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ENERGY TRANSFER IN ARTIFICIAL MEMBRANE SYSTEMS

SINGLET-SINGLET ENERGY TRANSFER FROM ALLOXAZINES TO ISOALLOXAZINES IN DIPAMITOYL PHOSPHATIDYLCHOLINE LIPOSOMES AND DIALKYLAMMONIUM CHLORIDE VESICLES *

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Summary

The singlet-singlet energy transfer from alloxazines to isoalloxazines has been investigated in dipalmitoyl phosphatidylcholine (DPPC) liposomes and dioctadecyltrimethylammonium chloride ($2C_{18}NC$) vesicles to clarify the role of the artificial membranes in the energy transfer phenomenon. The structures of the artificial membranes were divided into two types: the single-walled (sonicated DPPC) and the multi-compartment vesicles (unsonicated DPPC and sonicated $2C_{18}NC$). In the DPPC single-walled liposomes, the energy of the donor lost by quenching is efficiently transferred to the acceptor via the Förster-type dipole-dipole interaction. In the case of multi-compartment liposomes of DPPC, the mean distance between donor and acceptor is so small because the external surface of a bilayer is in the vicinity of the internal surface of another bilayer. As a consequence, efficiencies both of energy transfer and of energy loss were greater than those in single-walled liposomes. The fluid property of the $2C_{18}NC$ bilayer allowed the preferential collisional quenching. The marked reduction in the efficiencies of both energy transfer and energy loss were attributed to the elongation of donor-acceptor distances due to the increase of the size of liposome.

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Abbreviations: DPPC, dipalmitoyl phosphatidylcholine; $2C_{18}NC$, dioctadecyltrimethylammonium chloride; DOA, 1,3-dioctylalloxazine; DBA, 1,3-dibutylalloxazine; DDA, 1,3-didodecylalloxazine; CBIA, 3-cetyl-10-butylisoalloxazine; DBIA, 3,10-dibutylisoalloxazine; OBIA, 3-octyl-10-butylisoalloxazine; DMPC, dimyristoyl phosphatidylcholine.

Introduction

The electronic energy transfer in bio- and biomimetic membranes has been extensively studied from various aspects. In the case of photosynthesis, the light energy is collected by several kinds of pigment, such as carotinoids and chlorophylls, in the chloroplast thylakoid membrane, where the concentration of the so-called antenna chlorophylls reaches as high as 0.1 M [1]. The light energy caught by antenna pigments efficiently migrates to the reaction center without significant energy loss. Recently, many studies have been carried out on energy transfer between photosynthetic pigments in biomimetic systems, such as detergent micelles [2—4], bilayer lipid membranes [5], lipid monolayers [6—9], lipid films [10,11], and lipid vesicles (liposomes) [12—14] to clarify the mechanism of the light-harvesting process in vivo [15—17]. Liposomes, lipid bilayer vesicles, are convenient and excellent models of biomembranes [18—21]. Porter and co-workers [9] suggested that the hydrogen bonding between chlorophyll *a* and galactosyl diacylglycerols and/or the bulkiness of lipid head groups inhibits the concentration quenching of chlorophyll *a* fluorescence in liposomes. Mehreteab and Strauss [22] have reported that the rigidity of lipid bilayers prohibits the collisional quenching so that the energy of the donor efficiently migrates to the acceptor if the donor—acceptor mean distance (R_{DA}) is within the Förster's critical transfer distance (R_0).

Since the study on the energy transfer in membranes is very important not only for deducing the mechanism of the photosynthetic energy-harvesting process but also for investigating the function and physical properties of membranes [23—26], systematic study in this field should be carried out in more detail.

The present study deals with the singlet energy transfer from the 1,3-dialkylalloxazines to the 3,10-dialkylisoalloxazines in artificial membranes such as dipalmitoyl phosphatidylcholine (DPPC) liposomes and dialkylammonium chloride vesicles [27—34]. Two types of artificial membrane were used in the experiment: single-walled (sonicated DPPC) and multi-compartment vesicles (unsonicated DPPC and sonicated dioctadecyldimethylammonium chloride, $2C_{18}NC$). The major objects of this study are clarification of the effects of the membrane rigidity, as well as the geometry of the donor and acceptor molecules, on the energy transfer in artificial membranes.

