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Organization and dynamics of lipids in bovine brain coated and uncoated vesicles

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Abstract. Three characteristics have been demonstrated by the chemical analysis of bovine brain coated vesicles following removal of the coat proteins: a high protein content, a high cholesterol/lipid ratio and a high percentage of phosphatidylethanolamine amongst the phospholipids.

The study of lipid bilayer organization and dynamics has been performed using the fluorescent probes pyrene and parinaric acid (*cis* and *trans*). This has allowed the study of both lateral mobility and rotational motion in the lipid bilayer of the coated and uncoated vesicles.

Lateral mobility in the fluid phase of the lipid is slightly reduced by the presence of the clathrin coat, as indicated by the lower diffusion coefficient of pyrene in coated compared with uncoated vesicles.

At all temperatures from 6° to 30° C, solid-phase domains, probed by *trans* parinaric acid, coexist with fluid-phase domains in the lipid bilayer. The temperature dependence of the parinaric acid lifetimes and of their amplitudes strongly suggests that the solid phase domains decrease in size with temperature, both in coated and uncoated vesicles.

However, the difference in the value of the anisotropy at long times (r_∞), between coated and uncoated vesicles (a difference which is more pronounced for cis than for *trans* parinaric acid), indicates that the presence of the clathrin coat introduces disorder in the surrounding lipids, thus suggesting a possible role of the clathrin in the formation of the pits on the plasma membrane.

Key words: Clathrin coat, lipid dynamics, coated vesicles

Introduction

Coated vesicles are distinct organelles occurring in all eukaryotic cells whose function is the transfer of metabolites (or even membranes) either across or between cellular membranes (Pearse and Bretscher 1981; Roth and Woods 1982). Their formation is part of the normal cellular processes of endocytosis (Goldstein et al. 1979), exocytosis (Rothman et al. 1980) and membrane recycling (Heuser and Reese 1973). They are readily obtainable in their native form, and represent a good model for the study of biological membrane structure and dynamics. Recently developed procedures for their preparation (Nandi et al. 1982; Pearse 1982; Dickson et al. 1983) allow the isolation of homogeneous preparations of coated vesicles (Nossal et al. 1983). This is in contrast with the preparation of other biological membranes, e.g., plasma membranes, endoplasmic reticulum, mitochondria, which involve the disruption of cellular membranes by homogenization, sonication or other procedures in order to release the desired particulate (vesicular) membrane. The absence of contamination by other types of membrane is often questionable and, in addition, the reformed vesicles obtained from the native membrane represent only fragments of the original structure.

The aim of the present work is to describe the organization and dynamics of the membrane of coated vesicles and to study the influence of the protein coat on the properties of the membrane. Indeed, the polymerization of clathrin in certain areas of the plasma membrane, where ligands bind to clustered receptors, appears to be one of the first steps in the formation of coated pits (Salisbury et al.

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Abbreviations
CVs coated vesicles; UVs uncoated vesicles; TLC thin layer chromatography; DMSO dimethylsulfoxide; DPPC dipalmitoylphosphatidylcholine; *cis* Pna *cis* parinaric acid; α (9,11,13,15-*cis-trans-trans-cis*) octadecatetraenoic acid; *Trans* Pna *Trans* parinaric acid; β (9,11,13,15-all-*trans*) octadecatetraenoic acid

1980; Pearse and Bretscher 1981). A better understanding of the interaction of the coat with the membrane lipids could shed light on the mechanism of pit formation, which involves a reorganization of the lipids, especially cholesterol (Montesano et al. 1980), possibly changing the radius of curvature of the lipid bilayer. For this purpose fluorescent probes, which partition mainly in the lipids, have been used to study the dynamics of the membrane.

Materials and methods

Chemicals

Pyrene was purchased from Aldrich. The fluorescence emission spectrum of this product, recorded in DMSO, exhibits the characteristic fine structure in the pyrene monomer peak. Parinaric acid (*cis* and *trans*) was from Molecular Probes (Oregon). The molar extinction coefficients reported by Sklar et al. (1977a) were used to determine the concentration of the parinaric acid isomers.

Preparation of uncoated vesicles

The methods for the preparation of coated vesicles (CVs) and for the dissociation of the coat proteins to obtain uncoated vesicles (UVs) have previously been described (Nandi et al. 1982). They are modifications of existing methods (Pearse 1975; Keen et al. 1979; Woodward and Roth 1978). The uncoated vesicles were free of clathrin as demonstrated by SDS gel electrophoresis and by the measurement of protein/phospholipid molar ratio.

