrated the existence of the electrostatically stabilized aggregate (ESAg)[8] made up of oppositely charged long-chain molecules with 8—12 CH_2 groups in the concentration domain of 10^{-7}—10^{-5} mol/L. The question now is, since L*n*s bear zwitterionic charges, is there any such ESAg species formed when the probe is electrically charged? In order to answer this question, ω-[2-(α-naphthyl)-ethoxyl]-decyltrimethylammonium bromide (FP^+) and sodium ω-[2-(α-naphthyl) ethoxyl]-undecanoate (FP^-) were employed as cationic and anionic fluorescent probes respectively. We studied the effect of L*n*s on the fluorescent spectra of FP^+ and FP^-. No coaggregation was observed within the limit of L*n*s solubility and instrument detectability. This indicates that the L*n*s behave as neutral molecules even though they are zwitterionic in nature.

Fluorescent studies on deaggregation

In previous section we have demonstrated that the phospholipids and their analogues (L*n*s and P*n*s) are aggregators which can coaggregate with the monomeric probe molecules that possess a long hydrocarbon chain, meanwhile we observed that L*n*s can reduce (retard) the formation of Np-12 aggregate in a system with weaker SAgP. Since L*n*s and their analogues P*n*s either bear the electric charge or the hydrophilic hydroxyl group, they might act as deaggregators, which can break up or reduce the size of the aggregate or the coaggregate. Thus the interaction between L*n*s and P*n*s of different chain lengths with an aggregate were investigated by means of fluorescence spectroscopy.

As mentioned previously, the CAgC values for NP-12 as a fluorescent probe is 2.0×10^{-6} mol/L, in the Φ = 0.45 MeOH-H_2O system at 25 ℃. Therefore, the concentration of Np-12 was chosen to be larger than its CAgC values, *i.e.*, the NP-12 molecules in this system were in aggregated form, in order to study the deaggregating abilities of L*n*s and P*n*s.

Fig. 5 illustrates the fluorescence spectra of partly aggregated Np-12 ([Np-12] = 1.5×10^{-5} mol/L) in the absence and presence of graded concentrations of L16 in the Φ = 0.45 MeOH-H_2O system at 25 ℃. When L16 was added to the aggregated Np-12 solution, the excimer emission decreases and the monomer emission increases. These results indicate that the aggregated Np-12 has been broken up by the added L16, which acts as a deaggrgator, and monomeric Np-12 was released into the bulk of the solvent. The corresponding intensity values of monomer and excimer emission of Np-12 are given in Table 3. If we plot the I_e/I_m vs. [L16] (Fig. 6), the slope ρ of the straight line may be used as an indicator of the deaggragating ability of L16, as well as that of L12 or L8, because both behave similarly to L16. The results are summarized in Table 4, in which larger ρ values reflect greater deaggregating abilities. Examination of Table 4 reveals that: (1) the order of decreasing deaggregating ability is: L16 > L8 > L12; and (2) the greater the degree of the probe (Np-12) aggregation, *i.e.*, the greater the concentration of Np-12, the more obvious effect of deAgr's (L*n*s) on the aggregated Np-12; (3) another interesting observation is that the deaggregating ability of L12 is the smallest among L*n*s, less than that of the longer chained L16, even less than that of the shorter chained L8. However, no deaggregation was observed in the case of P*n*s, the analogues of L*n*s (Fig. 7).

