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Effect of Cholesterol on the Molecular Motion in the Hydrocarbon Region of Lecithin Bilayers Studied by Nanosecond Fluorescence Techniques[†]

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ABSTRACT: Effects of cholesterol on the dynamic structure of the hydrocarbon region of dipalmitoyllecithin vesicles were examined. Decays of the emission anisotropy and the fluorescence intensity of 1,6-diphenyl-1,3,5-hexatriene embedded in lecithin–cholesterol vesicles were measured over a temperature range of 10–60 °C. The emission anisotropy decreased rapidly with time and then leveled off. The rotational motion of the probe was analyzed by a model of wobbling diffusion confined in a cone. Cholesterol (10–50 mol %) decreased the cone angle in the liquid-crystalline phase and increased it in the gel phase. In the presence of 33 mol % cholesterol, the wobbling diffusion constant increased in the gel phase and changed little in the liquid-crystalline phase. The viscosity in the cone decreased in the gel phase and remained almost unchanged in the liquid-crystalline phase in the presence of 33 mol % cholesterol. The total fluorescence intensity followed a single exponential decay independently of the cholesterol content 0–50 mol %.

Since cholesterol is a major lipid component of many biomembranes, the understanding of its interaction with phospholipids is essential for the description of the hydrophobic environment in which various membrane processes occur.

Cholesterol has been reported to have a "dual effect on fluidity" of phospholipid bilayers. The ordered array of lipid acyl chains in the gel phase is fluidized by the addition of cholesterol, whereas in the liquid-crystalline phase cholesterol reduces the fluidity (Ladbrooke et al., 1968; Lippert & Peticolas, 1971; Oldfield & Chapman, 1971). At sufficiently high concentrations cholesterol abolishes the phase transition (Hinz & Sturtevant, 1972).

The term "fluidity" is, however, somewhat ambiguous and covers several aspects of the dynamic structure of membranes. Different experimental techniques have employed their own operationally defined parameters in order to describe the membrane fluidity. In spin-label or nuclear magnetic resonance studies, for instance, the use of the "order parameter", a time-averaged orientational order of lipid acyl chains, is widespread (Hubbel & McConnell, 1971; Seelig, 1977); with the steady-state fluoresence depolarization method, the "microviscosities" of various membranes were estimated by equating the membrane interior with a reference oil (Shinitzky 1971; Cogan et al., 1973).

In a previous paper (Kawato et al., 1977), we have emphasized the complex nature of the "fluidity" of membranes. Rotational motion of a fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH)[1] embedded in the bilayers of dipalmitoyllecithin (DPL) was examined by the nanosecond time-resolved fluorescence depolarization technique. Its motion was adequately described by a wobbling-in-cone model, where the rod-shaped DPH molecules were assumed to exhibit a uniform rotational diffusion within a confine of a cone. When temperature was raised through the phase transition, the cone angle increased abruptly upon melting of fatty acyl chains, whereas the rate of rotational diffusion (wobbling diffusion constant) increased continuously throughout the temperature range of 10–60 °C. The restriction of the probe rotation presumably comes from the anisotropic environment made of the lipid acyl chains, and the motion of the hydrophobic probe is expected to reflect the tumbling of acyl chains closely. Thus, a description of the dynamic structure of membranes requires at least two parameters, such as the cone angle which represents the static orientational constraint and the wobbling diffusion constant which characterizes the dynamic friction.

In the present paper, we have extended the previous study to explore the effect of cholesterol on the molecular motion in the hydrocarbon region of lecithin–cholesterol vesicles. Again DPH was used to probe its dynamic environment. Cholesterol exerted differential effect on the cone angle and the wobbling diffusion constant.