Chemical analysis of uncoated vesicles

Protein was determined by a modification of the Lowry procedure (Markwek et al. 1978). Phospholipid was determined by the measurement of phosphorus concentration, using the method of Ames and Dubin (1960). A chloroform-methanol procedure was used for the extraction and partition of phospholipids; the different phospholipids were separated by TLC according to Hess and Talheimer (1965). Gangliosides were determined by fluorimetric titration of the sialic acid (Hess and Rolde 1964), and cholesterol was determined by an enzymatic assay in the presence of Triton ×100 (Ott et al. 1982).

Incorporation of the fluorescent labels

To give a molar ratio of probe/lipid in the range 0.01–0.08, 1–10 µl of 1 mM pyrene in DMSO was added to the vesicle suspension. The parinaric acids were dissolved in ethanol and added to vesicle suspensions under nitrogen or argon; all solutions were maintained in a nitrogen or argon atmosphere. The probe/lipid molar ratio for the parinaric acids was of the order of $1/300$. With both pyrene and parinaric acid, controls were performed to check that no fluorescence arose from binding of the probe to the clathrin coat.

Fluorescence intensity measurements

Fluorescence intensity measurements were made on Perkin-Elmer MPF3 (NIH, USA) and Jobin Yvon JY3C (ELM, France) fluorimeters. Pyrene monomer and excimer emission were measured at 390 and 470 nm, respectively, with excitation at 346 nm. The emission of *cis* and *trans*parinaric acids was measured at 410 nm with an excitation wavelength of 320 nm.

Lifetime measurements

Fluorescence lifetimes for pyrene (monomer and excimer) and the parinaric acids were determined using a single-photon counting fluorometer equipped with an RCA photomultiplier (ELM, France). The electronic devices were an Ortec System 9200 coupled with an Ortec 6240 multichannel analyser interfaced with a PDP 10 computer. The optical system (Applied Photophysics, SP7) was equipped with two f/4 monochromators for excitation and emission wavelength. The temperature was controlled by circulating water from a thermostatted bath through a compartment surrounding the cell. This equipment and the methods of data analysis employed have been described elsewhere (Gallay et al. 1981). The lifetime for the pyrene excimer was measured using concentrated pyrene solutions in which the dimer is the predominant form.

Steady-state anisotropy

Parinaric acids. Fluorescence polarization measurements were performed with an SLM fluorimeter. Excitation and emission wavelengths were 320 and 410 nm, respectively. Fluorescence anisotropy *(r)* was obtained by making intensity measurements of the parallel and perpendicular components of the fluorescence emission, and using the equation

$$r = (I_v VG - I_v H)/(I_v VG + 2\, I_v H)$$

where G is an instrumental correction factor. The absorbance of the suspensions was always less than 0.15 and a blank was used for light-scattering corrections.

Anisotropy decay measurements

Time-resolved emission anisotropy was obtained with the Applied Photophysics system described above and elsewhere (Gallay et al. 1981; de Paillerets et al. 1981). The emission decay curves $I_vV(t)$ and $I_vH(t)$ of the sample, from which a blank was subtracted when needed, were collected successively for time periods in the range of 5–10 min, depending on sample intensity. Methods of calculation were as previously described (Vincent et al. 1982).

A series of measurement was also performed at Oregon State University, (Corvallis, USA) in collaboration with R. Ludescher. The equipment used similar electronics (ORTEC); the flash was a picosecond dye laser, coupled to an Apple II computer. Data analysis was performed by standard, non-linear least-squares regression techniques, or by the method of moments as described by Isenberg and Small (1982). A PDP 11 computer was used.

Pyrene diffusion measurements

According to Galla and Luisetti (1980) the formation of excited complexes (excimers) by a collision between an excited and an unexcited pyrene monomer is a diffusion-controlled process in the fluid state of the membrane. The intensity ratio, I'/I, (excimer intensity *(I')* at 470 nm and monomer intensity *(I)* at 390 nm) is then a measure of the collision rate of the pyrene molecules, i.e., of the lateral mobility of the probe within the membrane. The collision frequency is given by Galla and Hartmann (1980)

$$\nu_{col} = I'/I_{\varkappa} \times k_f/k'_f \times 1/\tau'$$

where k_f and k'_F are the rate constants for the radiative decay of the excited monomer and excimer, respectively. We have used a value of 0.10 for the ratio k_f/k'_F, as given by Galla and Sackmann (1974). τ' is the lifetime of the excited state dimer. $\varkappa$ was obtained from the ratio of intensities to quantum yields *(I/Q)* determined at low and high pyrene concentrations for the monomer and excimer, respectively.

$$\varkappa = I'/I \,/\, Q'/Q$$

Using a two-dimensional random walk model for the diffusion process, the jump frequency in a lipid membrane has been given (Galla et al. 1979) as

$$\nu_j = \langle n_s \rangle \, \nu_{col}$$

where $\langle n_s \rangle$ is the average step number between collisions and is given by

$$\langle n_s \rangle = 2/\pi\chi_{la} \times \ln 2/\chi_{la}$$

where χ_{la} is the mole fraction of pyrene in the phospholipid. The lateral diffusion coefficient is related to ν_j by the equation

$$D = 1/4 \times \nu_j\lambda^2$$

where λ, the jump length, was taken to be 8Å from the work of Galla and Sackmann (1974) on artificial membranes. We have calculated values of D for CVs and UVs from measurements of I', I, Q', and τ'.