Experimental Procedure

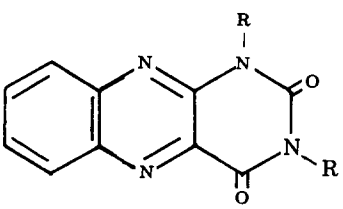
Materials

Synthetic dipalmitoyl-DL-α-phosphatidylcholine (DPPC) was used as received from Sigma Chemical Company (Grade I). The monohydrate of $2C_{18}NC$ was kindly provided by Kao Soap Co. Ltd., Tokyo and the purity of this surfactant was shown by elemental analysis to be satisfactory. Alloxazine was prepared by condensation reaction of *o*-phenylenediamine with alloxan according to the procedures in the literature [35]. The crude alloxazine was recrystallized from aqueous *N,N*-dimethylformamide to yield a pale yellow solid (94% yield): m.p. > 250°C. Analysis:

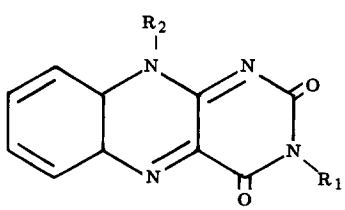
Calcd. for $C_{10}H_6N_4O_2$: C, 56.08; H, 2.82; N, 26.16%.

Found: C, 56.04; H, 2.81; N, 26.13%.

1,3-Dioctylalloxazine (DOA) was prepared by alkylation of alloxazine with octyl iodide. A mixture of 1 g alloxazine, 4 g octyl iodide, and 3 g K_2CO_3 in 200 ml *N,N*-dimethylformamide was stirred for 52 h at room temperature. K_2CO_3 was filtered off and *N,N*-dimethylformamide was evaporated at reduced pressure. The residue was dissolved into $CHCl_3$ and washed with 0.1 N NaOH. After drying on Na_2SO_4, $CHCl_3$ was evaporated at reduced pressure and the residue was recrystallized twice from acetonitrile to yield a pale yellow solid DOA (0.8 g, 50%): m.p. 103—104°C. 1,3-dibutyl- (DBA) and 1,3-didodecyl-alloxazines (DDA) were also prepared by dialkylation of alloxazine with corresponding alkyl iodides and purified according to the same procedures as DOA. The analytical data are summarized in Table I. 3-Butylisoalloxazine [36] and 3-cetyl-10-butylisoalloxazine (CBIA) [37] were prepared and purified according to the procedures in the literature. The method as used to obtain CBIA also afforded 3,10-dibutryl- (DBIA) and 3-octyl-10-butylisoalloxazines (OBIA). The analytical data of the isoalloxazines are also listed in Table I.



Alloxazine



Isoalloxazine

Single-walled liposomes

DPPC (7.3 mg, 0.01 mmol) and appropriate volumes of the stock solutions of the donor and acceptor in $CHCl_3$ were placed in a pyrex test tube and

TABLE I

ANALYTICAL DATA OF PREPARED ALLOXAZINES AND ISOALLOXAZINES

Yield (%) refer to the yields of alkylation of alloxazine and 10-butylisoallxazine with the corresponding alkyl iodides.

Compound	*R*	R_1	R_2	Yield (%)	m.p. (°C)	Analysis found (calcd.)		
DBA	C_4H_9			40	142—143	C, 66.31 (66.24)	H, 6.78 (6.79)	N, 17.24 (17.17)
DOA	C_8H_{17}			50	103—104	C, 71.30 (71.20)	H, 8.74 (8.73)	N, 12.92 (12.77)
DDA	$C_{12}H_{25}$			85	106—108	C, 74.04 (74.14)	H, 9.89 (9.88)	N, 10.12 (10.17)
DBIA		C_4H_9	C_4H_9	40	178—179	C, 66.11 (66.24)	H, 6.82 (6.79)	N, 17.04 (17.14)
OBIA		C_8H_{17}	C_4H_9	35	122—123	C, 68.70 (69.07)	H, 7.91 (7.91)	N, 14.26 (14.64)
CBIA		$C_{16}H_{33}$	C_4H_9	67	105—107	C, 72.82 (72.83)	H, 9.44 (9.37)	N, 11.07 (11.33)

$CHCl_3$ was removed by a stream of N_2. The residue was dried on silica gel under reduced pressure and added to 1—2 ml phosphate buffer (0.01 M, pH 7.0) containing 0.1 M NaCl. The solution was sonicated with a bath-type sonicator (Bransonic 12, 50 W) until the solution became almost clear. During sonication, the temperature of the sample solution was kept at 50—60°C and the sample solution was bubbled with N_2 gas. The single-walled liposome solution thus obtained was diluted by the same buffer solution to measure the fluorescence.

Multi-compartment liposomes

The $CHCl_3$ solution containing appropriate amounts of the components was put into a flask and subjected to rotary evaporation to form a thin film on the surface of the flask. The thin film was dispersed in the buffer solution by shaking with a Vortex mixer at 55—60°C.

Surfactant vesicles

Procedures for preparing $2C_{18}NC$ vesicles were essentially the same as in preparation of single-walled liposomes. Since the surfactant vesicles were prepared with difficulty in the presence of high concentrations of inorganic salts, water was used in place of the buffer solution. Kano et al. [34] have reported that the sonicated $2C_{18}NC$ dispersion forms the multi-compartment vesicles with 250—500 Å diameter. The gel/liquid phase transition temperature (T_c) of $2C_{18}NC$ has been determined to be 30—37°C by positron annihilation [34], fluorescence [31,34], and NMR measurements [31]. Therefore, the sonication of the $2C_{18}NC$ dispersion was carried out at 50—55°C, well above T_c.