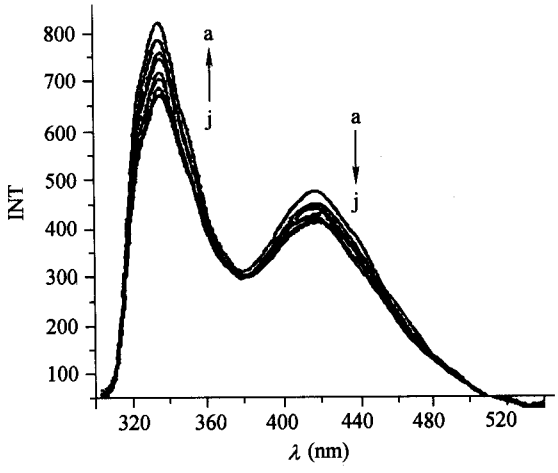


Fig. 5 Effect of P16 concentration on the fluorescence spectra of aggregated Np-12 in the Φ = 0.45 MeOH-H_2O system at 25 ℃, λ_{ex} = 280 nm, [Np-12] = 15.0×10^{-6} mol/L, [P16] (10^{-6} mol/L): a, 0; b, 1.2; c, 3.0; d, 5.4; e, 7.8; f, 10.2; g, 13.2; h, 16.2; i, 19.2; j, 24.0.

Table 3 Intensity values of monomer and excimer emission of Np-12 at different concentrations of L16 in $\Phi = 0.45$ MeOH-H_2O system at 25 ℃, [Np-12] = 1.5×10^{-5} mol/L.

	[L16] (10^{-6} mol/L)									
	0	1.2	3.0	5.4	7.8	10.2	13.2	16.2	19.2	24.0
$I_{337.7}$	676.89	680.50	684.12	691.35	709.43	723.90	749.21	763.67	788.98	825.14
$I_{419.1}$	478.02	452.70	449.09	445.47	441.86	431.01	427.39	423.78	416.55	412.93
I_e/I_m	0.71	0.67	0.66	0.64	0.62	0.60	0.57	0.55	0.53	0.50
$\rho(10^3$ mol^{-1}·L)[a]	8.28 ($r = 0.9901$)									

[a] Obtained by linear regression of I_e/I_m vs. [L16].

Table 4 Deaggregating abilities of L*n*s in terms of ρ values at different fixed concentrations of Np-12[a]

	L*n*s	[Np-12] (10^{-6} mol/L)						
		2.0	4.0	6.0	10.0	15.0	20.0	30.0
$\rho^b(10^3$ mol^{-1}·L)	L8		2.09	4.91	5.13	5.29	7.81	10.52
	L12		1.29	3.84	4.10	4.36	4.55	7.01
	L16	4.96	5.87	7.70	7.97	8.30	10.45	13.69

[a] In $\Phi = 0.45$ MeOH-H_2O system at 25 ℃. [b] Obtained by linear regression of I_e/I_m vs. [L*n*s].

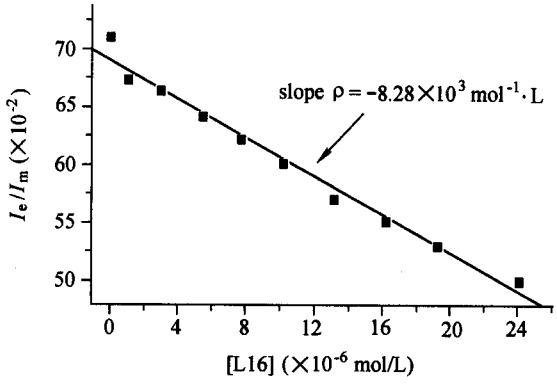


Fig. 6 Plot of the I_e/I_m vs. [L16] in the $\Phi = 0.45$ MeOH-H_2O system at 25 ℃, [Np-12] = 15.0×10^{-6} mol/L. Values of I_e/I_m are given in Table 3, correlation coefficient $r = 0.9939$.