Results

1. Chemical composition

The protein and lipid composition of UVs are given in Table 1. The high percentage of phosphatidyl ethanolamine, i.e., 25%, is noteworthy since it suggests that the membrane bilayer may be relatively rich in unsaturated fatty acyl chains (Trewehella and Collins 1973). The cholesterol/lipid molar ratio is relatively high, in agreement with the data of McGookey et al. (1983) on filipin-cholesterol complexes.

2. Pyrene emission fluorescence

a) Spectra. The emission spectrum of pyrene incorporated into the membrane of CVs is very similar to that found for UVs (inset Fig. 1). The spectrum is characterized by two well-separated maxima, at 388 and 468 nm, indicative of monomer and excimer emission, respectively (Galla and Sackmann 1974). It should be emphasized that the spectrum of the pyrene embedded in the vesicle suspension progressively loses its fine structure when the concentration of material is high and the monomer concentration is low because of extensive dimer formation.

Table 1. Protein and lipid composition of UVs (g/100 g)

Protein	60 ± 2
Phospholipid	14.0 ± 0.2
Glycolipid	4.00 ± 0.08
Cholesterol	4.0 ± 0.2
Undetermined	18

Molar ratio cholesterol/lipid = 0.57

Lipid composition of UVs (excluding cholesterol)

Phosphatidylcholine	40.0 ± 2
Phosphatidylethanolamine	25.0 ± 1.2
Phosphatidylinositol and serine	21 ± 1
Sphingomyelin	10.0 ± 0.5
Cerebrosides	3.00 ± 0.5
Gangliosides	1.00 ± 0.02

b) Effect of temperature. The temperature dependence of the ratio of excimer to monomer emission intensities, I'/I, has been measured from 5° to 55° C in both CVs and UVs (Fig. 1). There was a significant difference between the I'/I plot for CVs and that for UVs in the temperature range studied; the ratio of pyrene to lipid was the same for both types of vesicle. The change in slope from positive to negative, which occurs near 45° C in both UVs and CVs, indicates that an important structural transition of the membrane occurs at this temperature. The process was not reversible since a distinct hysteresis effect was observed when cooling solutions of UVs. Solutions of CVs became turbid above 50° C due to the denaturation of clathrin.

c) Effect of pyrene concentration. It has been shown with DPPC vesicles that a linear relationship between I'/I, and pyrene concentration is observed only in the fluid phase of the membrane. Below the transition temperature, linearity is no longer observed due to phase separation or pyrene clustering. A linear relationship therefore indicates a diffusion-controlled process and implies a fluid phase for the membrane structure (Galla and Sackmann 1974). Plots of I'/I vs pyrene concentration were found to be linear at both 25° and 45° C, for both CVs and UVs (Fig. 2). Measurements of the quantum yield of the excimer and of the monomer allows the determination of $\varkappa$, the proportionality constant between fluorescence intensity (at the maximum wavelength) and the quantum yield, from the equation

$$I'/I = \varkappa Q'/Q$$

A value of 0.7 was obtained.

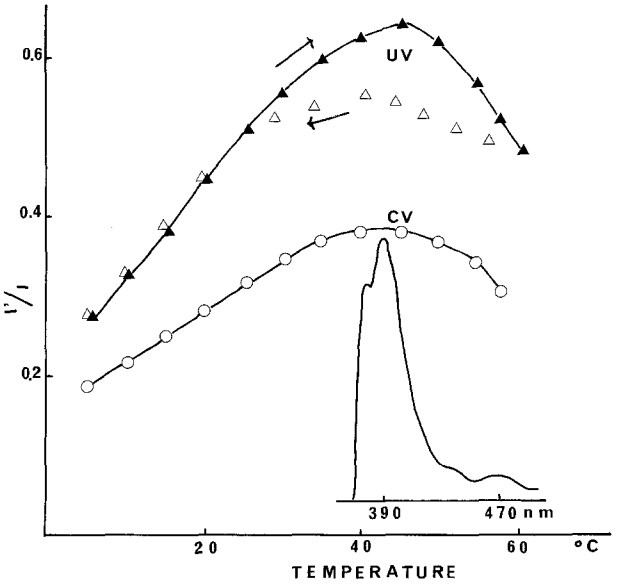

Fig. 1. Temperature dependence of the excimer to monomer fluorescence intensity ratio (I'/I) for pyrene incorporated into CVs ○ and UVs ▲ (heating curves), △ (cooling curve) the pyrene / lipid molar ratio was 0.03 for both types of vesicle. *Inset:* Emission spectrum of pyrene in CVs (2 μM)

d) Pyrene lifetimes. The lifetimes of the excited states of pyrene monomer and excimer at different temperatures (in CVs and UVs) are given in Table 2. The decay curves fit a single exponential. It is interesting to note that the lifetimes obtained for both monomer and excimer were shorter in coated than in uncoated vesicles, suggesting different interactions between the probe and the bilayer in the two types of vesicles.