Spectral measurements

Emission and excitation spectra were measured on a Shimadzu model RF-500 spectrofluorometer the cell compartment of which was kept at constant temperature. The fluorescence spectra were corrected by the use of the spectrum from a 1.0 μM solution of quinine sulfate in 0.1 N H_2SO_4. Determination of the degrees of fluorescence polarization (P) [38,39] and of the fluorescence quantum yields (Φ_f) [40] was carried out by the procedures described in the literature. These results are summarized in Table II. The absorption spectra were measured on a Shimadzu model UV-200 spectrophotometer using 1 cm optical path.

Determination of energy transfer efficiency

Fig. 1 shows the absorption and fluorescence emission spectra of DOA (donor) and OBIA (acceptor). All of the donor molecules (DBA, DOA and DDA) in lipid and $2C_{18}NC$ bilayers have absorption maxima at 327 ($\epsilon = 9 \cdot 10^3\ M^{-1} \cdot cm^{-1}$) and 389 nm ($\epsilon = 7 \cdot 10^3\ M^{-1} \cdot cm^{-1}$) and fluorescence maxima at 450 nm. The absorption maxima of the acceptors were 342 ($\epsilon = 9 \cdot 10^3\ M^{-1} \cdot cm^{-1}$) and 440 nm ($\epsilon = 1 \cdot 10^4\ M^{-1} \cdot cm^{-1}$). The longest wavelength absorption band of the acceptor well overlaps with the fluorescence spectrum of the donor, which indicates that the alloxazine-isoalloxazine system is suitable for investigation of the Förster-type resonance energy transfer. The sensitized emission was observed at the fluorescence maxima of the acceptors

TABLE II

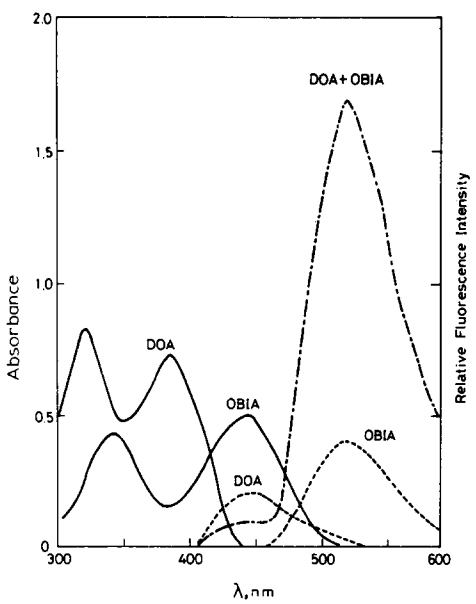
DEGREES OF FLUORESCENCE POLARIZATION (P) AND FLUORESCENCE QUANTUM YIELDS (Φ_f) OF ALLOXAZINES AND ISOALLOXAZINES IN DPPC SINGLE-WALLED LIPOSOMES

For determining P values, the fluorophores were excited at 388 nm for alloxazines and 440 nm for isoalloxazines. The emission intensities were followed at 440 nm for alloxazines and 500 nm for isoalloxazines. The measurements were carried out at 4°C. The Φ_f values were determined at 20°C.

Fluorophore	P	Φ_f
DBA	0.202 (0.038/MeOH)	
DOA	0.245	0.028
DDA	0.264	
DBIA	0.106	
OBIA	0.209	
CBIA	0.248	0.24

(520 nm).

The efficiency of energy loss of donor (ϕ_Q) and that of energy transfer (ϕ_{DA}) were determined by the methods described by Mehreteab and Strauss [22] with minor modifications. Fig. 2 illustrates the method of calculating the energy transfer efficiency. ϕ_Q is the quenching fraction of the donor fluor-



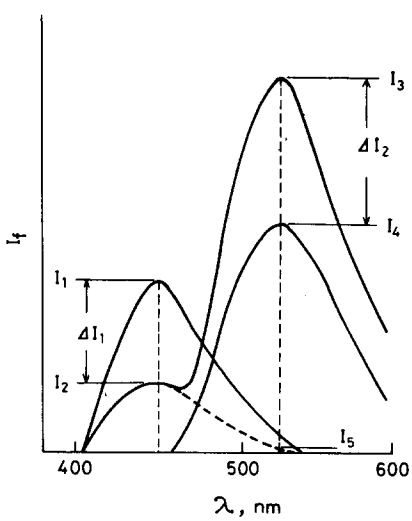


Fig. 1. Sensitization of fluorescence emission from OBIA by the use of DOA in DPPC single-walled liposomes at 4°C: absorption (———) and fluorescence emission spectra (- - - - - -) of DOA (10 μM) and OBIA (4 μM) independently incorporated into liposomes, and sensitized fluorescence emission spectrum of OBIA (· — · — ·). The sensitized emission was obtained by exciting a liposome solution ([DPPC] = 0.4 mM) containing DOA (10 μM) and OBIA (4 μM) at 380 nm. The fluorescence emission spectra of DOA and OBIA independently incorporated into DPPC liposomes ([DPPC] = 0.4 mM) were also obtained upon excitation at 380 nm.