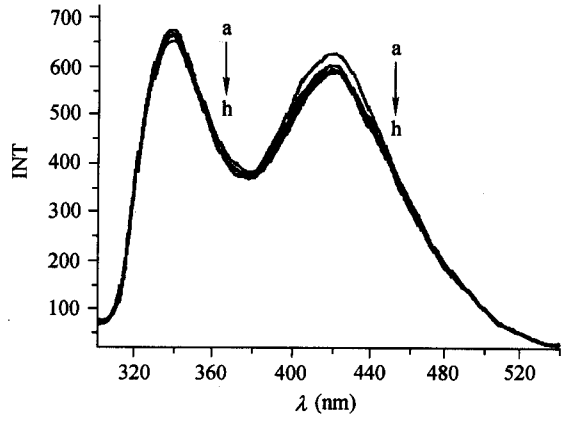


Fig. 7 Effect of P12 concentration on the fluorescence spectra of aggregated Np-12 in the $\Phi = 0.45$ MeOH-H_2O system at 25 ℃, $\lambda_{ex} = 280$ nm, [Np-12] = 15.0×10^{-6} mol/L, [P12] (10^{-6} mol/L): a, 0; b, 0.3; c, 0.6; d, 0.9; e, 1.2; f, 1.5; g, 2.0; h, 3.0.

On the other hand, we have compared the deaggregating abilities of L*n*s in a system with weaker SAgP, *i.e.*, $\Phi = 0.50$ MeOH-H_2O system at 25 ℃. Table 5 shows the deaggregating slopes (ρ) of L*n*s on the aggregate of Np-12. The deaggregating abilities of L*n*s greatly decrease with decreasing SAgP. It is known that increasing SAgP will increase the aggregating ability of Agr[16] and the degree of aggregation of the aggregate. Possibly, the decrease of the deaggregating ability of L*n*s might result from the decrease of both the degree of aggregation of the aggregate and the aggregating tendency of L*n*s in the system with weaker SAgP.

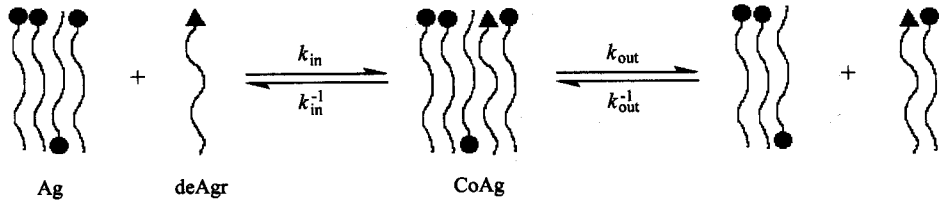
Table 5 Deaggregating ability of L*n*s in terms of Φ values at [Np-12] = 20.0×10^{-6} mol/L

	L16	L12	L8
ρ (10^3 mol^{-1}·L)	2.10	2.21	2.05
r (I_e/I_m vs. [L*n*s] plot)	0.9710	0.9863	0.9582

We have suggested that an effective HLI-driven deAgr might mainly operate by a mechanism depicted in a greatly simplified manner by Scheme 1,[7b] *i.e.*, the deAgr molecule can break up or reduce the size of an Ag or CoAg mainly by two steps, *i.e.*, (1) getting into the Ag (k_{in}) by virtue of the HLI between the long hydrocarbon chain(s) of the deAgr and the Agr molecules in the Ag, and (2) "grabbing" one or more Agr molecules in the Ag and carrying the latter out of the Ag (k_{out}) into the bulk of the solvent, and the latter release the Agr molecule in its monomeric form, by virtue of its hydrophilicity. We presume that the most effective deAgr should possess the best balance among its four rate constants, k_{in}, k_{in}^{-1}, k_{out} and k_{out}^{-1}. Some effective deaggregators have recently reported by our group.[11]

Based on the suggested mechanism of deaggregation described above (Scheme 1)[11] our present results might

Scheme 1 Hypothetical mechanism of deaggregation[11]