Experimental Procedures

Materials. DL-α-Dipalmitoyllecithin (Fluka Puriss grade), cholesterol (Tokyokasei Puriss grade), and 1,6-diphenyl-1,3,5-hexatriene (Aldrich Puriss grade) were used without further purification. Purity of the lipids was analyzed by thin-layer chromatography on silica gel G plates using chloroform, methanol, and 7 M ammonia as solvent in the volume ratio 230:90:15. Both lipids were determined pure by the presence of only one spot for each after staining with iodine.

Preparation of Samples. An appropriate amount of DPL–cholesterol mixture was dissolved in chloroform solution and was lyophilized under reduced pressure. The product was dispersed in phosphate buffer of ionic strength 0.6, pH 7.2, to yield a final concentration of 3 to 4.5 mg/mL. Fluorescence labeling was achieved by a 200-fold dilution of a solution of 1 mM DPH in tetrahydrofuran with a vigorously stirred lipid dispersion. The amount of dye added was equivalent to 1 molecule per 800 to 1600 molecules of lipids. Under these conditions, undesirable fluorescence depolarization due to the energy transfer between dye molecules was eliminated. Then, the dispersion was sonicated under nitrogen gas, for about 30 min in a jacketed vessel maintained above 40 °C with a 10-kcycle sonifier (Choonpa Kogyo Model USV-150V) at 50%

full power. The incorporation of DPH into vesicles was followed as an increase in fluorescence intensity. After a few hours above 40 °C, when the fluorescence intensity leveled off, the fluorescence measurements were performed.

To prepare centrifuged vesicles, the sonicated clear samples were centrifuged at 10 000g for 1 h, and the supernatant was used.

Fluorescence Measurements. Decays of the fluorescence polarization and the total fluorescence intensity were measured by a single photon counting fluorometer as described previously (Kinosita et al., 1976). The excitation wavelength used for DPH was 360 nm. Time courses of the emission from samples were analyzed into vertically and horizontally polarized components, $I_V(t)$ and $I_H(t)$, by an analyzer. All emission above 420 nm was collected through cut off filters (Toshiba UV-39 and Hoya L-42). The steady-state fluorescence measurements were performed by the same system after changing the light source to a 500-W xenon lamp.

Since samples of high concentrations of lipids (3–4.5 mg/mL) were used in the fluorescence decay measurements, the depolarization due to the scattered exciting and emitted lights could not be neglected. It was corrected according to the following method. The steady-state emission anisotropy, r^s, was measured under the same condition but at dilute concentration (0.3–0.4 mg/mL) where the scattering of exciting and emitted lights could be neglected. An experimental emission anisotropy decay curve, $r(t)$, was corrected so that its time-averaged value, $\langle r \rangle$, was equal to r^s where $\langle r \rangle$ is defined by

$$\langle r \rangle = \frac{\int \{I_V(t) - I_H(t)\}\mathrm{d}t}{\int \{I_V(t) + 2I_H(t)\}\mathrm{d}t} \tag{1}$$

Analysis. From observed intensities of I_V and I_H, the total fluorescence intensity, I_T, the difference between vertically and horizontally polarized components, I_D, and the emission anisotropy, r, were obtained as

$$I_T = I_V + 2I_H \tag{2}$$

$$I_D = I_V - I_H \tag{3}$$

$$r = I_D/I_T \tag{4}$$

Quantities $I_T(t)$, $I_D(t)$, and $r(t)$ are responses to the excitation pulse whose duration was finite. These quantities are related to $I_T^\delta(t)$ and $r^\delta(t)$, responses to a truly impulsive excitation expressed as $\delta(t)$, Dirac's delta function, by the following convolution equations:

$$I_T(t) = \int_0^t g(t')I_T^\delta(t - t')\mathrm{d}t' \tag{5}$$

$$I_D(t) = \int_0^t g(t')I_T^\delta(t - t')r^\delta(t - t')\mathrm{d}t' \tag{6}$$

where $g(t)$ is the response function of the apparatus. As the apparatus response function, we used $g'(\lambda_{em},t)$ which was the distribution function of the light intensity of flash measured at the central wavelength of the emission (Kawato et al., 1977). In order to determine $I_T^\delta(t)$ and $r^\delta(t)$, a curve-fitting procedure was performed, by assuming that they were expressed as sums of exponential functions:

$$I_T^\delta(t) = \sum_{i=1}^{N} I_i \exp(-t/\tau_i) \tag{7}$$

$$r^\delta(t) = \sum_{j=1}^{M} r_j \exp(-t/\phi_j) \tag{8}$$

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[1] Abbreviations used: DPH, 1,6-diphenyl-1,3,5-hexatriene; DPL, dipalmitoyllecithin.
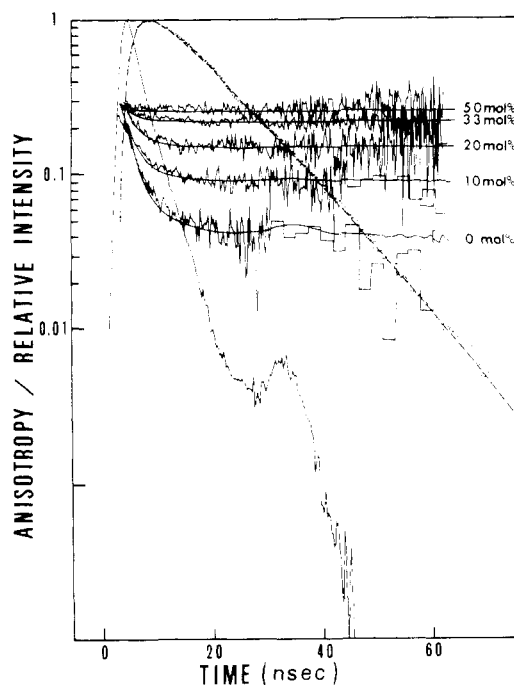
FIGURE 1: Time dependence of the emission anisotropy $r(t)$ for various cholesterol concentrations (zig-zag curves) and the total fluorescence intensity $I_T(t)$ for 50 mol % cholesterol (dots) at 49.5 °C of DPH in DPL-cholesterol vesicles. Thin solid line: the best fit curve of $r(t)$ according to eq 11. Thin dashed line: the best fit curve of $I_T(t)$ for a single exponential approximation (the fluorescence lifetime is 10.6 ns). Thin chain line: the apparatus response function $g'(\lambda_{em}, t)$. $\lambda_{em} = 455$ nm.

where I_i, r_j, τ_i, and ϕ_j are the parameters to be determined. The values of these parameters were so determined as to minimize the following quantities:

$$F_T \equiv \sum_{t_n} [\{I_T(t_n) - I_T{}^{cal}(t_n)\}^2/I_T(t_n)] \qquad (9)$$

$$F_D \equiv \sum_{t_n} [\{I_D(t_n) - I_D{}^{cal}(t_n)\}^2/I_D(t_n)] \qquad (10)$$

where $I_T{}^{cal}$ and $I_D{}^{cal}$ are the calculated fluorescence intensity and difference between vertically and horizontally polarized components, respectively. All calculations were performed on a Hitac 8800/8700 computer, using an iterative least-squares program.

Results

Time courses of the decay of the emission anisotropy, $r(t)$, and those of the fluorescence intensity, $I_T(t)$, of DPH embedded in DPL-cholesterol vesicles were measured over the temperature range 10-60 °C. Typical decays are shown in semilogarithmic plots in Figure 1. The $r(t)$'s of uncentrifuged vesicles were the same within experimental error as those of centrifuged ones when the effect of depolarization due to the scattering of exciting and emitted lights was corrected. As is seen in the figure, $r(t)$'s in all cases consisted of two phases: the initial rapidly decreasing phase and the second almost constant one. Therefore, the decays of the emission anisotropy were analyzed by setting