e) Diffusion coefficient. The collision frequency calculated as described above is given in Table 3. The diffusion coefficient for the probe is smaller in the lipids of CVs than it is in the lipids of UVs.

3. *Parinaric acid emission*

The *cis* and *trans* isomers of Pna are convenient probes to monitor membrane behaviour since they are naturally fluorescent, long-chain, unsaturated fatty acids which insert readily into membranes. Furthermore, they allow precise discrimination between solid and fluid phases of the lipid bilayer, since *trans* Pna partitions preferentially in the gel domains of the lipid, whereas *cis* Pna partitions equally in the gel and fluid domains (Sklar et al. 1977b).

Table 2. Lifetime (ns) of pyrene monomer and excimer fluorescence

Monomer [ns]			Excimer [ns]		
T (°C)	CVs	UVs	T (°C)	CVs	UVs
5.5	223	289	4.5	140	178
17.5	145	199	16.5	110	138
			25.0	92	121
29.5	109	132	30.0	86	116
37.0	96	112	37.0	72	99
45.0	83	97	45.0	58	82

Table 3. Jump frequencies and diffusion coefficients of pyrene in coated and uncoated vesicles[a]

T (°C)	CVs		UVs	
	ν_j	D	ν_j	D
25	0.19	3.04	0.24	3.84
30	0.23	3.68	0.28	4.48
40	0.30	4.80	0.36	5.76
45	0.37	5.92	0.44	7.04

[a] ν_j in units of 10^8/s D in units of $10^{-8} \cdot$ cm^2/s
$D = \nu_j \lambda^2/4 = \nu_j\ 16 \times 10^{-16}$ cm^2

The excitation and emission spectra of *trans* Pna in CVs are shown in Fig. 3. Similar spectra were observed for UVs. It appears that the two probes are embedded in the lipid bilayer in both types of vesicle in a similar way, since there are only minor differences in their spectra.

a) Fluorescence lifetime

Trans parinaric acid: The fluorescence decay of *trans* Pna in UVs and CVs is nearly identical and heterogeneous. Two lifetimes were found with the nanosecond flash and three with the laser picosecond flash, ranging from 6 to 60 ns at 6° C for both types of vesicle (Table 4). The temperature dependence of the lifetimes and amplitudes is nearly linear (Fig. 4).

Cis Parinaric acid: The fluorescence decay of this isomer differs slightly for the two vesicle types but is also heterogeneous with at least two lifetimes below 20° C. At 6° C, a long lifetime (31 for CVs and 39 for UVs) is present in both types of vesicle, with an amplitude of about 20% (Table 4). The amplitude of the short lifetime increases with temperature. At 30° C the amplitudes are similar for CVs and UVs. At 6° C, UVs exhibit a larger amplitude for the short lifetime component and a smaller amplitude for the long one, compared to CVs.

Therefore, for both isomers, the lipid bilayer of CVs and UVs is in an heterogeneous state at all temperatures measured. The presence of large fractions of short and long lifetimes at 6° C and below clearly indicates the presence of both solid and fluid

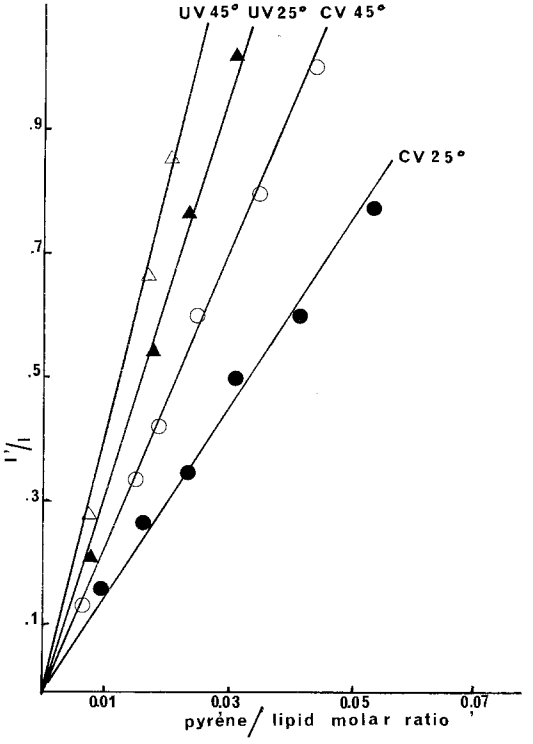

Fig. 2. Dependence of excimer to monomer fluorescence intensity ratio (I'/I) on pyrene/lipid molar ratio in CVs at 25° C ● and 45° C ○ and in UVs at 25° C ▲ and 45° C △

