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Lateral heterogeneity in human platelet plasma membrane and lipids from the time-resolved fluorescence of *trans*-parinaric acid

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Abstract. We have investigated the complex behaviour of the time resolved fluorescence intensity and anisotropy of *trans*-parinaric acid, incorporated into fragments of the plasma membrane of human platelets and in multibilayers of lipids extracted from that membrane. It is shown that the observation of anisotropies that increase at long times can be satisfactorily interpreted by assuming two populations of the fluorescence probe with distinct lifetimes, rotational relaxation times and order parameters. The heterogeneous probe distribution was correlated with a similar heterogeneity in the lipid composition of the bilayer, modulated by temperature. Below 35 °C an important fraction of the lipids of the plasma membrane are apparently in the form of solid-like domains (20% at 20 °C). However, in the physiological temperature range that solid/fluid heterogeneity is almost negligible. Since these effects were also observed in multibilayers of lipids from the platelet membrane, the formation of solid-like clusters appears to arise from lipid-lipid interactions only, and most probably involving cholesterol. These results support the previous finding of a lateral phase separation for temperatures less than 37 °C described by Gordon et al. (1983) in a spin-probe study of the platelet plasma membrane.

Key words: Platelets – Membrane heterogeneity – Bilayer – *Trans*-parinaric acid – Fluorescence decay

Introduction

The lipid component of biological membranes has frequently been assumed to form a physically homogeneous, well-mixed fluid bilayer at physiological temperatures. In recent years, many studies based on a variety of techniques have shown that a non-uniform distribution of lipids may give rise to specialized domains in the cell surface membrane (for a comprehensive review see Alvia et al. 1988). In a few cases, the lateral phase separation of lipids on a macroscopic scale can be observed (Wolf and Voglmayr 1984). More frequently, however, indirect evidence, obtained from calorimetry, photobleaching or spectroscopy, suggests the presence of microscopic clusters randomly disposed within predominantly more fluid lipids. In the case of the plasma membrane of human platelets there is some experimental evidence pointing to the presence of lateral lipid heterogeneity. Thus, from the changes in the ESR spectra of nitroxide-labelled platelet plasma membranes, Gordon et al. (1983) concluded that there are "quasicrystalline" cholesterol-rich lipid domains, that coexist with a fluid lipid pool up to ca. 37 °C. Aslanian et al. (1983), using Raman spectroscopy, reported a double thermotropic state transition of the platelet membrane lipids between 0°–7 °C and 13°–20 °C. However, the heterogeneity discovered by Gordon et al. (1983) was not apparent in that work. On the other hand, we have carried out a study of the order and dynamics of the platelet plasma membrane by determining the extent of the fluorescence depolarization of several lipophilic probes (Mateo et al. 1991). In that work it was observed that the temperature dependence of the DPH* order parameter (S) and apparent viscosity (η) of the lipid bilayer presented discontinuities in the interval of 36°–40 °C. This is precisely the temperature range where the lipid segregation ends according to Gordon and coworkers (1983). Based on this and other arguments, the changes observed in the slope of S and obtained from our DPH fluorescence polarization measurements in the platelet membrane were tentatively ascribed to the presence of lateral heterogeneity in the lipid distribution of the bilayer.

Since the fluorescence parameters of DPH are weakly sensitive to the density of its micro-environment (Barrow and Lentz 1985), this probe has occasionally been used to investigate the presence of solid-like domains in bilayers (e.g. Fiorini et al. 1988). However, DPH is not ideally suited for that purpose, because the partition coefficient

Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, dipalmitoyl-phosphatidylcholine; tPnA, *trans*-parinaric acid

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between the more fluid and gel-like regions is close to unity (Lentz 1989). Therefore, if the fraction of lipids with gel-like properties is small, the feeble effect on the photophysics of DPH would render it difficult to separate experimentally from that due to the dominant, fluid fraction. In contrast, the *trans* isomer of parinaric acid (tPnA) dissolves preferentially in lipid environments with gel-like properties (Sklar et al. 1977a, 1977b, 1979a, 1979b) and, in addition, some of its photophysical properties (absorption spectrum and fluorescence lifetimes) are sensitive to local density (Sklar et al. 1977b; Hudson et al. 1986; Hudson and Cavalier 1988). The investigation of lipid heterogeneity using tPnA is based on the assignment of the multiple discrete components of the fluorescence decay to distinct fractions of the bilayer. With this prerequisite, it is frequently possible to obtain an image of the bilayer consistent not only with the complex set of fluorescence lifetimes and amplitudes observed in natural membranes but also with the decay of the probe fluorescence anisotropy (Sklar et al. 1979a, 1979b; Schroeder 1983; Illsley et al. 1988; Van Paridon et al. 1988). It should be noted, however, that the analysis of the tPnA fluorescence decay in terms of a few discrete lifetimes has been considered potentially misleading, compared with one based on a continuous distribution of lifetimes (James et al. 1987). Moreover, Parasassi et al. (1984) have warned that this probe may not be suitable at all for distinguishing lipid segregation, based on the observation of a multiexponential fluorescence even in pure, homogeneous solvents.