$$r^\delta(t) = (r_0 - r_\infty) \exp(-t/\phi_1) + r_\infty \qquad (11)$$

where the limiting anisotropy, r_0, of DPH was taken as 0.395 (Kawato et al., 1977). Corresponding best fit curves are shown in thin solid lines in Figure 1. The $r(t)$'s were also deconvoluted by setting $M = 2$ in eq 8 (the two exponential approximation).
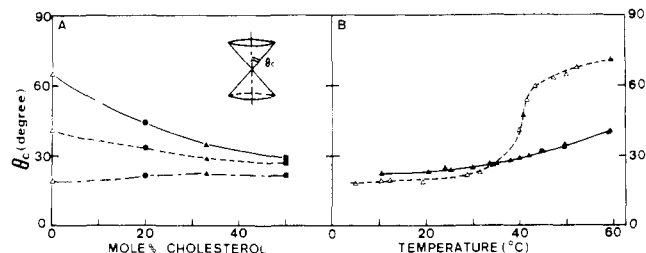

FIGURE 2: (A) The effect of cholesterol on the cone angle at different temperatures of DPH in DPL-cholesterol vesicles. ($- \cdot - \cdot -$) 10.5 °C, ($- \cdot -$) 40 °C, ($-$) 49.5 °C. Cholesterol concentrations are (△) 0, (○) 10, (●) 20, (▲) 33, and (■) 50 mol %. (B) Temperature dependence of the cone angle of DPH for 33 mol % cholesterol (▲) and for pure DPL vesicles (△). Experimental points (△) are taken from Kawato et al. (1977).

Under all conditions the calculated ϕ_2's were greater than 200 ns, thereby justifying using the simpler eq 11.

The presence of the finite residual anisotropy, r_∞, suggests that the rotation of the probe molecules in the DPL-cholesterol bilayers is restricted to a certain angle at least in the time range up to 200 ns. In the following, therefore, we attempt to interpret the anisotropy data in terms of the wobbling-in-cone model. We assume that the orientation of the major axis of DPH is confined within a cone of half angle θ_c around the normal of the membrane, and that the major axis wobbles uniformly in the cone with a diffusion constant D_w. A theoretical calculation (Kinosita et al., 1977) has shown that $r^\delta(t)$ for this model is closely approximated by the expression

$$r^\delta(t) = (r_0 - r_\infty) \exp(-D_w t/\langle \sigma \rangle) + r_\infty \qquad (12)$$

where $\langle \sigma \rangle$ is a constant that depends only on θ_c (see footnote 2) and θ_c is related with r_∞ by

$$\left\{ \frac{1}{2} \cos \theta_c (1 + \cos \theta_c) \right\}^2 = r_\infty/r_0 \qquad (13)$$

Since eq 12 has exactly the same form as eq 11, the motion of DPH in the DPL-cholesterol vesicles can be adequately described by the wobbling-in-cone model. In the case of restricted rotation, ϕ_1 in eq 11 represents the relaxation time with which excited DPH approaches the equilibrium distribution. In this case, in contrast to a free rotation, the inverse of the relaxation time does not necessarily express the rate of rotation. For example, even when the rate of rotation, D_w, does not change, ϕ_1 will be decreased by a decrease of θ_c, because of the relation $\phi_1 = \langle \sigma \rangle D_w{}^{-1}$ ($\langle \sigma \rangle$ is an increasing function of θ_c for $\theta_c \leq 70°$) (Kinosita et al., 1977).

The wobbling diffusion constant, D_w, and the cone angle, θ_c, under various conditions were determined from the experimentally obtained ϕ_1 and r_∞. The effect of cholesterol on the cone angle is illustrated in Figure 2A. In the liquid-crystalline phase at 49.5°C, the incorporation of cholesterol sharply decreased θ_c. In the gel phase at 10.5 °C, on the other hand, θ_c increased slightly as the cholesterol content was raised. An intermediate result was obtained in the phase transition region at 40 °C. The cone angle appears to converge to 30°, irrespective of temperature, at sufficiently high concentrations of cholesterol. The temperature dependence of θ_c in the presence of 33 mol % cholesterol is shown in Figure 2B. The cone angle gradually increased as temperature was raised, and the abrupt increase of θ_c at the transition in the absence of cholesterol was almost completely suppressed by the addition of cholesterol.

