BILAYER ACYL CHAIN DYNAMICS AND LIPID-PROTEIN INTERACTION

THE EFFECT OF THE M13 BACTERIOPHAGE COAT PROTEIN ON THE DECAY OF THE FLUORESCENCE ANISOTROPY OF PARINARIC ACID

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ABSTRACT Nanosecond fluorescence polarization anisotropy decay is used to determine the effect of the bacteriophage M13 coat protein on lipid bilayer acyl chain dynamics and order. The fluorescent acyl chain analogues *cis*- and *trans*-parinaric acid were used to determine the rate and extent of the angular motion of acyl chains in liquid crystalline (39°C) dimyristoylphosphatidylcholine bilayers free of coat protein or containing the coat protein at a protein:lipid ratio of 1:30. Subnanosecond time resolution was obtained by using synchrotron radiation as the excitation source for single photon counting detection. Previous measurements of Förster energy transfer from coat protein tryptophan to *cis*- or *trans*-parinaric acid have shown that these probes are randomly distributed in the bilayer with respect to the protein. The anisotropy decay observed for pure bilayers has the form of a rapid drop, followed by a nonzero constant region extending from roughly 3 ns to at least 12 ns. The magnitude of the anisotropy in the plateau region is simply related to the acyl chain order parameter. The effect of the M13 coat protein is to increase the acyl chain order parameter significantly while having only a small effect on the rate of angular relaxation. This behavior is rationalized in terms of a simple microscopic model. The order parameters for pure lipid and coat protein containing bilayers are compared to ^{2}H-NMR values.

INTRODUCTION

Time-resolved fluorescence polarization anisotropy decay has several unique features which make this technique particularly well suited for studies of anisotropic or restricted rotational diffusion. The time-dependent anisotropy, $r(t)$, provides a direct, real time measurement of reorientational motion in the 10^{-10} to 10^{-7} s time range. Anisotropic motion is revealed as multiple exponential components in the decay of $r(t)$. Restriction of the range of reorientational motion is revealed as a constant asymptotic value of $r(t)$. For biophysical applications, where very low illumination levels are mandatory and very reliable data may be needed, the single photon counting technique is the method of choice for obtaining the time resolved data. The time resolution of this fluorescence method has been extended into the subnanosecond range by the use of synchrotron radiation (Munro et al., 1979; Lopez-Delago et al., 1974) and high repetition rate, short pulse laser systems (see, for example, Koester and Dowben, 1977; Harris et al., 1977; Richardson et al., 1979). Reorienting motions which are faster than the time resolution of the system are revealed as a value of $r(t)$ evaluated at $t=0$, which is less than the value determined when there is no reorientational motion. This extends the range of the technique to times shorter than the intrinsic resolution.

This paper describes the application of this technique to the study of lipid-protein interactions. The reorientational motion being studied is that of the chromophore of the lipid analogue parinaric acid (PnA) (Wolber and Hudson, 1981; Kimelman et al., 1979; Sklar et al., 1977; 1975). The anisotropy decay for this chromophore in liquid crystalline (fluid) phase 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) bilayers has recently been presented (Wolber and Hudson, 1981). Similar results have been obtained for diphenylhexatriene using flashlamp excitation (Kawato et al., 1977). These studies have shown how fluorescence anisotropy measurements can separate motional rates from order. The anisotropy reached after a few nanoseconds remains constant for the remainder of the available time range. This asymptotic anisotropy is simply related to the acyl chain order parameter and the value

obtained from fluorescence may be compared to ^{2}H-NMR values (Wolber and Hudson, 1981; Heyn, 1979; Jähnig, 1979).

The effect of the addition of a protein to the lipid bilayer on acyl chain dynamics and order may therefore be determined by changes in the rate of decay of $r(t)$ and its asymptotic value. This study deals with the effect of the M13 bacteriophage coat protein on these two aspects of acyl chain motion. The coat protein of the filamentous M13 bacteriophage (equivalent to the fd protein) is a 50 residue polypeptide which can be easily reconstituted into synthetic 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) bilayers using a cholate detergent dilution method (Wickner, 1977; 1976). It has been shown that this method results in large, single-walled vesicular structures with coat protein spanning the bilayer as it does in vivo in the *E. coli* membrane (Wickner,1976). The amino acid sequence of this protein (Asbeck et al., 1969; Nakishima and Konigsberg. 1979) is remarkably modular, consisting of a highly acidic segment of 20 residues at the N-terminus, a sequence of highly hydrophobic residues (21–39), and a basic sequence of eleven residues at the C-terminus.

A previous study of this lipid-protein system using PnA (Kimelman et al., 1979) produced two observations which are relevant to the present study. The first is the demonstration that both isomers of PnA (see Materials and Methods) are randomly distributed in the plane of the fluid bilayer with respect to the coat protein. This was determined using Förster energy transfer from the single tryptophan of the coat protein to PnA in conjunction with the theory of energy transfer in two dimensions (Wolber and Hudson, 1979). The other relevant observation in this earlier work was that addition of the coat protein results in an increase in the steady-state polarization of PnA fluorescence from fluid bilayers. This observation, taken in combination with the known minimal perturbation of the fluorescence quantum yields of both probes upon addition of coat protein, means that the protein inhibits acyl chain motion on the ns timescale either by slowing down the rate of that motion or by decreasing its amplitude. The time resolved anisotropy results presented here can distinguish between these two possibilities.