In this work we discuss the use of the fluorescence of tPnA to investigate the thermally-dependent lipid segregation in the platelet plasma membrane proposed by Gordon et al. (1983) and glimpsed in our previous study with DPH derivatives (Mateo et al. 1991). We show that, even considering the criticisms mentioned above, the changes observed in the fluorescence lifetimes and the anisotropy relaxation times of tPnA incorporated into fragments of platelet plasma membrane and multibilayers made up from the membrane lipids, reveal two coexisting regions of distinct density, associated with the lipid component of the membrane.

Materials and methods

Materials

Concentrated solutions of 1,6-diphenyl-1,3,5-hexatriene (Sigma Chemical Co., USA) in N,N′-dimethylformamide and *trans*-parinaric acid (Molecular Probes, USA) in ethanol containing 1% butylated hydroxytoluene (BHT) were stored at −20 °C under an inert atmosphere. Dipalmitoyl-phosphatidylcholine (Sigma Chemical Co. USA) was used as received. The probes were incorporated into the biological samples following standard procedures (Mateo et al. 1991). Human platelets were usually isolated from out-dated platelet concentrates as described in Barber and Jamienson (1970). Control samples were also prepared immediately after the blood was drawn from normal donors. Purified fragments of the platelet plasma membrane and multilamellar vesicles made up from the lipids of the platelet plasma membrane were prepared as described before (Mateo et al. 1991).

Temperature dependence of the platelet membrane acid phosphatase and phosphodiesterase activity

The acid phosphatase activity in purified platelet plasma membrane fragments was assayed using p-nitrophenol phosphate, according to Linhardt and Walter (1965). The phosphodiesterase was assayed with bis-p-nitrophenol phosphate, following Koerner and Sinsheimer (1957). The effect of temperature on the specific activity of these enzymes was studied with the aid of a slightly modified "Arrhenius block" (Mobley and Green 1988) in the 15°–45 °C range.

Time-resolved fluorescence

Fluorescence experiments were performed using the time-correlated single-photon counting spectrometer described in the previous paper (Mateo et al. 1991). The emission of tPnA was excited with the 316 nm line of a N_2-filled flash-lamp and detected through a combination of long-pass KV (Schott Glaswerk, Germany) or interference (Oriel GmbH, Germany) filters. Polaroid HNP′B filters were placed in both channels. Lifetime experiments were carried out with the emission polarizer oriented at the magic angle (54.7°) relative to the (vertical) transmission axis of the excitation polarizer. The fluorescence collected in this way was analysed by iterative convolution using a local version of the non-linear least-squares method (Mateo et al. 1991) and fitted to a multiexponential function:

$$I(t) = \sum_i \alpha_i \cdot \exp(-t/\tau_i) \tag{1}$$

The fluorescence anisotropy function, $r(t)$, was determined by the global analysis method detailed in a previous article (Mateo et al. 1991). Two models of the anisotropy decay were fitted to the depolarization data:

(1) Restricted motion of a single rotor (lifetime and dynamic homogeneity):

$$r(t) = (r_0 - r_\infty)\left[\sum_{i=1}^{2} b_i \cdot \exp(-t/\Phi_i)\right] + r_\infty \tag{2}$$

The fitting parameters are the rotational correlation times (Φ_i), residual anisotropy (r_∞) and weighting factors (b_i). This approximation and the specific methods that allow an estimation of the probe's average relaxation time $\langle\Phi\rangle$ and order parameter S from Eq. (2) were discussed in the previous article (Mateo et al. 1991).