[2] In the previous paper (Kinosita et al., 1977), a denominator in eq 34 was omitted by mistake. Correct equation should read $\langle \sigma \rangle = (\Sigma_{i \neq \infty} A_i \sigma_i)/(1 - A_\infty)$.
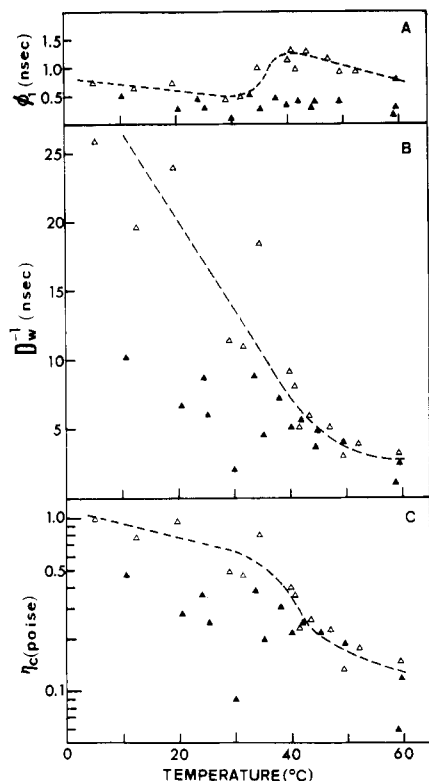
FIGURE 3: (A) The effect of cholesterol on the relaxation time of DPH in DPL-cholesterol vesicles. Cholesterol concentrations are (△) 0 and (▲) 33 mol %. (B) Temperature dependence of the inverse of wobbling diffusion constant D_w^{-1} of DPH for 33 mol % cholesterol (▲) and for pure DPL vesicles (△). (C) Temperature dependence of the viscosity in the cone for 33 mol % cholesterol (▲) and for pure DPL vesicles (△). Experimental points (△) are taken from Kawato et al. (1977).

The relaxation time, ϕ_1, and the inverse of the wobbling diffusion constant, D_w^{-1}, in the presence (33 mol %) and absence of cholesterol are plotted against temperature in Figures 3A and 3B. Cholesterol decreased the relaxation time to less than 0.6 ns over the temperature range 10-60 °C. The values of D_w^{-1} were rather widely scattered because of the small ϕ_1 values. The scatter allows only a qualitative conclusion: in the presence of cholesterol, D_w^{-1} decreases appreciably below the phase transition, while D_w^{-1} changes little above the transition.

The viscosity in the cone, η_c, which represents the dynamic friction against the wobbling motion, was estimated from the following equation:

$$\eta_c = \frac{kT}{6D_w V_e f} \tag{14}$$

where V_e and f denote the effective volume and the shape factor of the probe, k is the Boltzmann's constant, and T is the absolute temperature. The $V_e f$ of DPH was taken as 1.7×10^{-22} cm^3, a value estimated in liquid paraffin (Kawato et al., 1977). Figure 3C shows that η_c for 33 mol % cholesterol is smaller than that in pure DPL in the gel phase and that η_c for 33 mol % is almost the same as that in pure DPL in the liquid-crystalline phase.