MATERIALS AND METHODS

Coat protein (CP) was prepared from M13 bacteriophage (gift of Dr. W. Wickner) according to Knippers and Hoffman-Berling (1966), and reconstituted into DMPC vesicles (Sigma Chemical Co., St. Louis, MO) by the cholate dilution method (Racker et al., 1975; Wickner, 1976, 1977; Kimelman et al., 1979). The coat protein is known to be completely oriented in such preparations. The *cis* and *trans* isomers of parinaric acid, all *trans* 9,11,13,15-octadecatetraenoic acid (tPnA), and cPnA, the *cis, trans, trans, cis* isomer of tPnA, were prepared and stored as described (Sklar et al., 1977; Wolber and Hudson, 1981). Vesicle suspensions were labeled with aliquots of a 2 mM ethanolic solution of PnA, at a temperature at least 5°C above the DMPC phase transition (23°C).

Time-resolved fluorescence decays were measured with a modified Ortec time resolved fluorometer at the Stanford Linear Accelerator

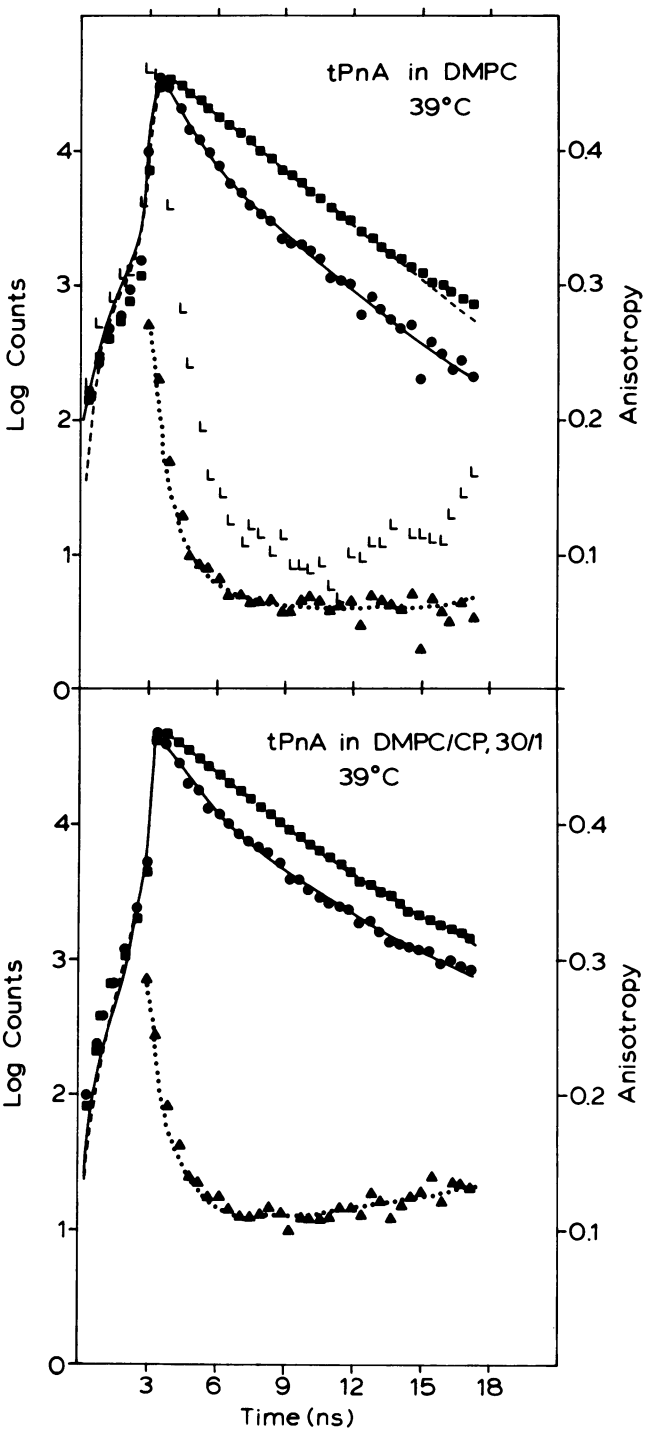

FIGURE 1 Total and difference intensity decays for tPnA in DMPC (upper panel) and CP/DMPC, 30/1 (lower panel), at 39°C. Fits to the difference (—) and total (- -) decays, as well as every third difference (- ● -) and total (- ■ -) decay experimental point are shown. Also plotted are the fit (· · ·) and experimental (- ▲ -) values for the undeconvoluted anisotropy, $R(t)$ (see right hand, linear ordinate scale). Every third point of the excitation pulse shape for all decays (L) is plotted logarithmically in the upper panel.