Fig. 2. Scheme for interpreting the determination of the efficiencies of energy loss (ϕ_Q) and of energy transfer (ϕ_{DA}). The detail and definition of I_1—I_6 are described in the text.

escence when the donor and acceptor coexist within a vesicle. ϕ_Q (%) is defined in Eqn. 1:

$$\phi_Q = \frac{I_1 - I_2}{I_1} \cdot 100 \tag{1}$$

where I_1 and I_2 are the fluorescence intensities of donor at 440 nm in the absence and presence of acceptor, respectively, when the donor was excited at 380 nm. The energy transfer efficiency (ϕ_{DA}) is calculated from the sensitized fluorescence intensity of acceptor. Since ϕ_{DA} is defined by

$$\phi_{DA} = \frac{\text{(number of sensitized acceptor molecules)}}{\text{(number of excited donor molecules)}} \tag{2}$$

ϕ_{DA} (%) is correlated with the fluorescence intensities of the acceptor in the presence and absence of donor by the following equation [22]:

$$\phi_{DA} = \frac{(I_3 - I_4 - I_5)/I_4}{A_D/A_A} \cdot 100 \tag{3}$$

I_3 is the observed fluorescence intensity of acceptor at 520 nm when coexisting donor molecules are excited at 380 nm. I_4 is the fluorescence intensity of acceptor at 520 nm upon exciting acceptor at 380 nm in the absence of donor. I_5 is the fluorescence intensity of donor at 520 nm when the donor molecules are excited at 380 nm in the presence of acceptor. Since I_5 cannot be observed directly, it was calculated by Eqn. 4;

$$I_5 = \frac{I_2 \cdot I_6}{I_1} \tag{4}$$

where I_6 is the fluorescence intensity of donor at 520 nm when the donor molecules are excited at 380 nm in the absence of acceptor. A_A and A_D are the absorbances of acceptor and donor at 380 nm, respectively.

Results and Discussion

Energy transfer in single-walled liposomes

The efficiencies of energy transfer (ϕ_{DA}) and of energy loss of the donors (ϕ_Q) in the alloxazine-isoalloxazine system were determined in DPPC single-walled liposomes well below and above the T_c of DPPC (T_c of DPPC is 41°C [20]). The results are listed in Table III. Several important features are noticed here, as summarized in the following:

(1) both ϕ_Q and ϕ_{DA} increased with increasing alkyl chain lengths of the acceptors;

(2) the most effective energy donor is DOA, which has intermediate alkyl chain lengths;

(3) in the case of DBIA, which has two short alkyl chains, the ϕ_{DA}-values are considerably smaller than the ϕ_Q values;

(4) in the cases of OBIA and CBIA, ϕ_{DA} differed slightly from ϕ_Q; and

(5) in most cases, both ϕ_Q and ϕ_{DA} are significantly reduced above T_c.

The low energy transfer efficiencies for DBIA may be ascribed to the water-soluble nature of this fluorophore. The small value in the degree of DBIA

TABLE III

EFFICIENCIES OF ENERGY LOSS (ϕ_Q) AND OF ENERGY TRANSFER (ϕ_{DA}) OF VARIOUS DONOR-ACCEPTOR SYSTEMS IN DPPC SINGLE-WALLED LIPOSOMES

The donor molecules ([donor] = 10 μM) incorporated into DPPC single-walled liposomes ([DPPC] = 0.4 mM) were excited at 380 nm in aqueous phosphate buffer solutions (0.01 M, pH 7.0) containing 0.1 M NaCl at 4°C. The sensitized fluorescence emission of acceptors ([acceptor] = 4 μM) were followed at 520 nm. The ϕ_Q and ϕ_{DA} values were determined by using Eqns. 1 and 3, respectively.

	Temperature (°C)	DBIA		OBIA		CBIA	
		ϕ_Q	ϕ_{DA}	ϕ_Q	ϕ_{DA}	ϕ_Q	ϕ_{DA}
DBA	4	13	7	30	30	37	30
	20	9	2	25	29	30	30
	50	8	0	14	14	18	23
DOA	4	21	11	41	39	56	50
	20	15	10	41	40	52	46
	50	14	3	16	21	30	30
DDA	4	7	2	25	22	31	31
	20	4	2	17	25	28	30
	50	14	2	21	23	20	27

fluorescence polarization (P) in DPPC liposomes (see Table II) supports this interpretation. Since DBIA molecules are found in the bulk phase, as well as in the lipid bilayer, it is quite likely that the excitation energy is lost by the collision between DBIA molecules. In good agreement with this expectation, the ϕ_Q values were considerably larger than the ϕ_{DA} values in the case of DBIA. On the basis of the P values (see Table II), all fluorophores except for DBIA seem to be incorporated into lipid bilayers, so that their rotational motions are strongly hindered. Under these circumstances, the ϕ_{DA} values are fairly well in agreement with the ϕ_Q values, which indicates that the excitation energies of the donors were transferred to the acceptors without collisional quenching.