be rationalized as follows. All species incorporated in the deaggregating process are in a dynamic equilibrium. The most effective deAgr should possess the best balance among its four rate constants. Otherwise, when K_{in} ($K_{in} = k_{in}/k_{in}^{-1}$) is much larger than K_{out} ($K_{out} = k_{out}/k_{out}^{-1}$), it is easy for the deAgr to get into the Ag, but difficult to get out, the net effect is coaggregation; when K_{in} is much less than K_{out}, the deAgr can not readily get into the Ag to grab the aggregated molecules, then deaggregation is also not realized. The four rate constants are expected to be effected by multiple factors, *e.g.*, the length and foldability of the chain, hydrophilicity, lipophilicity as well as the SAgP of the system. For L*n*s, their amphiphilic structures with zwitterionic seem to favor the balance toward deaggregation. It is worthy to note that the double character of aggregation and deaggregation exhibited in L*n*s might shed a new light on understanding the mechanism of the membrane-directed antitumor,[1] antiviral[2] and antifungal[3] activities of phosphatidylcholines and their analogues. It might be the aggregating tendency that drives the phospholipid to attach the membrane and then it might be the deaggregating ability of the phospholipid that fluidize the cell membranes. But the desirable pharmacological effects could be realized only when the two interactions reach an optimal balance.

In a previous work we have noticed that the deaggregating ability of the deAgr increases as the degree of aggregation of the probe increases.[11] In the present work, if we plot the slop ρ vs. [Np-12] for each L*n*s, three similar curves are obtained (Fig. 8).

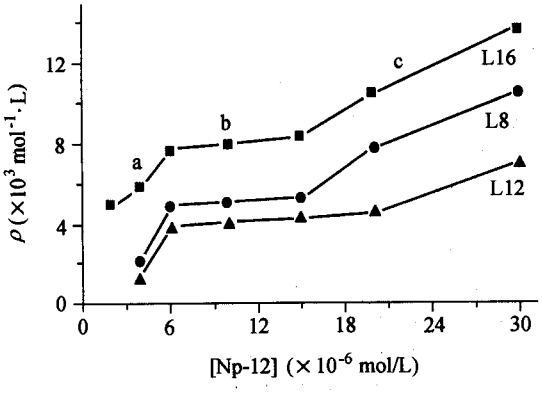


Fig. 8 Dependence of the deaggregating abilities (ρ) of the DeAgr on the degree of aggregation of the probe. Plots of ρ vs. [Np-12] in the $\Phi = 0.45$ MeOH-H_2O system at 25 ℃.

As shown in Fig. 8, the deaggregating abilities of L*n*s certainly depend on the concentration of aggregated Np-12. Recently we have found[17] that the degree of aggregation increased as the aggregator concentration increased, and it is mainly a consequence of the increase in the average aggregate number (N), but not a consequence of the increase in the number of aggregates. But when the degree of aggregation increases further, the number of aggregates will also increase with the increasing aggregator concentration. Thus, we suspect that the three phases of deaggregating ability might correspond to the changed structures, *e.g.*, changes in the average aggregate number (N) of the Np-12 aggregates while the total degree of aggregation of Np-12 is increasing. In the first region (a), the N values is small and the number of Np-12 molecules in each aggregate is increasing as the concentration of Np-12 increases, *i.e.*, the size of the aggregate is growing and the opportunity of a deAgr molecule to enter and grab one or more probe (Np-12) molecule(s) in the aggregate is increased, thus the opportunity for the deAgr molecule to carry these probe molecules into the bulk of the solvent increases too. In the second region (b), although both N and the number of Np-12 aggregates are increasing, the relative increase in N becomes smaller than its increase in phase (a), so that a smaller ρ value emerges, or the deaggregating abilities of L*n*s do not change much while the concentration of Np-12 increases. In the third region (c), the number of the aggregate is quickly increasing, therefore, the possibility for deAgr molecules to get into the aggregate quickly increases too. Consequently, the ρ value becomes larger again.