Decays of the total fluorescence intensity, $I_T(t)$, were analyzed by setting $N = 1$ or 2 in eq 7. A typical $I_T(t)$ for 50 mol % cholesterol is shown in Figure 1 together with the best single-exponential fit in thin broken line. The best fit curve for a double exponential approximation was almost completely the same as that for the single exponential approximation. Over the temperature range 10-60 °C, the fluorescence intensity
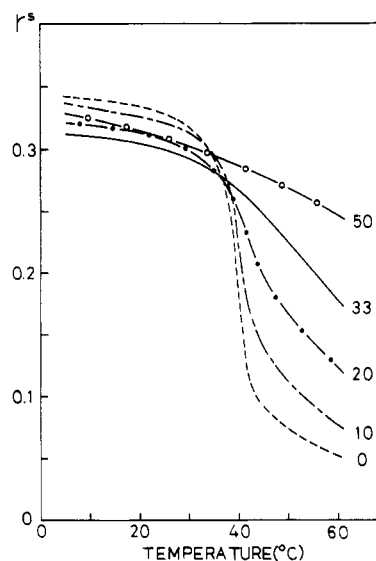

FIGURE 4: The effect of cholesterol on the temperature dependence of the steady-state emission anisotropy r^s of DPH in DPL-cholesterol vesicles. Cholesterol concentrations are (- - -) 0, (— · —) 10, (— ● —) 20, (—) 33, and (— O —) 50 mol %. Deviations of experimental points from the curves were within ±0.003 in the gel phase and within ±0.002 in the liquid-crystalline phase. Temperature interval of experimental points of each r^s curve was about 2 °C.

TABLE I: Fluorescence Lifetime (ns) of DPH in DPL-Cholesterol Vesicles.[a]

temp (°C)	mol % cholesterol				
	0	10	20	33	50
10.5	10.2	9.4	9.7	9.1	10.0
24.0	11.0			9.8	
33.5	11.1			10.0	
40.0	9.8			9.5	
44.5	9.0			9.7	
49.5	8 4	8.7	9.8	9.5	10.6
59.0	7.6			9.1	

[a] The lifetimes for pure DPL vesicles are taken from Kawato et al. (1977).

followed a single exponential decay both in the presence and in the absence of cholesterol (0 to 50 mol %). As shown in Table I, the lifetime was changed by the presence of cholesterol. Below the phase transition at 10.5 °C cholesterol decreased the lifetime slightly, and above the transition at 49.5 °C the lifetime increased in the presence of cholesterol. In the presence of 33 mol % cholesterol, the lifetime was almost constant ranging between 9.1 and 10.0 ns over the whole temperature range.

The steady-state emission anisotropy, r^s, of DPH in DPL-cholesterol vesicles was measured as a function of temperature (Figure 4). The value of r^s decreased in the gel phase ("fluidization") and increased in the liquid-crystalline phase ("crystallization") up to 33 mol % cholesterol. When the cholesterol content was raised further, r^s increased over the whole temperature range. The phase transition observed in r^s almost vanished at 33 mol % cholesterol. Each of the decay data concerning the effect of cholesterol was collected within 24 h after preparation, and the temperature profiles shown in Figure 4 remained unchanged during this period.

Discussion

The decays of emission anisotropy observed were biphasic and did not approach zero at long time after the excitation.

Similar results were reported by Chen et al. (1977) for DPH in L-α-dimyristoyllecithin vesicles and by Dale et al. (1977) for DPH in egg lecithin vesicles. In general, the presence of the finite residual anisotropy, r_∞, can be interpreted by either of the following models. (1) The probe undergoes the rapid rotation restricted in a cone formed by the lipid acyl chains (Kawato et al., 1977; Dale et al., 1977; Lakowicz & Prendergast, 1978). (2) There are two classes of probes: one population consists of trapped probe molecules that cannot rotate appreciably in the time scale of the measurement, and the other consists of free-rotating probe molecules (Weber et al., 1976). We consider that the first model is more plausible for the interpretation of the present observations because of the following reasons. (1) For pure DPL vesicles, ϕ_1 was increased by the phase transition (Figure 3A). In the mixed population model, this finding would indicate the unlikely case of a slower motion in the free rotating population in spite of the "fluidization". On the other hand, this is easily explained by the wobbling-in-cone model because the abrupt increase in θ_c overcomes the increase in D_w (Kawato et al., 1977). (2) Fluorescence polarization experiments on oriented multilayers have shown that below the phase transition the major axis of DPH is oriented along the normal of the membrane (Andrich & Vanderkooi, 1976). (3) Many spin label or nuclear magnetic resonance studies (Israelachvili et al., 1975; Seelig, 1977) have indicated an orientational order of lipid acyl chains in bilayers suggesting a highly anisotropic environment for the rod-shaped DPH molecules. Although conclusive evidence awaits further investigations, we tentatively adopt the view of the restricted rotation.