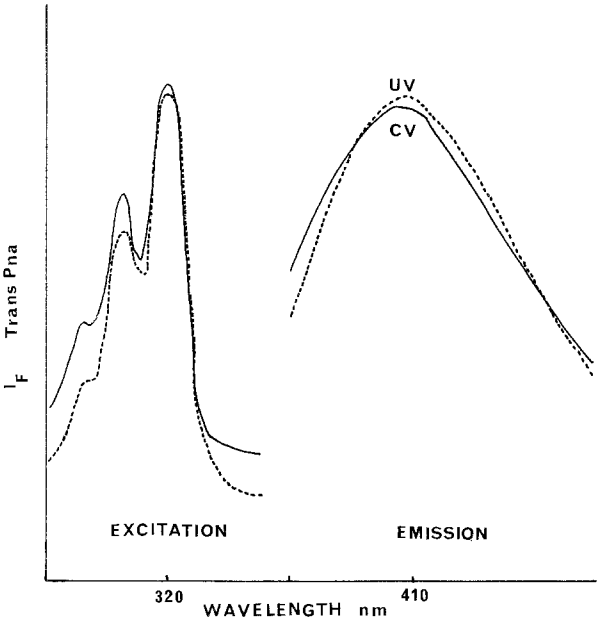

Fig. 3. Excitation and emission spectra of *trans* Pna in CVs and UVs. For excitation spectra, emission was at 410 nm. For emission spectra, excitation was at 320 nm. CVs, *trans*Pna (———) UVs, *trans* Pna (– – –)

Table 4. Lifetimes (τ) of the excited state of *cis* and *trans* Pna in CVs and UVs at different temperatures

T (°C)	τ (ns) *cis* Pna						τ (ns) *trans* Pna					
	CVs			UVs			CVs			UVs		
	τ_1	τ_2	τ_3	τ_1	τ_2	τ_3	τ_1	τ_2	τ_3	τ_1	τ_2	τ_3
6	31.5	16.4	5.9	39.0	18.1	6.0	53.8	22.0	6.8	56.5	21.4	6.0
18	20.0	9.1	2.5	18.5	4.7	0.2	33.0	12.2	3.75	35.0	13.0	3.5
30	11.5	4.0	0.1	12.5	4.1	0.2	23.0	8.2	2.4	23.6	8.8	2.3

[a] The numbers are averages from three series of experiments made with different preparations of CVs and UVs

lipid domains at this temperature; this is also true at 30° C as probed by the *trans* Pna. The *cis* Pna does not see the solid phase, which is in agreement with the fact that the *trans* isomer partitions preferentially in the gel phase. The linearity of the temperature dependence of ln τ_1 (long life time) for *trans* Pna suggests that the lipids are organized in separate solid domains, which decrease in size with increasing temperature. The lifetimes and amplitudes of both isomers are insensitive to the presence of the clathrin coat.

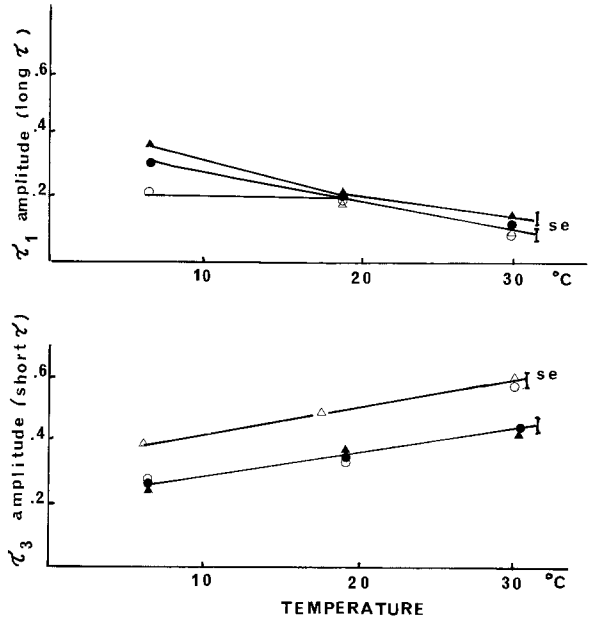

Fig. 4. Variation in lifetime amplitudes for *trans* and *cis* Pna in CVs and UVs as a function of temperature. CVs *cis* Pna ○; CVs *trans* Pna ●; UVs *cis* Pna △; UVs *trans* Pna ▲

b) Anisotropy decays. The analysis of the decay curves (Fig. 5 and Table 5) for the anisotropy is made very difficult, for both isomers, due to the presence of more than two components in the exponential term. However, the short time behaviour of the probes in both CVs and UVs is very similar, with a very fast decay. At all temperatures studied the amplitude of this fast decay is larger in CVs than in UVs. This leads, at long times, to a significantly lower anisotropy (r_∞) for the CVs than for the UVs. The cis isomer is even more sensitive to the presence of the clathrin coat around the lipid vesicle with a lower value or r_∞. Both probes therefore indicate that the clathrin coat introduces more disorder in the lipid bilayer.