Summary and conclusion

In the present paper we have used α-naphthylethyl lauryl ether (Np-12) as the fluorescent probe to study the hydrophobic-lipophilic interactions of phosphatidylcholines (L*n*s) and their analogues 1,2-diacyl-*sn*-glycerol-3-phosphoric acid bromoethyl ester (P*n*s) in $\Phi = 0.45$ MeOH-H_2O system at 25 ℃, and have found that the L*n*s possess smaller tendencies to aggregate or coaggregate with other molecules than P*n*s, and the orders of decreasing coaggregating tendencies are: L12 > L16 > L8; P12 ~ P16 > P8. A more interesting observation is that the L*n*s are also demonstrated to be deaggregators if they interact with an aggregated probe. In other words, they seem to possess double character, *i.e.*, they can play the roles of both the aggregator and the deaggregator. The order of decreas-

ing deaggregating abilities is: L16 > L8 > L12. The amphiphilic zwitterionic structure of L*n*s is believed to be the origin of their deaggregating abilities. The double character shared by L*n*s might provide insights into the mechanism of phospholipids' membrane-directing activities.

The chain-length effect and chain-foldability effect, together with the hypothetical chain length-matching effect, have been invoked to rationalize our results. Both the aggregating tendency and deaggregating ability of L*n*s have been shown to decrease as the SAgP of the system decreases. Our results also support our previously proposed scheme for the mechanism of deaggregation.

Experimental

Materials

Analytical pure grade NaCl was used without further purification. L*n* and P*n* ($n = 8$, 12, 16) were prepared generally according to Ref. 18, the synthetic route is shown in Scheme 2. The fluorescence probe Np-12 and FP^+ and FP^- were prepared respectively according to ref. 19 and by methods reported elsewhere.[8c] All of the synthesized substrates were identified by ^1H NMR, IR and elemental analysis.

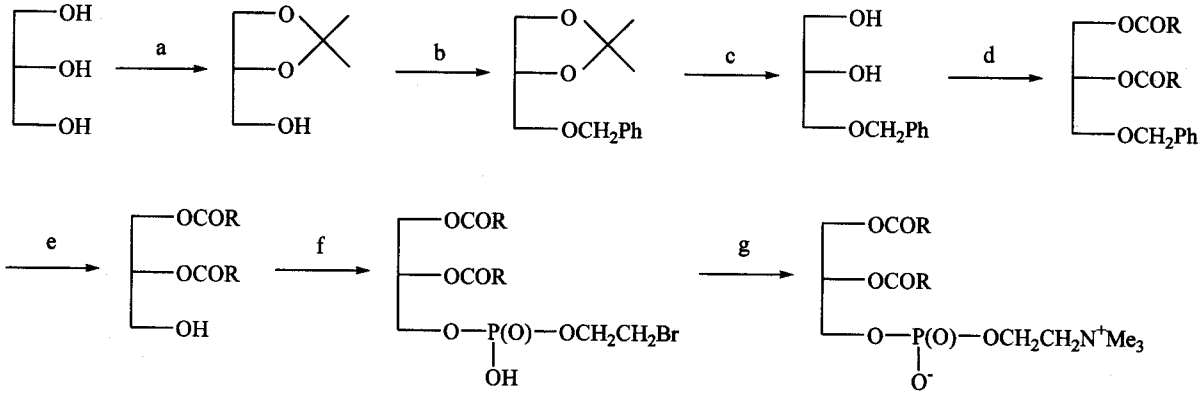
Solvent

Water was twice distilled and methanol was redistilled from HPLC-grade one. Spectral experiments were carried out in the $\Phi = 0.45$ and 0.50 mixtures of MeOH and water containing 0.37 mol/L NaCl.

General procedure for fluorescence measurement

Steady state fluorescence spectra of Np-12 were measured on a Perkin-Elmer Luminescence Spectrometer LS 50 in the 45:55 (V/V, $\Phi = 0.45$) and 50:50 (V/V, $\Phi = 0.50$) MeOH-H_2O systems at 25 ℃, by using the excitation wavelength of 280 nm and monitoring the intensity of monomer emission at wavelength of 337 nm and excimer emission at 420 nm.

Scheme 2



R = *n*-C_7H_{15}; *n*-$C_{11}H_{25}$; *n*-$C_{15}H_{31}$

Reagents and conditions: a) acetone, dry HCl; b) 50% NaOH, BnCl, cat. TEBA; c) 10 % HOAc; d) RCOCl, Py; or RCOOH, DCC; e) 10% Pd-C, H_2; f) 1. $BrCH_2CH_2OP(O)Cl_2$; 2. 0.1 mol/L KCl; g) Me_3N.