Center (SLAC), as described (Munro et al., 1979; Wolber and Hudson, 1981). Vertically polarized synchrotron radiation (320 nm) was used for excitation, and the intensities polarized parallel ($I_{VV}(t)$) and perpendicular ($I_{VH}(t)$) to the excitation polarization were measured at 90°C to the excitation beam. The measured decays were combined to give the total decay,

$$F(t) = I_{VV}(t) + 2I_{VH}(t), \tag{1a}$$

and the difference decay,

$$D(t) = I_{VV}(t) - I_{VH}(t). \tag{1b}$$

The total and difference decays measured are convolutions of the delta-function excitation response functions $f(t)$ and $d(t)$ with the excitation pulse shape. The functions $f(t)$ and $d(t)$ have been modeled by sums of exponentials; the details of the deconvolution process are described elsewhere (Wolber, 1980; Wolber and Hudson, 1981). Scaling factors C_f and C_d have been removed from $f(t)$ and $d(t)$, so that $f(0)=d(0)=1$. The ratio $C_d/C_f = r(0)$ is the fit approximation to r_0, the zero-time (or limiting) anisotropy. The fit approximation to $r(t)$ is given by

$$r(t) = r(0)d(t)/f(t). \tag{2}$$

The test function used to fit $d(t)$ assumes that $r(t)$ decays to a nonzero asymptote [i.e. the longest lifetime in $d(t)$ is fixed equal to the longest lifetime in $f(t)$]. This asymptote, $r_\infty^{(fit)}$, is given by

$$r_\infty^{(fit)} = \lim_{t\to\infty} r(t). \tag{3}$$

Alternatively, the undeconvoluted anisotropy $R(t) = D(t)/F(t)$ may be used to calculate $r_\infty^{(tail)}$ by averaging several values in the asymptotic time range.

The kinetic and order properties measured have been quantitated as the order parameter (S_{fit} or S_{tail}) and the ensemble average inverse rotational correlation time $\langle\phi^{-1}\rangle$, respectively (Kinosita et al., 1977):

$$|S| = |\langle(3\cos^2\theta - 1)/2\rangle| = (r_\infty/r_0)^{1/2}, \tag{4a}$$

$$\langle\phi^{-1}\rangle = 6D_w = -\lim_{t\to\infty}\frac{d}{dt}[r(t)/r_0]. \tag{4b}$$

In Eq. 4a, θ is the angle between the PnA emission transition dipole and the local bilayer normal. In Eq. 4b, D_w is the "wobbling diffusion coefficient." In both equations, brackets $\langle\rangle$ denote ensemble averaging, and r_0 has been assumed to be 2/5, the theoretical maximum.

RESULTS

The time-resolved difference and total intensity decays of tPnA in DMPC and CP/DMPC, 1/30 (both at 39°C) are shown in Fig. 1. The experimental and fit values of the undeconvoluted anisotropy $R(t)$ are also plotted. Decays for cPnA in the same samples are shown in Fig. 2. The fit decay forms, and the values of the initial decay rates $\langle\phi^{-1}\rangle$, order parameters S, and limiting anisotropies $r(0)$ calculated from the fits are tabulated in Table I.

It is obvious from Figs. 1 and 2 that the ratio of the difference and total decays for all samples decays rapidly at short times [indicating a rapid initial decay of $r(t)$] and approaches a nonzero asymptote at long times. In the case of tPnA in CP/DMPC, 1/30, the ratio of the difference and total decays actually falls to a value below the asymptote and then increases. This peculiar behavior indicates the presence of multiple environments with different total and difference decays (see Discussion). All of the features shown by $R(t)$ are also shown by the fit approximations to $r(t)$, plotted in Fig. 3. In addition, the deconvoluted anisotropy decays extrapolate to values of