(2) Restricted motion of two independent rotors (lifetime and dynamic heterogeneity). This model accounts for the possibility of a probe being localized in two different environments A and B, resulting in two sub-populations of the emitting species with independent fluorescence, $I_i(t)$, and anisotropy, $r_i(t)$, decay laws. The fraction of the total

fluorescence, $I(t)$, contributed by species i at any time is given by:

$$f_i(t) = \alpha_i \cdot I_i(t)/I(t) \quad (3)$$

and the (average) anisotropy by:

$$r(t) = f_A(t) \cdot r_A(t) + f_B(t) \cdot r_B(t) \quad (4)$$

To reduce the number of free parameters, the anisotropy function of each sub-population was defined by a single relaxation time and a (positive) residual anisotropy. The initial anisotropy at $t = 0$ was considered to be the same for the two populations and was not fixed (Ruggiero and Hudson 1989b). The computed parameters presented below correspond to fittings that passed the standard statistical criteria (Ameloot and Hendrickx 1982) and yielded reduced χ^2 values in the range of 1.0–1.3. Since the limitations of this "associative" model as well as its use in the analysis of tPnA fluorescence lifetimes and anisotropy, both in synthetic bilayers and in natural membranes, have been discussed before in great depth (Ludescher et al. 1987; Ruggiero and Hudson 1989b; Calafut et al. (1989) they will not be repeated here.

Results

Temperature effects on tPnA fluorescence in isolated platelet plasma membrane fragments and multibilayers of membrane lipids

The time-resolved fluorescence intensity and anisotropy of tPnA in fragments of the platelet plasma membrane at 20 °C are shown in Fig. 1. An important observation is that the anisotropy decays in a few nanoseconds to a minimum value and then increases at longer times. This remarkable behaviour has been found, to a varied extent, throughout the temperature range examined here (20°–44 °C). Moreover, the same phenomenon was observed when the fluorescent probe was incorporated into multibilayers of plasma membrane lipids. Since the initial anisotropy r_0 of tPnA is very close to the theoretical value for parallel absorption and emission moments (Sklar et al. 1977a, 1977b), the upward curvature in the anisotropy decay is a strong indication of rotational heterogeneity with association of fluorescence lifetime components (Ludescher et al. 1987).

An additional observation supporting this is the blue shift (2.5 nm) of the fluorescence excitation spectrum of tPnA in membrane fragments from 15° to 43 °C. This may result from the well-known sensitivity of the absorption spectrum of the probe to the density of its microenvironment (Sklar et al. 1977b). Therefore, a detailed analysis of the lifetime components of tPnA in fragments of the plasma membrane and in vesicles of membrane lipids in the temperature interval from 20° to 44 °C was carried out (Table 1). The emission was fitted to a decay function described by three lifetimes, that in the two preparations have similar values and weighting factors: a short lifetime (1–2 ns) with a small amplitude, an intermediate- and a long-lifetime component. The long lifetime of tPnA in membrane fragments shows a strong temperature dependence, while the intermediate and short lifetimes change much less over this temperature range (Fig. 2). The variation of the amplitudes with temperature is of particular interest. The amplitude of the long lifetime component is strongly dependent on temperature (Table 1), decreasing rapidly in the 24°–35 °C range. However, as the temperature is further increased, the rate of change of this amplitude is almost negligible. On the other hand, the amplitudes associated with the intermediate and short lifetimes display the opposite behavior, increasing steadily from 24° to 35 °C and levelling-off in the 35°–43 °C range.

The changes in the amplitudes of the three lifetime components when the probe is incorporated into multibilayers of platelet lipids closely reproduce those just described for the natural membrane (Table 1 and Fig. 3). These data show that the striking thermal dependence of the pre-exponential factors must be correlated with the lipid composition of the plasma membrane and fairly independent of protein-lipid interactions. In addition, this

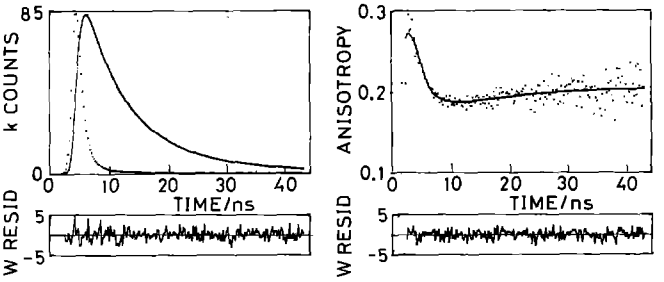

Fig. 1. Time-resolved fluorescence intensity and anisotropy of *trans*-parinaric acid in isolated fragments of platelet plasma membrane at 37 °C. See Tables 1 and 2 for the fitting parameters of the overlayed functions. The goodness of fit is demonstrated by the weighted residuals plot