References

1 (a) Lohmeyer, M.; Bittman, R. *Drugs Future* **1994**, *19*, 1021.
(b) Houlihan, W. J.; Lohmeyer, M.; Workman, P.; Cheon, S. H. *Med. Res. Rev.* **1995**, *15*, 123.

2 (a) Kucera, L. S.; Iyer, N.; Leake, E.; Raben, A.; Modest, E. J.; Daniel, L. W.; Piantadosi, C. *AIDS Res. Hum. Retroviruses* **1990**, *6*, 491.
(b) Kucera, L. S.; Krugnerhigby, I. A.; Iyer, N. P.; Goff, D. H.; Edwards, T. V.; Neufeld, J. A.; Anand, I. S.; Pucket, S.; Morris-Natschke, S. L.; Piantadosi, C. *J. Cell Biochem.* **1993**, *17e*, 16.

3 (a) Bierer, D. E.; Gerber, R. E.; Jolad, S. D.; Ubillas, R. P.; Randle, J.; Nauka, E.; Latour, J.; Dener, J. M.; Fort, D. M.; Kuo, J. E.; Inman, W. D.; Dubenko, L. G.; Ayala, F.; Ozioko, A.; Obialor, C.; Elisabetsky, E.; Carlson, T.; Truong, T. V.; Bruening, R. C. *J. Org. Chem.* **1995**, *60*, 7022.
(b) Bierer, D. E.; Dubenko, L. G.; Litvak, J.; Gerber, R. E.; Chu, J.; Thai, D. L.; Tempesta, M. S.; Truong, T. V. *J. Org. Chem.* **1995**, *60*, 7646.

4 (a) Noseda, A.; Godwin, P.; Modest, E. *Biochem. Biophys. Acta* **1988**, *945*, 92.
(b) Meyer, K. L.; Maraco, Jr., C. J.; Morris-Natschke, S. L.; Ishaq, K. S.; Piantadosi, C. *J. Med. Chem.* **1991**, *34*, 1377.

5 Gennis, R. B. *Biomembranes: Molecular Structure and Functions*, Springer-Verlag, New York, **1989**.

6 (a) Tanford, C. *The Hydrophobic Effect: Formation of Micells and Biolotical Membranes*, 2nd Ed., John Wiley & Sons, New York, **1980**.

(b) Ben-Naim, A. *Hydrophobic Interactions*, Plenum Press, New York, **1980**.
(c) Blokziji, W.; Engberts, J. B. F, N. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1545.

7 (a) Jiang, X.-K. *Acc. Chem. Res.* **1988**, *21*, 362.
(b) Jiang, X.-K. *Pure Appl. Chem.* **1994**, *66*, 1621.

8 (a) Jiang, X.-K.; Ji, G.-Z.; Wang, J.-S. *Chin. Chem. Lett.* **1991**, *2*, 813.
(b) Jiang, X.-K.; Ji, G.-Z.; Wang, J.-S. *Chin. Chem. Lett.* **1992**, *3*, 231.
(c) Jiang, X.-K.; Wang, J.-S. *Chin. J. Chem.* **1993**, *11*, 472.

9 (a) Jiang, X.-K.; Ji, G.-Z.; Nie, J.; Zhang, J.-T. *Chin. J. Chem.* **1991**, *9*, 559.
(b) Jiang, X.-K.; Zhang, J.-T.; Tong, Z.-H. *Chin. Chem. Lett.* **1992**, *3*, 231.
(c) Jiang, X.-K.; Wang, J.-S. *Chin. J. Chem.* **1993**, *11*, 187.
(d) Zhang, J.-T.; Jiang, X.-K.; Ji, G.-Z.; *Chin. J. Chem.* **1994**, *12*, 277.