The energy transfer from DOA to OBIA in DPPC single-walled liposomes was studied in further detail by the use of temperature effects. The temperature effects were observed with ϕ_Q, ϕ_{DA}, P values for the donor in the absence of acceptor, the corresponding values for the acceptor in the absence and presence of the donor, and fluorescence intensities of the donor and acceptor in the absence of the partner (Fig. 3A). In the temperature range investigated, the ϕ_{DA} values were always close to the ϕ_Q values. The collisional quenching in the DOA-OBIA system, therefore, can be excluded from the consideration. The binding sites of the donor and acceptor molecules within lipid bilayers can be discussed on the basis of the P values (Fig. 3A (ii)) and fluorescence intensities (Fig. 3A (iii)). The fluorescence intensities of the donor and acceptor in DPPC single-walled liposomes decreased continuously with increasing temperature. This result indicates that neither donor nor acceptor form self-aggregates in lipid bilayers. The P values of DOA were not influenced by the temperature, while those of OBIA gradually decreased above the T_c of DPPC. The rotational mobility of the fluorophore with two long alkyl chains (DOA) seems to be restricted even if the surrounding alkyl chains of DPPC melt at the temperature above T_c. On the other hand, the rotational motion of OBIA, which has one

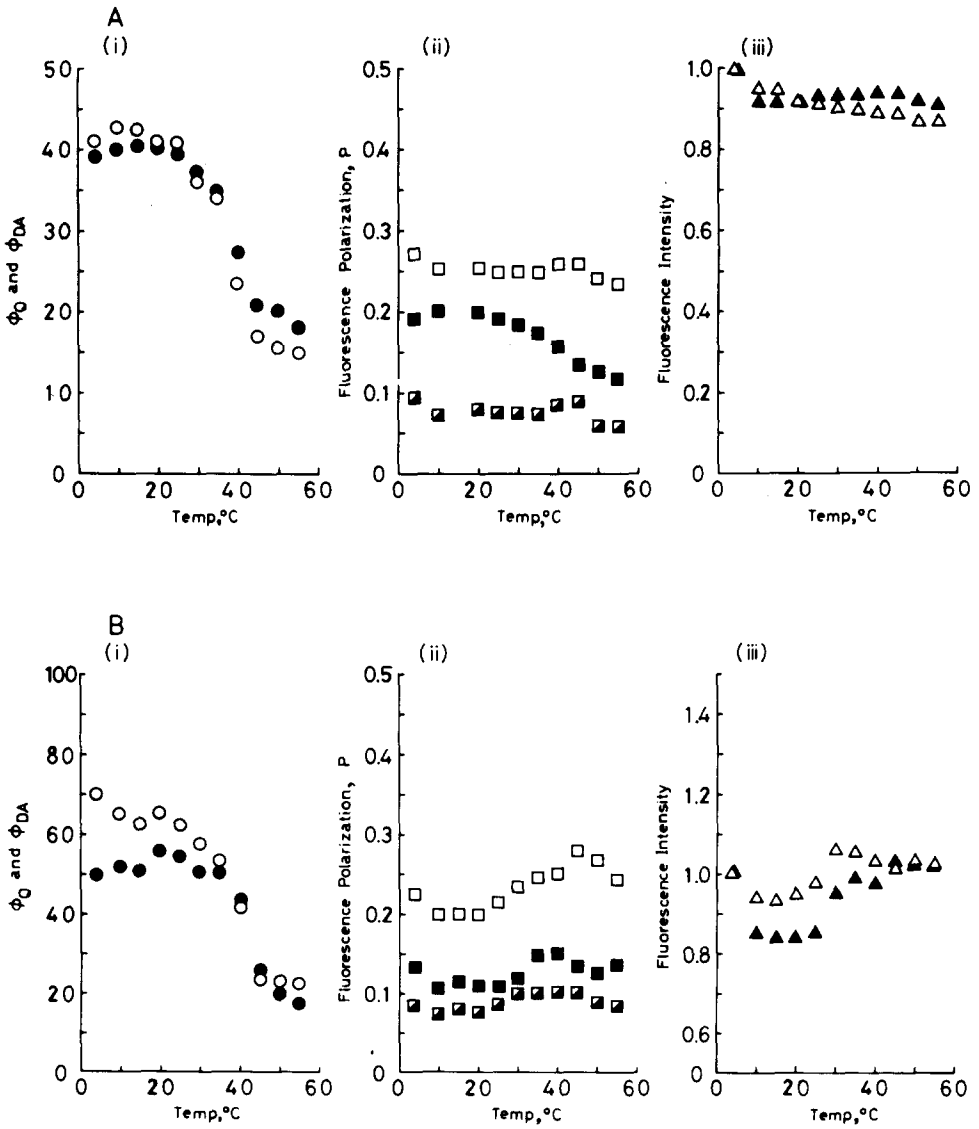


Fig. 3. Sensitization of the OBIA fluorescence emission by DOA in DPPC single-walled (A) and multicompartment liposomes (B): [DPPC] = 0.4 mM, [DOA] = 10 μM, and [OBIA] = 4 μM. i. The efficiencies of the energy loss of DOA excited state (ϕ_Q, ○) and of the energy transfer (ϕ_{DA}, ●) as a function of temperature. ii. The degrees of fluorescence polarization of DOA (□), OBIA (■), and sensitized OBIA (◪). iii. The relative fluorescence intensities of DOA (△) and OBIA (▲), independently incorporated into liposomes, at 440 and 500 nm, respectively. The experiments were carried out in 0.01 M phosphate buffer (pH 7.0) containing 0.1 M NaCl.

long alkyl chain, is accelerated above T_c. As Fig. 3A(i) clearly shows, the temperature profiles on ϕ_Q and ϕ_{DA} were typical sigmoidal curves, where both values were rapidly reduced from 40% to less than 20% as the temperature exceeded T_c. P values of sensitized acceptor smaller than those of acceptor alone indicate that the energy transfer from DOA to OBIA proceeds via the Förster-type dipole-dipole resonance interaction [8,11,41].

The efficiency of the Förster-type energy transfer drastically varies with the donor–acceptor distance as predicted by the following equation [42,43];

$$k_{D\text{-}A} = \frac{1}{\tau_0}\left(\frac{R_0}{R_{DA}}\right)^6 \tag{5}$$