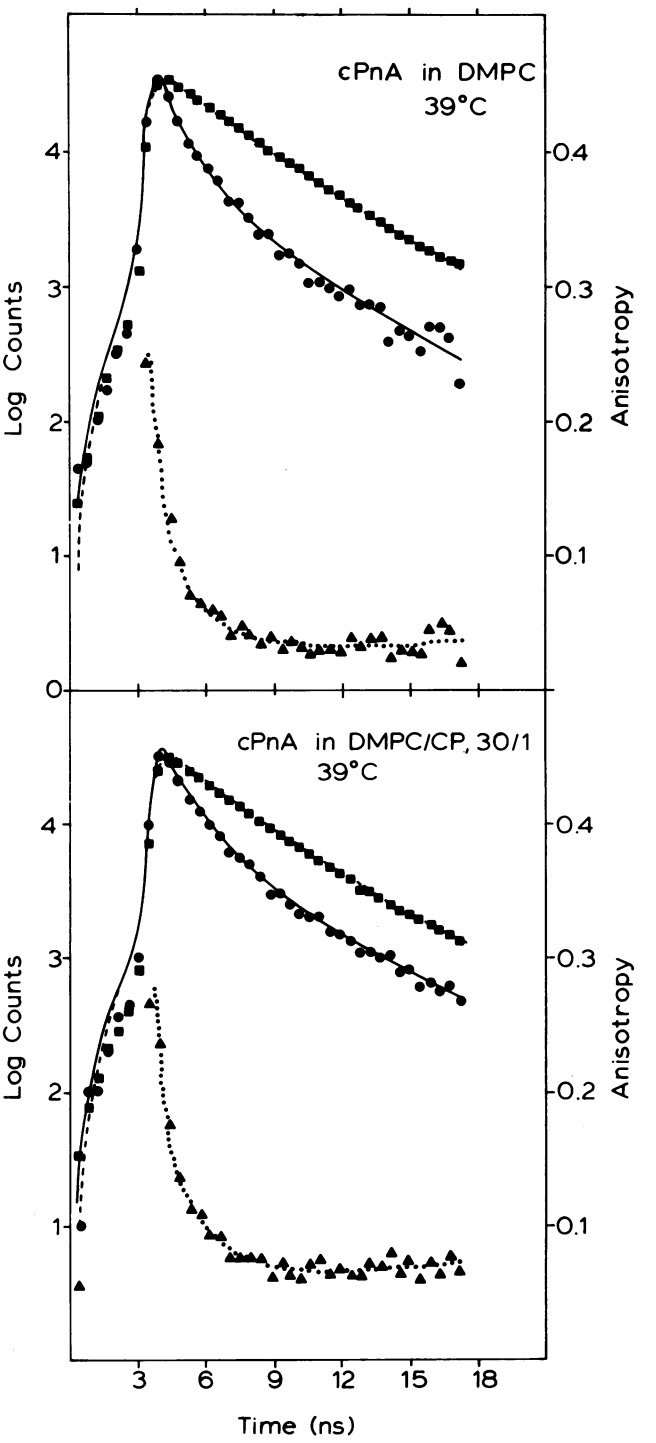

FIGURE 2 Total and difference decays for cPnA in DMPC (upper panel) and CP/DMPC, 30/1 (lower panel), at 39°C. All lines and symbols are as in Fig. 1.

TABLE I
DECONVOLUTED DATA FITS AND CALCULATED RESULTS

probe	CP/DMPC	$d(t)$	$f(t)$	$\langle\phi^{-1}\rangle$	S_{fit}	S_{tail}	$r(0)$	$\bar{\tau}$
				(*GHz*)				(*ns*)
tPnA	0/30	$0.583e^{-t/0.17}$ $+0.270e^{-t/1.02}$ $+0.147e^{-t/3.15}$	$e^{-t/3.15}$	3.50	0.39	0.38	0.41	3.15
tPnA	1/30	$0.503e^{-t/0.32}$ $+0.351e^{-t/1.44}$ $+0.146e^{-t/4.74}$	$0.621e^{-t/2.22}$ $+0.379e^{-t/4.74}$	1.18	0.57	0.61	0.35	3.18
cPnA	0/30	$0.622e^{-t/0.18}$ $+0.310e^{-t/1.11}$ $+0.068e^{-t/4.20}$	$0.263e^{-t/2.15}$ $+0.737e^{-t/4.20}$	3.28	0.30	0.30	0.38	3.66
cPnA	1/30	$0.555e^{-t/0.21}$ $+0.353e^{-t/1.38}$ $+0.092e^{-t/4.91}$	$0.529e^{-t/2.46}$ $+0.471e^{-t/4.91}$	2.50	0.44	0.44	0.39	3.61

$r(0)$ very close to 2/5, indicating that the early time decays are accurately represented (Wolber and Hudson, 1981).

The calculated quantities in Table I demonstrate several points. The large increase in static fluorescence anisotropy seen upon addition of CP to DMPC (Kimmelman et al., 1979) is primarily due to an increase in the asymptotic anisotropy r_∞ (and, therefore, the order parameter S); the initial decay rate $<\phi^{-1}>$ also decreases upon addition of CP to DMPC. The average lifetimes $\bar{\tau}$ (i.e., preexponential weighted average of the fit lifetimes) for both isomers of parinaric acid are not affected by the addition of CP, in agreement with quantum yield measurements (Kimelman et al., 1979). Finally, the results of repeated fits using different numbers of data points, and the results of "condition tests" (Wilkinson, 1972) both indicate that the double exponential fits to the total decays of cPnA in DMPC and