10 (a) Brown, G. H.; Wolken, J. J. *Lipid Crystals and Biological Structures*, Academic Press, New York, **1979**. Chapter 12.
(b) Small, D. M. *Arteriosclerosis* **1988**, *8*, 103.

11 Zhang, J.-T.; Ji, G.-Z; Jiang, X.-K. *Langmuir* **1994**, *10*, 122.

12 (a) Tyrrel, D. A.; Heath, T. D.; Colly, C. M.; Pyman, B. E. *Biochem. Biophys. Acta* **1976**, *457*, 259.
(b) Eibl, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 257.
(c) *Phospholipids: Biochemical, Pharmaceutical, and Analytical Considerations*, Eds: Hanin, I.; Pepeu, G., Plenum Press, New York, **1990**.

13 (a) Menger, F. M.; Portony, C. F. *J. Am. Chem. Soc.* **1968**, *90*, 1875.
(b) Knowles, J. R.; Parson, C. A. *J. Am. Chem. Soc.* **1971**, *93*, 3017.

14 (a) Jiang, X.-K.; Hui, Y.-Z.; Fei, Z.-X. *J. Am. Chem. Soc.* **1987**, *109*, 5862.
(b) Tong, Z.-H.; Xu, C.-B. *Acta Chim. Sinica* **1988**, *46*, 68 (in Chinese).
(c) Tung, C.-H; Wang, Y.-M. *J. Am. Chem. Soc.* **1990**, *112*, 6322.

15 Zhang, J.-T.; Nie, J.; Ji, G.-Z.; Jiang, X.-K. *Langmuir* **1994**, *10*, 2814.

16 (a) Jiang, X.-K.; Hui, Y.-Z.; Fan, W.-Q. *J. Am. Chem. Soc.* **1984**, *106*, 3839.
(b) Jiang, X.-K.; Ji, G.-Z.; Luo, G.-L. *Chin. J. Chem.* **1991**, *9*, 448, 453.
(c) Jiang, X.-K.; Li, X.-Y.; Huang, B.-Z. *Proc. Indian Acad. Sci. (Chem. Sci.)* **1987**, *98*, 409, 423.
(d) Zhu, M.-Y.; Zhang, T. X.-Y.; Zeng, J. H.; Jiang, X.-K. *Langmuir* **1997**, *13*, 3603.
(e) Tu, E. B.; Ji, G.-Z.; Jiang, X.-K. *Langmuir* **1997**, *13*, 4234.

17 (a) Jiang, X.-K.; Zhang, J.-T.; Tong, Z.-H. *Chin. Chem. Lett.* **1992**, *3*, 675.
(b) Zhang, J.-T.; Tu, B.; Ji, G.-Z.; Jiang, X.-K. *Chin. Chem. Lett.* **1993**, *4*, 879.

18 (a) Malkin, T.; EL Shurbagy, M. R. *J. Chem. Soc.* **1936**, 1634.
(b) Overman, L. E.; Robertson, G. M.; Robinchard, A. R. *J. Am. Chem. Soc.* **1991**, *113*, 2605.
(c) Howe, R. J.; Malkin, T. *J. Chem. Soc.* **1951**, 2666.
(d) Ioannou, P. V.; Dodd, G. H.; Golding, B. T. *Synthesis* **1979**, 939.
(e) Neises, B.; Steglich, W. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 522.
(f) Hebert, M.; Beck, A.; Lennock, R. B.; Just, G. *J. Org. Chem.* **1992**, *57*, 1777.
(g) Heathcock, C. H.; Ratcliffe, R. *J. Am. Chem. Soc.* **1971**, 93, 1755.
(h) Eible, V. H.; Arnold, D.; Weltzien, H. U. *Leibigs Ann. Chem.* **1967**. *709*, 226.

19 (a) Feit, P. W. *J. Med. Chem.* **1964**, *7*, 14.
(b) Kates, M.; Chan, T. H.; Stanacev, N. Z. *Biochemistry* **1963**, *2*, 394.

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