where $k_{D\text{-}A}$ is the rate constant for dipole-dipole energy transfer, τ_0 is the fluorescence lifetime of donor in the absence of acceptor, R_{DA} is the mean distance between donor and acceptor, and R_0 is the critical transfer distance. On the basis of Eqn. 5, the sharp decrease in ϕ_Q and ϕ_{DA} above the T_c of the lipid bilayer should be ascribed to the increase in R_{DA}, when the phase changes from gel to liquid crystalline state.

The critical transfer distance (R_0) is given by the following equation [42–45];

$$R_0^6 = \frac{9000K^2\Phi_f \ln 10}{128\pi^5 n^4 N}\int \frac{f_D(\nu)\,\epsilon_A(\nu)}{\nu^4} \tag{6}$$

where K^2 is an orientation factor, usually taken as 2/3 for random orientation, Φ_f is the absolute fluorescence quantum yield of the donor, n is the refractive index of the medium *, N is Avogadro's number, and $f_D(\nu)$ and $\epsilon_A(\nu)$ are the spectral distributions of emission of donor and of absorption of acceptor, respectively, on a wave-number scale. The overlap integral

$$J = \int \frac{f_D(\nu)\,\epsilon_A(\nu)}{\nu^4}\,d\nu$$

can be determined graphically. By the use of the Φ_f value for DOA (0.028), the R_0 value for the DOA-OBIA system is estimated to be 21 Å.

If the energy donor and acceptor are randomly distributed in lipid bilayer (model I), an approximately equivalent situation may be obtained allocating these compounds to the central zone of the lipid bilayer [22]. In this case, the mean donor–acceptor distance (R_{DA1}) is represented as

$$R_{DA1} = \frac{2}{3}\left(\frac{S}{2\pi X_A}\right)^{1/2} \tag{7}$$

where S is an area per lipid molecule in the bilayer and X_A is the mole ratio of acceptor to phospholipid. On the other hand, if the fluorescent moieties of both donor and acceptor are located only at the surfaces of the bilayer (model II), R_{DA} may be calculated by the following equation:

$$R_{DA2} = \frac{2}{3}\left(\frac{S}{\pi X_A}\right)^{1/2} \tag{8}$$

* In this case, the refractive index of water (n = 1.334) was used in calculating the R_0 value. When both donor and acceptor are randomly located in the lipid bilayer, it is appropriate to use the average refractive index of the lipid bilayer itself. However, there is no information about the refractive index of the lipid bilayer. Then the influence of the refractive index on the calculated R_0 value was examined in several cases. Lipid molecule possesses both alkane and alcohol moieties, the refractive indexes of which are larger than that of water. Then the calculations of the R_0 values have been made by using 1.4 and 1.45 as the upper limit for the refractive indexes of the lipid bilayer. The calculated R_0 values were 20.3 Å for n = 1.4 and 19.9 Å for n = 1.45, which are not so far from the R_0 value (21 Å) calculated by using the refractive index of water.