Since the thickness of rod-shaped DPH approximates one acyl chain of a lipid molecule, each probe molecule presumably replaces one acyl chain in the bilayer structure. Since the hypothetical cone around DPH would be filled with lipid acyl chains, the tumbling of the DPH rod occurs as a neighboring acyl chain(s) wobbles out of the cone. Thus, the restricted rotation of DPH directly reflects the thermal motion of lipid acyl chains.

Previous reports concerning the effect of cholesterol on the lipid bilayers can be summarized as the "dual effect on fluidity of lipids": cholesterol increases the mobility of lipid acyl chains in the gel phase, but decreases it in the liquid-crystalline phase (Oldfield et al., 1971; Oldfield & Chapman, 1972). However, when the mobility of DPH was decomposed into two components, θ_c and D_w, the wobbling diffusion constant did not show the "dual effect": D_w increased below the phase transition and changed little above the transition in the presence of cholesterol. Since the motion of lipid acyl chains is expected to be similar to the restricted rotation of DPH as discussed above, the tumbling rate of acyl chains would not show the "dual effect". Thus the accurate picture of the "dual effect on fluidity of lipids" appears to be complicated. The effect of cholesterol on the cone angle would be called the "dual effect": the addition of cholesterol decreased θ_c greatly in the liquid-crystalline phase, but slightly increased it in the gel phase. Therefore, the reported changes in the fluidity in the presence of cholesterol mainly reflect the change of the cone angle.

The differential effect of cholesterol on θ_c and D_w may be explained by a difference between two regions of cholesterol molecule. A cholesterol molecule has a bulky rigid steroid nucleus and a relatively narrow flexible tail. Cholesterol is largely immersed in the hydrocarbon core of the bilayer, with its methyl chain terminus near the bilayer center and its hydroxyl group near the water interface (Franks, 1976). The long axis of the cholesterol molecule is perpendicular to the bilayer plane (Engelman & Rothman, 1972; Franks, 1976). Thus, a Corey–Pauling–Koltun model tells us that the terminal methyl portion of the lipid acyl chain (adjacent to the narrow flexible tail) will be allowed greater conformational freedom than the rest (adjacent to the bulky rigid steroid nucleus).

The difference between these two regions of cholesterol molecule may cause the differential effect upon the motion of fatty acyl chains. In the gel phase, the addition of cholesterol disturbs the packing of lipid acyl chains (Engelman & Rothman, 1972), and may chiefly increase the conformational freedom and the tumbling rate of lower half of chains which are adjacent to the narrow tails. As a result of these changes of acyl chains, the cone angle and the wobbling diffusion constant of DPH might increase below the phase transition. In the liquid-crystalline phase, the range of swinging motion of the upper half of fatty acyl chains will be suppressed by the bulky rigid steroid nuclei of cholesterol. Therefore, the cone angle decreases. No decrease of the tumbling rate of DPH may reflect that the wobbling rate of DPH strongly depends on the tumbling motion of lower half of lipid acyl chains which is much less hindered by the addition of cholesterol (Stoffel et al., 1974). Or the wobbling rate, D_w, may strongly depend on the rapid rotational isomerization of acyl chains which is not appreciably affected by the addition of cholesterol above the transition (Godici & Landsberger, 1975). Cholesterol and DPL may mix unideally in sonicated vesicles which have small radii of curvature (Lentz et al., 1976a,b). Investigation of this possibility may be required before a detailed picture of the differential effect is obtained.