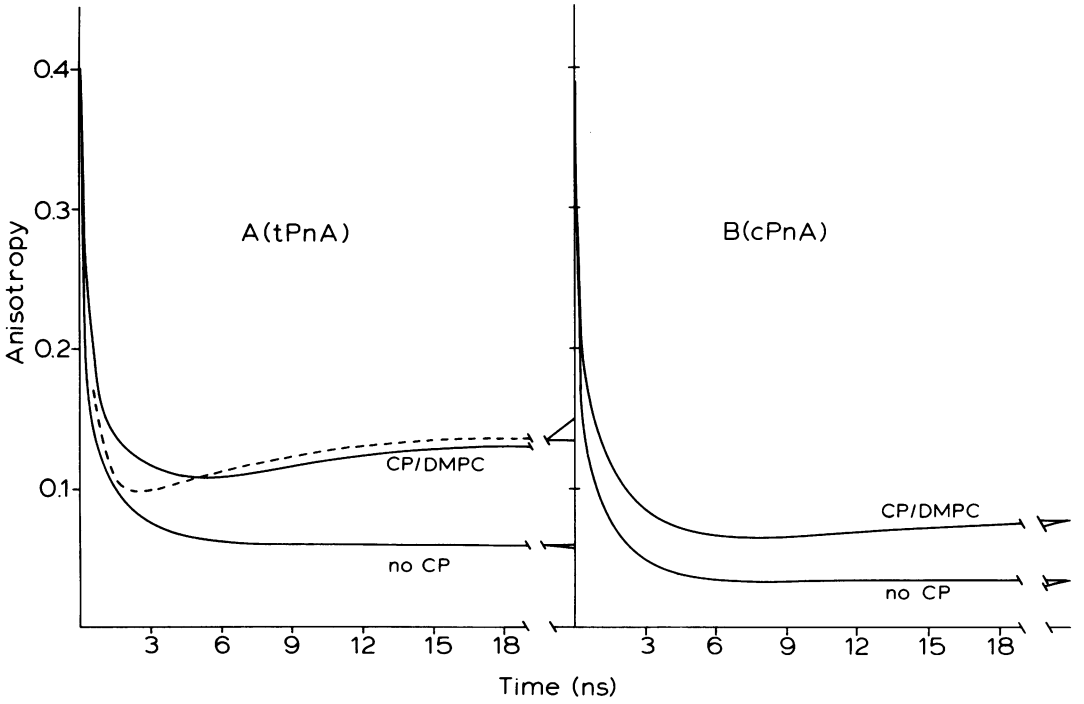

FIGURE 3 Deconvoluted anisotropy decays. Panel *A* shows d econvoluted decays (see Eq. 2 and Table I) for tPnA in DMPC and CP/DMPC, 1/30 (—). Values of $r_\infty^{(fit)}$ and $r_\infty^{(tail)}$ are denoted by the intersections of horizontal and diagonal lines, respectively, with the right margin. Also shown is a model anisotropy decay (---) of the form $r(t) = \Sigma_{i-1}^{2}\{[r_0 - r_\infty^{(i)}]e^{-t/\phi}\}\chi_i e^{-t/\tau_i}/\Sigma_{i-1}^{2}\chi_i e^{-t/\tau_i}$. The values of the preexponential weights χ_i and the lifetimes τ_i ($\tau_2 > \tau_1$) were those for tPnA in the presence of CP (Table I), the value of r_0 was 0.35, the values of $r_\infty^{(1)}$ $r_\infty^{(2)}$ were the same as $r_\infty^{(fit)}$ for tPnA in the absence and presence of CP (Table I), and ϕ was 0.52 ns, the harmonic mean of the rotational correlation times for tPnA in DMPC. The decay clearly shows a minimum below r_∞, and simulates the case of two environments which differ only in their values of r_∞ and τ. Panel *B* plots $r(t)$ for cPnA, as in panel *A*.

both PnA isomers in CP/DMPC are not well determined (i.e., cooperative distortions of the fit parameters will yield fits nearly as good). It is most probable that in these systems there exists a spectrum of lifetimes. This sort of behavior is seen in other fluid lipids (Wolber, 1980; Wolber and Hudson, 1981). Therefore, the individual fit parameters may not have much physical significance, even though the parameters calculated from the fits do have physical significance.

It should be noted that all of the important physical effects at long times are seen in the raw data, and that the effects at short times, which must be calculated from the deconvoluted decays, can be checked by examining the calculated value of $r(0)$, and comparing to the measured value of r_0 for PnA (very close to 2/5, the theoretical maximum). In spite of the ill-conditioned nature of the fitting problem, the forms of $f(t)$ and $d(t)$ extracted from the data are good numerical fits to the real forms of $f(t)$ and $d(t)$, inasmuch as they pass consistency checks at short and long times, they correctly predict the observed static fluorescence results, and they provide excellent fits to the observed decays.

DISCUSSION

The addition of CP to fluid DMPC substantially increases the order sensed by both isomers of PnA. The effects seen raise two questions: what fraction of the lipid is perturbed by CP, and what is the cause of the ordering effect? To answer these questions, we must clearly define what is being measured.

The anisotropy decay $r(t)$ is an average over all fluorophore environments. The weighting coefficients in this average will be time dependent if the fluorophore lifetime depends upon environment. The initial decay of $r(t)$ for tPnA in CP/DMPC to a value below $< r_\infty^{(\mathrm{fit})} >$, followed by a rise to this asymptotic value (Fig. 3 *A*), indicates that there are at least two species with different lifetimes present, and that the species with the higher quantum yield has a larger intrinsic value of r_∞ (i.e., its environment is more ordered). A simulated decay for this situation is shown in Fig. 3 *A*. This behavior agrees with that seen for tPnA in pure lipids: ordered environments produce high quantum yields (Wolber and Hudson, 1981).

The $r(t)$ decay for cPnA does not show a pronounced minimum at intermediate times, indicating less enhancement of the cPnA quantum yield by CP. This difference between cPnA and tPnA is in agreement with pure lipid results. Extensive measurements with cPnA and tPnA indicate that the relative quantum yield for tPnA increases 4.9% upon addition of CP to fluid DMPC, while the relative quantum yield for cPnA remains constant within the 1% experimental error (Wolber, 1980).

This constancy of the cPnA lifetime implies that r_∞ for cPnA is the average of r_∞ values for all environments, weighted by the fractions of cPnA in each environment. Thus, the simplest expression for r_∞ is

$$r_\infty = f_P r_\infty^{(P)} + (1 - f_P) r_\infty^{(NP)}, \quad (5)$$

where f_P is the fraction of cPnA in a perturbed environment, $r_\infty^{(P)}$ is the value of r_∞ in that environment, and $r_\infty^{(NP)}$ is the value of r_∞ for cPnA in pure DMPC. Eq. 5 can be rearranged to give

$$f_P = [r_\infty - r_\infty^{(NP)}]/[r_\infty^{(P)} - r_\infty^{(NP)}]. \quad (6)$$

The question now becomes how to calculate $r_\infty^{(P)}$ for cPnA. One reasonable method is to assume that $r_\infty^{(P)}$ is the same for cPnA and tPnA. This is expected if the motion of a PnA molecule near a CP molecule is restricted to the same range, regardless of the PnA isomer. An alternative assumption is that the values of $r_\infty^{(P)}$ for cPnA and tPnA are in the same ratio as the values of $r_\infty^{(NP)}$ for the two isomers. This assumes that the value of $r_\infty^{(NP)}$ for cPnA is lower due to the existence of an additional depolarization mechanism for cPnA, and that this mechanism is not affected by CP; i.e., the order parameter for cPnA is the order parameter for tPnA times the order parameter for the additional mechanism (Petersen and Chan, 1977).