Table 1. Fluorescence decay parameters of *trans*-parinaric acid in fragments of platelet plasma membrane, multibilayers of the native lipids of that membrane and of dipalmitoylphosphatidylcholine (DPPC)

Sample	T/°C	A_1	τ_1 (ns)	A_2	τ_2 (ns)	A_3	τ_3 (ns)
Membrane fragments	24	0.48	16.7	0.40	5.2	0.12	1.0
	27.6	0.37	15.5	0.44	6.0	0.19	1.5
	32.8	0.29	13.0	0.46	5.7	0.25	1.3
	34.6	0.26	12.5	0.51	5.4	0.23	1.3
	36.8	0.24	10.7	0.49	5.0	0.27	1.8
	38.5	0.24	11.5	0.53	4.9	0.23	1.1
	40	0.22	10.0	0.52	4.5	0.26	1.3
	41.4	0.21	9.5	0.53	4.4	0.26	1.3
	43.2	0.19	9.0	0.54	4.1	0.27	0.9
Native lipids	7.2	0.63	31.2	0.27	9.2	0.09	1.7
	20.3	0.42	20.2	0.43	8.1	0.15	1.7
	27.4	0.29	16.0	0.51	7.7	0.20	2.1
	35	0.25	12.0	0.56	5.7	0.19	1.6
	38	0.23	10.4	0.55	5.0	0.22	1.2
	40.4	0.25	9.0	0.55	4.2	0.20	0.9
	43.6	0.18	8.8	0.56	4.5	0.26	1.5
DPPC	20	0.77	49.6	0.19	16.9	0.04	1.6
	49.5	0.05	18.0	0.55	3.3	0.40	1.5

Lifetimes ±0.3 ns; fractional amplitudes ±0.02

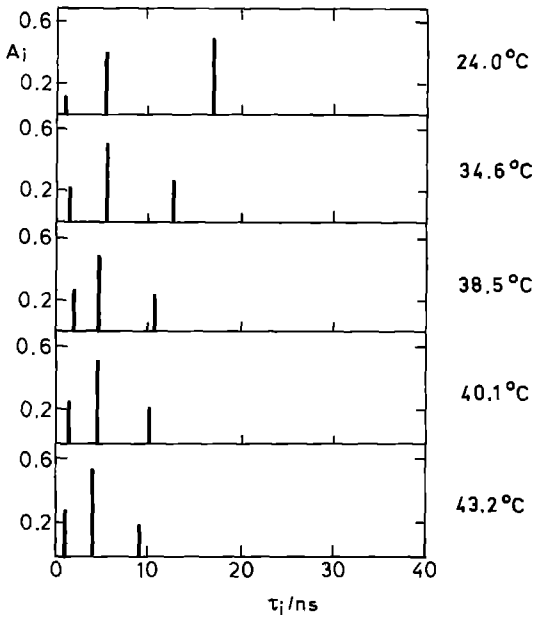

Fig. 2. The variation of the fluorescence lifetimes τ_i and fractional amplitudes $A_i = \alpha_i / \sum_{i=1}^{3} \alpha_i$ of *trans*-parinaric acid at the indicated temperatures in fragments of the plasma membrane from human platelets

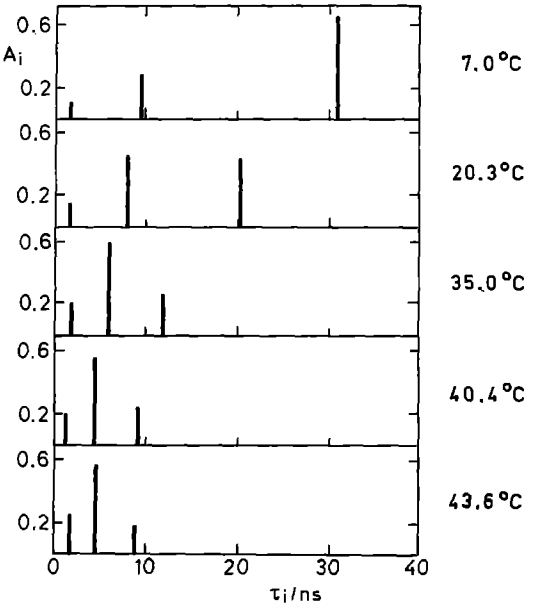

Fig. 3. The variation of the fluorescence lifetimes and fractional amplitudes of *trans*-parinaric acid at the indicated temperatures in multibilayers of lipids from the platelet plasma membrane