The decays of fluorescence intensity were expressed by single lifetimes over the cholesterol content 0–50 mol %. This may indicate that all DPH molecules embedded in lecithin–cholesterol vesicles are surrounded by the similar environment. This finding of single lifetimes may be interpreted as that there are no distinctly separated phases such as pure lecithin and lecithin–cholesterol complex phases (Ladbrooke et al., 1968; Darke et al., 1972; Engelman & Rothman, 1972; Hinz & Sturtevant, 1972), or that the exchange rate between these two phases, if there is such, is much faster than the fluorescence lifetime. The lifetime of DPH decreased in the gel phase and increased in the liquid-crystalline phase in the presence of cholesterol. Therefore, the environment of the probe appears to be changed by cholesterol. Double exponential decays of the fluorescence intensity were reported for DPH embedded in dimyristoyllecithin or egg lecithin vesicles (Chen et al., 1977; Dale et al., 1977). In the present stage, we cannot explain the difference between these results and our finding concerning the number of decay components.

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Purification, Characterization, and Amino Acid Sequence of Rat Anaphylatoxin (C3a)[†]

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ABSTRACT: C3a anaphylatoxin derived from the third component of complement has been isolated from rat serum and its complete amino acid sequence determined. A three-step purification procedure was employed that consisted of gel filtration on Sephadex G-100, followed by chromatography of the anaphylatoxin-containing pool on carboxymethylcellulose. A subsequent separation on DEAE-Sephadex resolved C3a from minor contaminating peptides. Biological studies have shown that purified rat anaphylatoxin is approximately twice as active as human or porcine C3a when tested for smooth-muscle contraction. In addition to the active form of rat anaphylatoxin, a serum carboxypeptidase B inactivated form of C3a ($C3a_{des-Arg}$) was purified from rat serum and utilized in subsequent structural studies. Sequence analysis of rat C3a was facilitated by a long automated Edman degradation which established the first 55 residues of the anaphylatoxin. Overlapping peptides were generated by cyanogen bromide and trypsin, and the resultant fragments were sequenced by either automated or manual Edman procedures. The primary structure of rat C3a is 70% identical to the previously determined structures of human and porcine anaphylatoxin. Antisera raised to the purified rat peptide do not cross-react immunologically by Ouchterlony analysis with either human or porcine C3a.

Studies of anaphylatoxins from a number of species have established that this peptide serves as a mediator of the local inflammatory response (Hugli and Müller-Eberhard, 1978). Micromolar concentrations of C3a induce histamine release from isolated mast cells (Dias da Silva and Lepow, 1967; Johnson et al., 1975) and produce smooth-muscle contraction (Dias da Silva et al., 1967; Cochrane and Müller-Eberhard, 1968). In addition, C3a has pronounced effects on vascular permeability (Dias da Silva and Lepow, 1967; Wuepper et al., 1972). The isolation and characterization of anaphylatoxins from both human and porcine sera revealed that the two peptides have essentially identical biological properties (Hugli et al., 1975b). Structural studies have shown a 70% homology in amino acid sequence between porcine and human C3a, suggesting that similarities in biological activities result from homologous primary structures (Hugli, 1975; Corbin and Hugli, 1976). Both peptides require a carboxy-terminal arginine residue for biological activity and are rapidly inactivated by carboxypeptidase B digestion (Hugli et al., 1975b). In spite of these chemical similarities, immunological studies demonstrate no visible cross-reactivity between porcine and human C3a by Ouchterlony analysis, suggesting that key antigenic determinants differ between the two anaphylatoxins.

This report extends the biological and structural characterization of anaphylatoxins to rat C3a. Studies on rat anaphylatoxin grew out of our initial efforts to purify rat somatomedin. The somatomedins are a family of insulin-like peptides which exhibit growth-promoting effects in a variety of tissues (Daughaday et al., 1972; Van Wyk et al., 1974). It was