These two assumptions yield values for f_P of 0.44 and 1.01, respectively. The second value cannot be correct, since it contradicts the observation from the $r(t)$ data that at least two environments are present, as well as the observation that raising the concentration of CP from 1/60–1/30 more than doubles the enhancement of the static anisotropy (Kimelman et al., 1979), which does not make sense if the ordering saturates at 1/30 CP/DMPC. The value of $f_P = 0.44$ translates into six to seven lipids perturbed per monolayer per CP (CP spans the bilayer). If the CP is about the size of a lipid, this amounts to one boundary layer. If the value of r_∞ for cPnA is in fact skewed towards $r_\infty^{(P)}$, the number of perturbed lipids is overestimated by Eq. 6. Therefore, it is likely that the perturbation of fluid DMPC by CP is restricted to the layer of lipid contacting the protein.

The microscopic source of the order enhancement is most easily understood by considering the data for tPnA. Fluorescence from tPnA can be depolarized by three motions: introducing *gauche* bonds in the acyl chain above the chromophore; rotating a bent molecule about the average (i.e, fully extended) axis; and swinging the entire molecule, which changes the average axis. Studies on pure lipids indicate that the last motion is probably not important (Wolber and Hudson, 1981). A little thought shows that the second motion is also unimportant, since the introduction of a *gauche* bend changes the fluorophore emission transition dipole direction by 60°, very close to the "magic angle" of 55°, which produces complete depolarization. Rotation about the average axis affects only bent molecules, which are probably already completely depolarized.

The above argument can be quantitated. Since the polyene chromophore of PnA is stiff and fully extended (Wolber and Hudson, 1981), the transition dipole of PnA

points along the acyl chain director at the "hinge" at C9 (the director $\hat{\mathbf{q}}$ is a unit vector pointing along the sum of the C—C bond vectors into and out of C9). Six directors (not counting signs) along the six edges of a tetrahedron are possible. Let $\hat{\mathbf{q}}_1$ be the director for the fully extended chain, and let $\hat{\mathbf{q}}_6$ be the one director perpendicular to $\hat{\mathbf{q}}_1$. Then $\hat{\mathbf{q}}_2$, $\hat{\mathbf{q}}_3$, $\hat{\mathbf{q}}_4$, $\hat{\mathbf{q}}_5$ are at 60° $\hat{\mathbf{q}}_1$.

If a director $\hat{\mathbf{q}}$ exists with probability p_i, then the contribution to r_∞ by a molecule which absorbs a photon with director q_i and emits a photon after the director changes to $\hat{\mathbf{q}}$ is $p_i p_j r_{ij}$, where r_{ij} is the intrinsic anisotropy tropy of the $i \rightarrow j$ transition. The value of r_∞ is the sum of all such contributions:

$$r_\infty = \sum_{i=1}^{6} \sum_{j=1}^{6} p_i p_j r_{ij}. \tag{7}$$

The value of $r_{ij} = r_{ji}$ depends upon the values of i and j, and upon the degree of angular freedom, $\Delta\phi$, of the acyl chain about the axis $\hat{\mathbf{q}}_1$. A formula for r_{ij} may be derived[1] by applying the addition theorem for spherical harmonics (Kinosita et al., 1977):

$$r_{ij} = r_0\left[\frac{1}{4}\left(3\cos^2\theta_i - 1\right)(3\cos^2\theta_j - 1)\right.$$
$$+ 12\sin\theta_i\cos\theta_i\sin\theta_j\cos\theta_j\cos\psi_{ij}\sin^2(\Delta\phi/2)/(\Delta\phi)^2$$
$$\left. + \frac{3}{4}\sin^2\theta_i\sin^2\theta_j\cos(2\psi_{ij})\sin^2\Delta\phi/(\Delta\phi)^2\right], \tag{8}$$

where $\cos\theta_i = \hat{\mathbf{q}}_1 \cdot \hat{\mathbf{q}}_i$, $\sin\theta_i = (1\cos^2\theta_i)^{1/2}$, and $\cos\psi_{ij} = \hat{\mathbf{V}}_i \cdot \hat{\mathbf{V}}_j$, where $\hat{\mathbf{V}}_i = \vec{\mathbf{V}}/|\vec{\mathbf{V}}|$, and

$$\vec{v}_i = (\hat{\mathbf{q}}_i \cdot \hat{\mathbf{q}}_6)\hat{\mathbf{q}}_6 + [\hat{\mathbf{q}}_i \cdot (\hat{\mathbf{q}}_1 \times \hat{\mathbf{q}}_6)](\hat{\mathbf{q}}_1 \times \hat{\mathbf{q}}_6). \tag{9}$$

The conformations leading to $\hat{\mathbf{q}}_6$ are improbable on energetic grounds, because the acyl chain is perpendicular to the bilayer normal and at least two *gauche* bonds are required. We therefore assume $p_6 = 0$, and furthermore that $p_{2\text{-}5}$ are all equal. If we rename $p_1 = p_t$, we may construct models based on the single parameter p_t, the probability that the director at C9 is that for the fully extended chain. For the cases of $\Delta\phi = 0, \pi$, and 2π, the expressions for $r_\infty/r_0 = S^2$ are

$$r_\infty/r_0 = 1/64(9p_t - 1)^2 \quad (\Delta\phi = 2\pi) \tag{10a}$$

$$r_\infty/r_0 = 1.31p_t^2 - 0.37p_t + 0.06 \quad (\Delta\phi = \pi) \tag{10b}$$

$$r_\infty/r_0 = 1/32(45p_t^2 - 18p_t + 5) \quad (\Delta\phi = 0). \tag{10c}$$

These three expressions are graphed in Fig. 4. As expected, the value of p_t calculated for tPnA in DMPC does not depend strongly on $\Delta\phi$, and this insensivity becomes equality for the higher value of S^2 encountered in the presence of CP. The values of p_t corresponding to S_{fit} for tPnA ($\Delta\phi = 2\pi$) are 0.47 and 0.62 in the absence and presence of CP.