behaviour is independent of the probe concentration, incubation time and frequency range of the fluorescence spectrum selected with the emission filters. It is therefore interpreted here as originating from the heterogeneous distribution of the probe in bilayer regions of different density. This assumption, which is crucial for the following analysis of the anisotropy data, is further supported by the fluorescence decay of DPH, which is expected to be homogeneously distributed (Lentz 1989), in the same samples (Mateo et al. 1991). Though, the fluorescence

Table 2. Parameters from the fit of an associative model to the fluorescence anisotropy decay of *trans*-parinaric acid in fragments of platelet plasma membrane and multibilayers of lipids from that membrane

Sample	T/°C	Φ_A (ns)	r_∞^A	Φ_B (ns)	r_∞^B	$r(0)$	$\bar{r}$*
Membrane fragments	20	2.4	0.246	0.2	0.182	0.40	0.238
	25.4	2.1	0.232	0.2	0.179	0.39	0.225
	35.4	2.1	0.207	0.5	0.155	0.23	0.201
	37	2.6	0.215	0.7	0.154	0.30	0.197
	39.0	2.3	0.202	0.8	0.141	0.30	0.193
	42.7	1.4	0.197	0.3	0.144	0.40	0.183
Native lipids	25.8	1.3	0.202	0.5	0.165	0.31	0.198
	35.2	1.1	0.176	0.2	0.134	0.36	0.168
	38	1.4	0.178	0.6	0.132	0.30	0.156
	41.2	1.1	0.153	0.7	0.128	0.40	0.154

Relaxation times ±0.4 ns; residual anisotropy ±0.005

* $\bar{r}$ is the measured steady-state emission anisotropy (Mateo et al. 1991) corresponding to $\int_0^\infty \sum_{i=1}^{n} f_i(t) \cdot r_i(t)\, dt \Big/ \int_0^\infty \sum_{i=1}^{n} f_i(t)\, dt$ (see Eqs. 1, 3 and 4)

kinetics are very similar in the two phases and do not exhibit much temperature dependence in the range considered, which, together with the lack of strong phase partioning ($K_p \approx 1$) should result in a very weak dependence of its photophysics on temperature. This is precisely what it is observed for the DPH fluorescence in platelet plasma membrane fragments: lifetimes and amplitudes remain virtually unchanged in the 25°–45 °C temperature range.

According to this background, the decay of the tPnA anisotropy in the platelet membrane and lipids was analysed with the simple associative model 2 (see Methods), by assuming that the longest lifetime represents the decay of the probe in environment A while the intermediate and shorter lifetimes pertain to species in B. This choice is based on the thermal dependence of the amplitudes of the three lifetime components in the membrane and lipids (Figs. 2–3) and in the way the probe lifetimes distribute in DPPC bilayers below and above the phase transition temperature (41.5 °C), as determined from our own data (Table 1) and from previous studies in synthetic bilayers of this and other lipids (Wolber and Hudson 1981; Hudson et al. 1986; Ruggiero and Hudson 1989b). The other alternative lifetime associations or the simple homogeneous model 1 failed to yield statistically adequate fits to the experimental anisotropy decays. The data from Table 2 show that the two probe sub-populations in membrane fragments are characterized by quite different values of the rotational relaxation time and residual anisotropy. Although the uncertainty in the time constants is rather large (due to a partial correlation with the initial anisotropy), these results indicate that the longer fluorescence lifetime is coupled to the lipid population (A) having a more rigid environment and higher structural order. The residual anisotropy r_∞^A at 20 °C is approximately 20% less than the value measured with this probe in the gel phase of synthetic lipid bilayers close to the phase transition temperature, while that of the B environ-