Discussion

The chemical analysis of UVs prepared by dissociation of the coat proteins of the CVs (mainly clathrin and the "light chains") indicates that 60% of the

Table 5. Limit anisotropy values (r_∞) of CVs and UVs at different temperatures

T °C	CVs		UVs	
	cis Pna	*Trans* Pna	*cis* Pna	*Trans* Pna
6	0.140	0.220	0.190	0.240
18	0.150	0.220	0.200	0.240
30	0.140	0.200	0.190	0.230

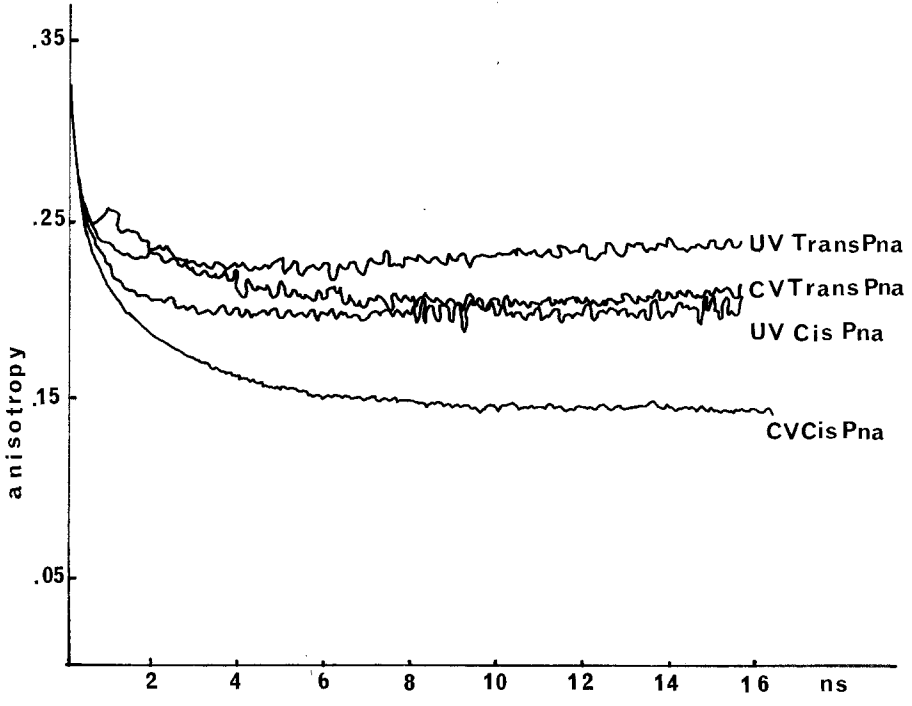

Fig. 5. Experimental anisotropy decay curves for *cis* and *trans* Pna in CVs and UVs at 18° C

weight of the UVs is due to the proteins. The phospholipid composition is similar to that reported previously for bovine brain CVs (Altstiel and Branton 1983; Pearse 1976) except that the ratio of cholesterol to phospholipid is considerably larger, i.e., 0.57, compared to 0.34 found by Altstiel and Branton, and 0.1 by Pearse. The cerebroside and ganglioside levels are reported for the first time. The cholesterol content is lower than that of either bovine myelin (Norton and Autiliot 1966) or guinea-pig brain synaptic vesicles (Eichberg et al. 1964), but is high compared to that of organelles from non-neural organs, such as the adrenal cortex (Gallay et al. 1981). It has been shown by Montesano et al. (1980) that the coated pit regions of 3T3 cells have a much lower content of cholesterol than surrounding areas of the plasma membrane. However, it was subsequently reported that uncoated vesicles contain cholesterol (McGookey et al. 1983). The redistribution of cholesterol during the formation of CVs could play an important role in coated vesicle formation.