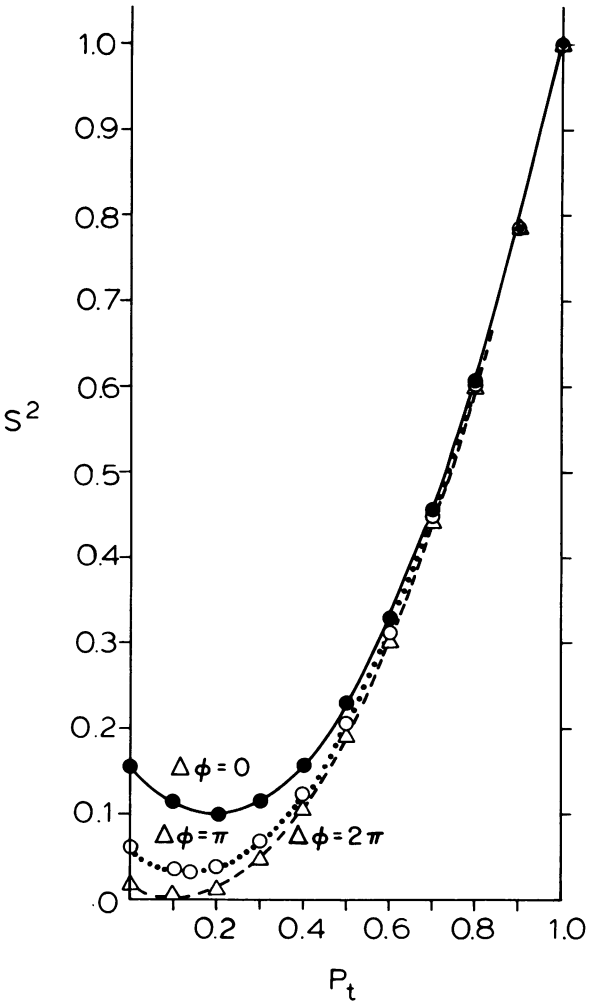

FIGURE 4 S^2 calculated for different values of $\Delta\phi$, as a function of p_t, the probability of an all trans director at C9 in tPnA. Curves are plotted for $\Delta\phi = 2\pi$ (-Δ-, Eq. 10*a*), $\Delta\phi = \pi$ (-O-, Eq. 10*b*), and $\Delta\phi = 0$ (- ● -, Eq. 10*c*).

A similar qualitative argument holds for cPnA, although the bent cPnA chromophore makes the argument difficult to quantitate. In summary, the primary effect of CP on nearby lipids appears to be restriction of the set of conformations PnA can adopt. This interpretation explains the lack of any significant effect of CP on the initial anisotropy decay rate $<\phi^{-1}>$ (Table I).

Our answers to the questions of amount of lipid perturbed and nature of the perturbation must be regarded as preliminary. A complete dose-response study must be done to learn the whole truth. However, the preliminary data are consistent with a model in which CP perturbs lipid by acting as a solid, passive pillar in the bilayer, blocking acyl chain motions in touching lipids. This interpretation rationalizes the difference between our results

[1]Wolber, Paul K., and Bruce S. Hudson. Unpublished results.

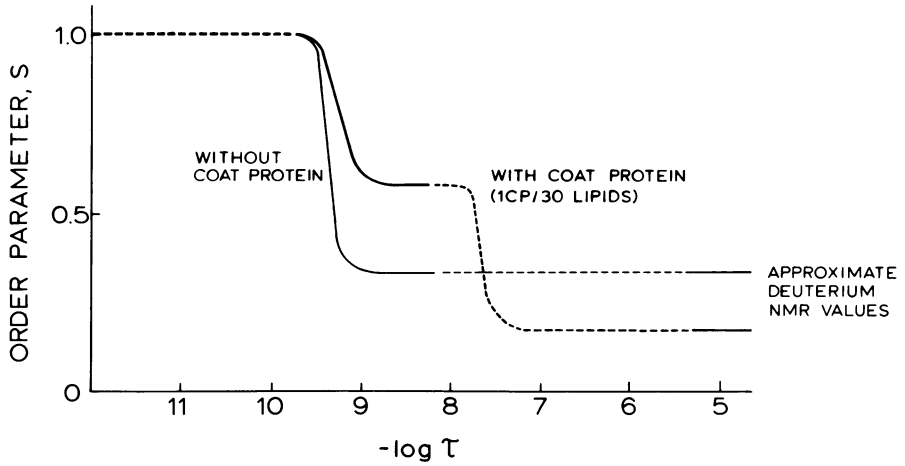

FIGURE 5 A schematic illustration of the time dependence of the frequency-amplitude order parameter S. The solid curve segments are derived from tPnA fluorescence polarization anisotropy measurements. The ^{2}H-NMR order parameters shown at the right are estimates from pure lipid and CP containing bilayers (see Wolber and Hudson, 1981; Oldfield et al., 1978).