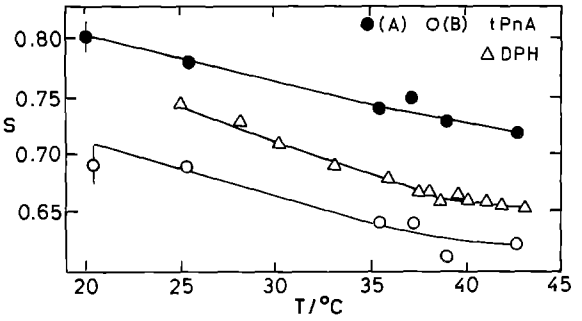

Fig. 4. The order parameters of DPH and *trans*-parinaric acid as a function of temperature in fragments of human platelet plasma membrane. For DPH, the time-resolved anisotropy was analysed with the homogeneous model 1 (see Methods) while for t-PnA, the associative model 2 was used, assuming that the fluorescent species was partitioning between solid-like (*A*) and fluid (*B*) environments

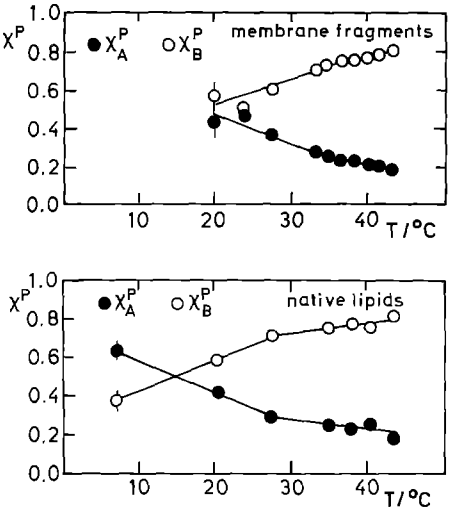

Fig. 5. The temperature dependence of the fraction of *trans*-parinaric acid in the solid-like (*A*) and fluid (*B*) regions, in platelet plasma membrane fragments (*upper panel*) and in multibilayers of the membrane native lipids (*lower panel*)

ment r_∞^B is typical of fluid phases in those conditions. The qualitative differences between the two sub-populations are preserved in the multibilayers of membrane lipids. It is interesting to note that in the latter preparation the "solid-like" fraction A appears more fluid and less ordered than in the plasma membrane. This indicates that the absence of the membrane protein component (and/or possibly the natural lipid asymmetry) results in a higher mobility and lower residual anisotropy of the probe, as observed previously using DPH (Mateo et al. 1991).

The anisotropy data of Table 2 may be expressed in the form of tPnA order parameters S in the two environments of the plasma membrane, as a function of temperature (Fig. 4). For comparison with the DPH homogeneous analysis (model 1 in Methods), S_{DPH} in the same preparation was also included.

Discussion

The main finding of the analysis of the fluorescence kinetics and depolarization of tPnA in the platelet samples is that the association of the long lifetime with a more dense environment (A) provides a consistent interpretation of the temperature dependence of the complex lifetime and anisotropy decay data. This is independent of whether the probe fluorescence is described by discrete lifetimes or by a continuous distribution, as favoured by James et al. (1987), because even in this last case the long-lifetime tail would be correlated with the more dense microenvironment, as was noted by Ruggiero and Hudson (1989a). In addition, the measurements of the fluorescence lifetimes of tPnA in DPPC bilayers (Table 1) showed that the specific approximation used here is capable of isolating the kinetic components of the emission associated with the gel and liquid-crystalline phases of the bilayer.

Therefore, to explain the fluorescence properties of tPnA in the platelet plasma membrane it is necessary to assume that two distinct regions coexist with a large difference in lateral density. The fraction of probe in each of them (X_A^P, X_B^P) can be estimated from the fractional fluorescence amplitudes $\left(A_i=\alpha_i \Big/ \sum_{i=1}^{3} \alpha_i\right)$: $X_A^P=A_3$; $X_B^P=A_1+A_2$, provided that the radiative rate constants are the same (Sklar et al. 1977a). The thermal dependence of the probe fraction (Fig. 5a) indicates that, for temperatures close to 20 °C, almost 50% of the probe is contained in the solid-like region. As the temperature increases, the situation changes sharply and at 35 °C only a minor fraction of tPnA stays in the solid-like phase. This specific probe partition is not affected by a further temperature increase in the physiological range (35–43 °C). The relative amount of each phase (X_A, X_B) in the platelet membrane can then be estimated from the fraction of tPnA in each and its partition coefficient $K_P^{A/B}$ in these samples. This coefficient for the solid/fluid distribution in synthetic lipid bilayers (Sklar et al. 1979a, b) and in rat liver plasma membranes (Schroeder 1983) takes values in the range of 3 to 5. Using such values for the platelet membrane, a simple calculation indicates that for temperatures near 20 °C, 20% of the bilayer lipids shows solid-like properties. A most remarkable finding is that the same pattern was observed in the lipid vesicles (Fig. 5b), where the unique thermal dependence of the solid- and fluid-like fractions is reproduced, in spite of complete randomization of the native lipids. The conclusion is that the observed lateral heterogeneity and its peculiar thermal dependence are a consequence of the lipid composition of the bilayer and do not derive from the presence of membrane proteins or the native lipid asymmetry. Apparently, the main effect that might be produced by these factors is the increase noted in the packing density of the solid- and fluid-like domains of the plasma membrane as compared with the separated lipid multibilayers.