Pyrene is a fluorescent molecule which forms excimers in membranes. The emission bands of the monomer and the dimer are well separated, allowing the simultaneous measurement of the two fluorescent species. Since it has been shown (Galla and Hartmann 1980) that the variation in the ratio of the fluorescence intensities of the monomer and excimer, as a function of probe concentration in the lipid, is a measure of the jump frequency and therefore of the lateral diffusion of the probe, this parameter has been measured in both CVs and UVs. The values obtained for the diffusion coefficient at several temperatures indicate that the coat perturbs the membrane and decreases the lateral mobility of pyrene. If we compare our data with that obtained with model vesicles of pure DPPC or DPPC containing different amounts of cholesterol (Galla and Sackmann 1974), the decrease in D corresponds to the addiditon of about 5% cholesterol. Furthermore the D values obtained for CVs are near that of a membrane with a relatively high cholesterol content, e.g. erythrocyte membranes. In membranes of low cholesterol content, such as sarcoplasmic reticulum, the D value is near that of UVs (Vanderkooi and Collis 1974).

Since the data for the lifetime of the excited state of the monomer and the dimer are best fitted by a single exponential, the distribution of the probe appears to be homogeneous in both types of vesicle. The values obtained correspond to a distinctly non-polar environment ($\tau > 100$ ns until 37° C).

The lifetimes of the excited state of the pyrene dimer as well as that of the monomer are significantly smaller for the CVs than for the UVs. This suggests that the presence of the coat proteins changes the organization of lipid and gives rise to a more polar environment for the probe, perhaps involving the membrane proteins.

The data obtained with the two isomers of parinaric acid shed further light on the lipid organization of CVs and UVs. As previously reported, the *trans* Pna partitions preferentially in the solid state domains, while *cis* Pna partitions equally in the fluid and solid domains of the lipids in systems where such domains coexist (Sklar et al., 1977b). The *cis* isomer exhibits also a preferential localization in the lipids surrounding the proteins (Kimmelman et al. 1979). Therefore, the lifetime analyses indicate the presence of coexisting solid and fluid lipid domains from 6° to 36° C, the solid phase decreasing in size with temperature. It should be noted that such a lipid organization is quite insensitive to the presence of the clathrin coat.

The anisotropy decay analysis confirms that the dynamics of the probes is not modified by the coat since the short time behaviour (fast decay) is the same in CVs and UVs. On the other hand, the amplitude of this fast decay is larger in CVs and about twice as high for *cis* as for *trans* Pna, leading to a lower anisotropy value at long time (r_∞) in the whole temperature range studied. This result indicates a disordering effect of the coat on the lipid bilayer. This effect is more important when probed by the *cis* than by the trans Pna, in agreement with a disordering effect due to protein, since the cis isomer partitions more in the lipids surrounding the proteins. According to Tecoma et al. (1977), in their study with *Escherichia coli* membranes, 50% by weight of proteins does not affect the ability of the probe to monitor lipid dynamics. Therefore, the effect of the coat is not due to the interaction of the probe with the coat proteins, but actually to modifications in the state of the lipids.

The data reported here can be understood if clathrin, when bound as a coat, interacts with one (or more) membrane-bound proteins to immobilize them and thereby reduce the lateral mobility of adjacent lipids in the fluid phase of the membrane. This can be seen in the reduced diffusion coefficient of pyrene. Furthermore, the interaction of the clathrin basket around the vesicle with one of the membrane proteins (Unanue et al. 1981) introduces more disorder in the surrounding lipid, as shown by the anisotropy value at long time.

In such lipids domains, probed mainly by *cis* Pna, the coat appears to slow down the decay rate, possibly by introducing a new component in the anisotropy decay. Due to the short time of residence of each lipid around a protein, the disordering effect of this molecule can only be measured with short time scale (nanosecond) methods, such as fluorescence. These data are in good agreement with the results obtained by Wolber and Hudson (1981) in experiments on the incorporation of the M13 coat protein

into dimyristoyl phosphatidylcholine vesicles. The observed influence of the clathrin coat upon lipid domains might allow some form of selection to take place in coated pits.

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References

Altstiel L, Branton D (1983) Fusion of coated vesicles with lysosomes: measurement with a fluorescent assay. Cell 32: 921–929

Ames BN, Dubin DT (1960) The role of polyamines in the neutralization of bacteriophages deoxyribonucleic acid. J Biol Chem 235: 769–775

Dickson RB, Beguinot L, Hanover JA, Richert ND, Willingham MC, Pastan I (1983) Isolation and characterization of a highly enriched preparation of receptosomes (endosomes) from a human cell line. Proc Natl Acad Sci USA 80: 5335–5339

Eichberg J, Whittaker VP, Dawson RMC (1964) Distribution of lipids in subcellular particles of guinea pig brain. Biochem J 92: 91–96

Galla HJ, Hartmann W (1980) Excimer forming lipids in membrane research. Chem Phys Lipids 27: 199–219

Galla HJ, Luisetti J (1980) Lateral and transversal diffusion and phase transitions in erythrocyte membranes, an excimer fluorescence study. Biochim Biophys Acta 596: 108–117