To estimate the R_{DA} values by using models I and II, one must know the area per phospholipid (S) of the DPPC single-walled liposome. Unfortunately, there is no information about the detailed morphology of the DPPC liposomes. Watts et al. [46] have reported the vesicle parameters of dimyristoyl phosphatidylcholine (DMPC) single-walled liposome as a function of temperature. They found that the area per lipid molecule (S = 45.3 Å^2 at 15°C) increases drastically above the T_c of DMPC (S = 74.7 Å^2 at 30°C) *. Since the structure of the DMPC molecule is analogous to that of the DPPC molecule except for the alkyl chain length, the S values of DMPC may be used for the DPPC system. Applying these parameters, the R_{DA1} and R_{DA2} values for the DOA-OBIA-DPPC system were calculated as listed in Table IV with [DPPC] = 0.4 mM and [OBIA] = 4 μM. Eqn. 5 can be transformed to Eqn. 9, which provides the energy transfer efficiency ($\phi_{DA}^{calcd.}$) from the R_{DA} and R_0 values:

$$\phi_{DA}^{calcd.} = \frac{R_{DA}^{-6}}{R_{DA}^{-6} + R_0^{-6}} \tag{9}$$

where R_0 is the critical transfer distance for the DOA-OBIA system, 21 Å. Table IV also shows the $\phi_{DA}^{calcd.}$ values corresponding to R_{DA1} and R_{DA2} together with the experimentally obtained ϕ_{DA} ($\phi_{DA}^{exper.}$) and R_{DA} ($R_{DA}^{exper.}$) values. $R_{DA}^{exper.}$ values were calculated by using Eqn. 9.

The experimentally obtained donor—acceptor distance ($R_{DA}^{exper.}$) is just between R_{DA1} and R_{DA2}. Considering the approximations involved in the calculations, one should not take the fact too seriously. However, taking into consideration of the somewhat hydrophilic property of the alloxazine and isoalloxazine moieties, it may be reasonable to consider that these fluorescent moieties are statistically located at the region of the lipid bilayer close to the polar head groups of the liposome.

Energy transfer in multi-compartment liposomes

The energy transfer characteristics are somewhat different in single- and multi-compartment liposomes. The results obtained in the DPPC multi-compartment liposomes are shown in Fig. 3B. One of the remarkable differences is that both ϕ_Q and ϕ_{DA} in multi-compartment liposomes below T_c are greater than those in single-walled liposomes. The structure of multi-compartment liposome is constructed by repeated accumulation of lipid bilayer shells and external surface of one lipid bilayer is close to internal surface of the next bilayer [18—21]. The X-ray and neutron diffraction studies [47—49] have shown that the distance between polar head groups of neighboring bilayers of multilayer lamella is approx. 20 Å, which is not so far from the critical transfer distance of the alloxazine-isoalloxazine system. Then the Förster-type energy transfer may occur between lipid bilayers. As a consequence, the mean distance between donor and acceptor molecules in the multi-compartment liposome may be less than that in the single-walled liposome, which provides efficient energy transfer conditions in the former system. It is also noticed that the ϕ_{DA} values below T_c are considerably smaller than the ϕ_Q values in the case of the multi-compartment liposomes. As shown in Fig. 3A(ii) and 3B(ii), the

* T_c of DMPC is 23°C.

TABLE IV

EXPERIMENTALLY AND THEORETICALLY OBTAINED DONOR-ACCEPTOR DISTANCES AND ENERGY TRANSFER EFFICIENCIES FOR DOA-OBIA SYSTEM IN DPPC SINGLE-WALLED LIPOSOMES

The mean donor—acceptor distances (R_{DA1} and R_{DA2}) below and above T_c of DPPC were calculated from Eqns. 7 and 8 by using the vesicle parameters of DMPC single-walled liposomes reported in Ref. 46. The $\phi_{DA}^{calcd.}$ values were calculated by Eqn. 9, which also provided $R_{DA}^{exper.}$ values when $\phi_{DA}^{exper.}$ values and 21 Å value of R_0 were used.

Temperature	R_{DA1} (Å)	$\phi_{DA}^{calcd.}$ (%)	R_{DA2} (Å)	$\phi_{DA}^{calcd.}$ (%)	$R_{DA}^{exper.}$ (Å)	$\phi_{DA}^{exper.}$ (%)
$T < Tc$	17.9	72.2	25.3	24.6	22.3	41
$T > T_c$	23.0	36.6	32.5	6.8	27.7	16

degrees of fluorescence polarization (P) of both DOA and OBIA in multi-compartment liposomes are smaller than those in single-walled liposomes. The small P values in multi-compartment liposomes seem to be ascribed to the energy migration between donor molecules and/or between acceptor molecules. The fact that increasing the temperature causes an increase of the P values and of the fluorescence intensities of donor and acceptor in the absence of the corresponding partners (see Fig. 3B(ii) and (iii)) also suggests self-quenching of donor and/or acceptor excited state in multi-compartment liposomes. The excited state of OBIA (acceptor) produced by the energy transfer from DOA (donor) may be self-quenched as noticed in the reduction of ϕ_{DA}.