In the case of DPH, since the fluorescence lifetimes in the solid and fluid phases do not differ appreciably (Barrow and Lentz 1985) and, in addition, the relative concentration of the probe in the A and B environments is similar ($K_P^{A/B} \approx 1$), the r_∞ values can be approximated as $X_A^P \cdot r_\infty^A + X_B^P \cdot r_\infty^B = X_A \cdot r_\infty^A + X_B \cdot r_\infty^B$. Thus, the observed r_∞ DPH and the order parameter derived therefrom would be expected to take intermediate values between

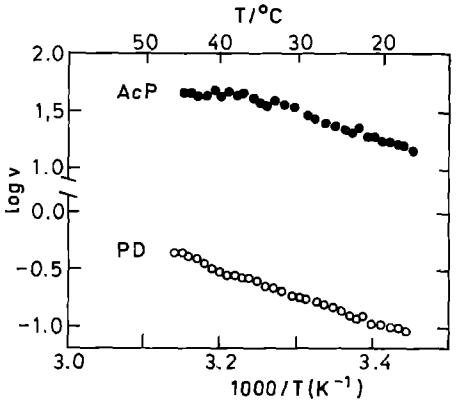

Fig. 6. Arrhenius plots of acid phosphatase (•) and phosphodiesterase (o) activity in isolated plasma membranes from human platelets, expressed as μmol product/mg protein/h

those of the tPnA, and that is what is observed in the temperature range studied here (Fig. 4).

These temperature-dependent changes in the molecular organization of the platelet membrane lipids are expected to influence the activity of integral membrane enzymes. In this regard, it is striking that the temperature range where platelet aggregation velocity is maximal (Gear 1982) is precisely the range over which the lipid hetrogeneity described here is minimal. More specifically, Gordon et al. (1983) noted that the Arrhenius plot of the activity of acid phosphatase in platelet membrane fragments presents a break at 35 °C. We extended these measurements by determining the activity of the acid phosphatase and also that of the phosphodiesterase in platelet membrane fragments (Fig. 6) by means of a thermal gradient device (see Methods), that allows the measurement of the activity at one-degree intervals. Our results for the acid phosphatase essentially reiterate those obtained before (Gordon et al. 1983; Mobley and Green 1988) at a higher level of specific activity, showing a marked increase in the heat of activation for temperatures below 35 °C where the solid-like lipid domains become more important. In contrast, the heat of activation of the phosphodiesterase in the same experimental conditions remains unaltered. Hence, the observed changes in the acid phosphatase are very unlikely to be due to artifacts of the technique and do reflect an important modification of its interaction with the membrane in the temperature range examined.

In conclusion, the data presented here confirm the existence of the thermotropic lipid phase separation at temperatures of 37 °C and lower proposed by Gordon et al. (1983). Moreover, our experiments in lipid vesicles demonstrate that those authors were correct in suggesting that the observed effects were due to the lipid component of the membrane only. On the other hand, we found no evidence for the thermotropic state transition at 17 °C in the platelet membrane lipids proposed by Aslanian et al. (1983).

The composition of the solid-like domains that occur in the lipid bilayer of the platelet plasma membrane is unknown. By comparison with the behavior of spin probes in cholesterol-enriched and in normal rat liver plasma membranes, Gordon et al. (1983) attributed the platelet bilayer heterogeneity to formation of cholesterol-rich and -poor domains, a plausible hypothesis taking into account the relatively high cholesterol/phospholipid molar ratio (0.74) of the platelet plasma membrane. However, further experimental work involving the monitoring of these fluorescence or spin-probes in platelet plasma membrane and in membrane lipids with modified lipid composition is necessary for an understanding of the molecular interactions responsible for the heterogeneity.

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