and ^{2}H-NMR results (Oldfield et al., 1978) which indicate that CP slightly decreases the order in fluid DMPC. Our results apply to a timescale of 10^{-8} s, where local perturbations are measured, since the lipid molecules do not translationally diffuse on this timescale. An ^{2}H-NMR experiment, on the other hand, is conducted on a timescale of 10^{-4}–10^{-5} s, and the lipid molecules sample all of the environments available. The results of fluorescence and NMR are compatible if CP increases the angular heterogeneity of environments, increases the order of some environments, and does not appreciably slow translational diffusion in fluid lipid. This comparison of fluorescence and ^{2}H-NMR measurements (Fig. 5) can be made for bilayers with or without CP. The hypothetical relaxation process which appears to exist in the protein-containing bilayer may be in the timescale accessible to modern fluorescence methods.

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DISCUSSION

Session Chairman: Patricia C. Jost *Scribe*: William J. Pjura

Jost: We'll begin with three questions from an anonymous referee: First, "In the data analysis you assume $r(0) = 0.40$. Hence, what is the point of listing fitted $r(0)$ values in Table I? In addition, $r(0)$ may be 0.40, but the observed value is likely to be dependent on the instrument and the alignment of its optics. Have you determined $r(0)$ for the fluorophores in your instrument?"

HUDSON: We have done this in our apparatus by collecting data for viscous solutions. The value of $r(0)$ is found to be 0.40. Values for $r(0)$ for the bilayer experiments are reported in our paper (Table I) because the values (0.35 to 0.41) demonstrate that we are not missing any part of the decay.

JOST: The other questions are (*a*) "The *cis* and *trans* isomers of parinaric acid yield distinct values for the limiting anisotropy ($r\infty$). Thus it appears that you may be measuring the order parameters of the probes and not of the membranes. How does this affect your analysis of the data, and your comparison of your order parameters with those found by NMR?" (*b*) "At 39°C the DMPC vesicles are above their phase-transition temperature. What is the effect of M13 coat protein when $T<T_c$? What is the effect on the order parameter of an unsaturated lipid like DOPC?"

HUDSON: The first question concerns experiments in the absence of protein where the asymptotic anisotropy observed for *cis* parinaric acid has a lower asymptotic anisotropy than that observed for *trans* parinaric acid. We believe that is simply due to the fact that *cis* parinaric acid has an extra depolarizing motion consisting of a rotation of the chain about its axis in addition to the other motions which occur. That means that if we're making comparisons to NMR experiments we should, in this case, compare to an oleic acid-like chain with the deuterium in position 9 rather than to a stearic acid-type chain with a deuterium in this position.

With respect to the second question, we haven't looked at any unsaturated lipids. We have looked at the low temperature phase primarily by static fluorescence measurements. The effect that we see is a small decrease in the order parameter when coat protein is in gel-phase lipids. Changes in static anisotropy are weighted in favor of species with higher quantum yield. DMPC bilayers containing coat protein appear to have a short lifetime component associated with the protein. This environmental lifetime effect results in a decreased importance for the perturbed region near the protein, and therefore the decrease in the order parameter may be larger than the observations suggest.

FEIGENSON: The state of aggregation of coat protein could influence the effect of the protein on the lipid-motional state. Have you determined whether the coat protein in your preparations was in the α-form or in the (polymeric) β-form?

HUDSON: We believe that the protein is in the monomeric state on the basis of an energy transfer experiment involving the tryptophan in the hydrophobic segment of this protein, reported in Kimelman et al. (1979. *Biochemistry.* 18:5874–5880). The fluorescence of this tryptophan acts as a label for measuring the distribution of the parinaric acid chromophore with respect to the protein. We get two pieces of information from this. The first is that in the quenching experiment, the decrease in tryptophan fluorescence as parinaric acid is added can be quantitatively described using the assumption that the parinaric acid and the protein are randomly distributed in the bilayer. If the protein were extensively aggregated, there would be tryptophan residues that would be too far from lipids to be quenched by parinaric acid. This does not exclude the possibility of dimers or trimers in the sample. There are no large aggregates.

FEIGENSON: I believe that the protein could still be aggregated and still be surrounded by lipid.

HUDSON: Yes, that is possible. In fact that is probably what happens at low temperatures. When you freeze the acyl chain the system probably phase-separates into one in which the proteins surrounded by ~20 acyl chains phase-separate from a pure lipid phase. We know that there is lipid surrounding that protein because we can still quench tryptophan. Phase-transition data (Kimelman et al., 1979) show that at high protein:lipid ratio the sharp transition of the pure lipid disappears. This indicates to us that there is no separate phase of pure lipid at high temperatures. Also, the static anisotropy is roughly proportional to the protein content.

FEIGENSON: Do you know whether your protein is 50% β-pleated sheet or 50% α-helical?

HUDSON: No, we haven't done the CD measurements.

FEIGENSON: But don't you know whether your protein is 50% α-helical or not? The conformation of the protein could affect the lipid that's around it.

HUDSON: Yes, it could.

WOLBER: While we can't say absolutely that we don't have aggregation problems in the fluid phase, we can make relevant probabilistic arguments. These involve the use of the two isomers of parinaric acid. The natural product, which is *cis-trans-trans-cis*, shows a slight preference for fluid lipids over gel-phase lipids. The *all-trans* isomer, which can be made from the natural product, shows a marked preference for