Energy transfer in surfactant vesicles

Recently, several groups have been developing the preparation and characterization of surfactant vesicles formed upon sonication of aqueous dispersions of dialkyldimethylammonium halides [27—34,50] and dihexadecylphosphate [51,52]. Sonic dispersal of $2C_{18}NC$ results in the formation of stable bilayer vesicle with radii of 150 ± 5 Å and bilayer thickness of 50 ± 5 Å [34]. Electron microscopy indicates that the multi-compartment vesicles of $2C_{18}NC$ are constructed from several bilayers. Energy transfer may provide useful information about the membrane fluidity and function of the surfactant vesicles. Fig. 4 shows the efficiencies of energy transfer (ϕ_{DA}) and of energy loss (ϕ_Q) in the DOA-OBIA-$2C_{18}NC$ vesicle system as a function of temperature, together with the degrees of fluorescence polarization (P) of DOA, OBIA, and sensitized OBIA in $2C_{18}NC$ vesicles. The most remarkable thing in this system is that the ϕ_{DA} values are significantly smaller than the ϕ_Q values. In contrast with the case of the DPPC multi-compartment liposome, the P values of the acceptor (OBIA) in $2C_{18}NC$ vesicle continuously decrease with increasing temperature; hence it cannot be concluded that the self-quenching of sensitized OBIA results the reduction of ϕ_{DA}. The collisional quenching of the excited donor (DOA) by the acceptor (OBIA) affords the most plausible explanation for the smaller ϕ_{DA} values. The electrostatic repulsion between positively charged head groups of the surfactant vesicle may provide a loose membrane structure which increases the probe mobility. This suggestion is consistent with the results

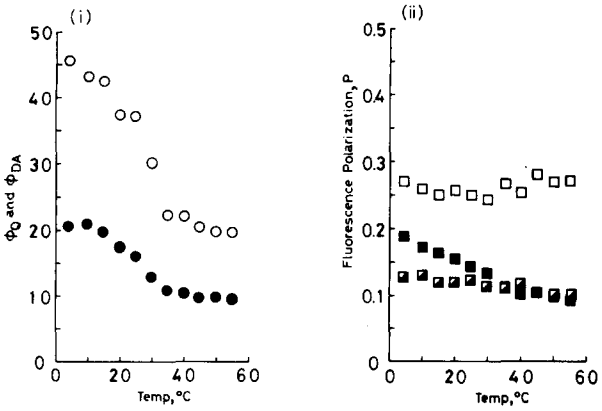


Fig. 4. Sensitization of the OBIA fluorescence emission by DOA in $2C_{18}NC$ multi-compartment vesicles: [$2C_{18}NC$] = 0.4 mM, [DOA] = 10 μM, and [OBIA] = 4 μM. i. ϕ_Q (○) and ϕ_{DA} (●) as a function of temperature. ii. The degrees of fluorescence polarization of DOA (□), OBIA (■), and of sensitized OBIA (◪). The experiments were carried out in water without buffer agents or NaCl.

obtained by Czarniecki and Breslow [52]. Three types of motion are expected with the probes; (1) lateral, (2) rotational, and (3) transversal. Judging from the P values, the rotational mobility of DOA and OBIA in $2C_{18}NC$ is restricted in the same order as that in DPPC single-walled liposomes. For the present, it is suggested that the lateral and/or transversal mobilities of the fluorophores cause the collisional quenching of the DOA excited state. The decrease in ϕ_Q and ϕ_{DA} around 20—35°C is associated with the phase transition temperature of $2C_{18}NC$ vesicles. Kano et al. [34] have measured the phase transition temperature of $2C_{18}NC$ vesicle as 30—37°C by means of positron annihilation and several other methods.

Concluding remarks

The efficiency of the singlet energy transfer from the alloxazines to isoalloxazines is affected markedly by the temperature and the structure and fluidity of the artificial membranes. Below the phase transition temperature (T_c), the singlet energy of the donor transfers to the acceptor via the Förster resonance mechanism without other bimolecular deactivation process in DPPC single-walled liposomes. Above T_c, the mean donor—acceptor distance in single-walled liposome increases with the vesicle size and the energy transfer efficiency decreases drastically. The energy transfer is enhanced in the multi-compartment liposome of DPPC. This is ascribed to the fact that the mean donor—acceptor distance is shortened by the participation of the neighboring lipid bilayer.

In the comparision with the DPPC liposomes, the energy transfer efficiency in the surfactant vesicle is significantly low in spite of the efficient energy loss. The looseness of the membrane structure due to the electrostatic repulsion between the head groups of the surfactant vesicle may cause the high lateral and/or transversal mobilities of the fluorophore (or fluorophores) which is responsible for the collisional quenching of the donor excited state.

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