Galla HJ, Sackmann E (1974) Lateral diffusion in the hydrophobic region of membranes: use of pyrene excimer as optical probes. Biochim Biophys Acta 339: 103–115

Galla HJ, Hartmann W, Theilen U, Sackmann E (1979) On two dimensional passive random walk in lipid bilayers and fluid pathways in biomembranes. J Membr Biol 48: 215–236

Gallay J, Vincent M, de Paillerets C, Rogard M, Alfsen A (1981) Relationship between the activity of the 3β hydroxysteroid dehydrogenase from bovine adrenal cortex microsomes and membrane structure. J Biol Chem 256: 1235–1241

Goldstein HL, Anderson RGW, Brown M (1979) Coated pits, coated vesicles and receptor mediated endocytosis. Nature 279: 679–685

Hess HH, Rolde E (1964) Fluoremetric assay of sialic acid in brain gangliosides J Biol Chem 239: 3215–3220

Hess HH, Talheimer C (1965) Microassay of biochemical structural components in nervous tissues. Extraction and partition of lipids and assay of nucleic acids. J Neurochem 12: 193–204

Heuser JE, Reese TS (1973) Evidence for recycling of synaptic vesicles membranes during transmitter release at the frog neuromuscular junction. J Cell Biol 57: 315–344

Isenberg I, Small EW (1982) Exponential depression as a test of estimated decay parameters. J Chem Phys 77: 2799–2805

Keen HJ, Willingham MC, Pastan IH (1979) Clathrin and coated vesicles proteins: immunological characterization. Cell 16: 303–312

Kimmelman D, Tecoma ES, Wolber PK, Hudson BS, Wickner WT, Simoni RD (1979) Protein-lipid interactions. Studies of the M13 coat protein in dimyristoylphosphatidylcholine vesicles using parinaric acid. Biochemistry 18: 5874–5880

Markwek MAK, Haas SM, Bieber LL, Talbert NE (1978) A modification of the Lowry procedure to simplify protein determination in membranes and lipoprotein samples. Anal Biochem 87: 206–210

McGookey DJ, Fagerberg K, Anderson RGW (1983) Filipin-cholesterol complexes form in uncoated vesicles membrane derived from coated vesicles during receptor mediated endocytosis of low density lipoproteins. J Cell Biol 96: 1273–1278

Montesano R, Perrelet A, Vassali P, Orci L (1980) Absence of Filipin-cholesterol complexes from large coated pits of cultured cells. Proc Natl Acad Sci USA 76: 6391–6395

Nandi PK, Irace G, Van Jaarsveld PP, Lippoldt RE, Edelhoch H (1982) Instability of coated vesicles in concentrated sucrose solutions. Proc Natl Acad Sci USA 79: 5881–5885

Norton WT, Autiliot LA (1966) The lipid composition of purified bovine brain myelin. J Neurochem 13: 213–217

Nossal R, Weiss GH, Nandi PK, Lippoldt RE, Edelhoch H (1983) Sizes and mass distribution of clathrin coated vesicles from bovine brain. Arch Biochem Biophys 226: 593–603

Ott P, Bingeli Y, Brodbeck Y (1982) A rapid and sensitive assay for determination of cholesterol in membrane lipid extracts. Biochim Biophys Acta 685: 211–213

Paillerets de C, Gallay J, Vincent M, Rogard M, Alfsen A (1981) Membrane lipid dynamics and enzymatic activity in bovine adrenal microsomes. Biochim Biophys Acta 664: 134–142

Pearse BMF (1975) Coated vesicles from pig brain: purification and biochemical characterization. J Mol Biol 97: 93–98

Pearse BMF (1976) Clathrin: a unique protein associated with intracellular transfer of membranes by coated vesicles. Proc Natl Acad Sci USA 73: 1255–1259

Pearse BMF (1982) Coated vesicles from human placenta carry ferritin, transferrin and immunoglobulin G. Proc Natl Acad Sci USA 79: 451–455

Pearse BMF, Bretscher MS (1981) Membrane recycling by coated vesicles. Annu Rev Biochem 50: 85–101

Roth TF, Woods JW (1982) In: Differentiation and function in hematopoietic cell surfaces. AR Liss, New York, USA, pp 163–181

Rothman JE, Bursztyn-Pettegrew H, Fine HE (1980) Coated vesicles transport newly synthesized membrane glycoproteins from endoplasmic reticulum to plasma membrane in two successive stages. J Cell Biol 86: 162–171

Salisbury JL, Condeelis JS, Satir P (1980) The role of coated vesicles microfilaments and calmodulin in receptor mediated endocytosis by cultured B lymphoblastoid cells. J Cell Biol 87: 132–141

Sklar LA, Hudson BS, Petersen M, Diamond J (1977a) Conjugated polyene fatty acids as fluorescent probes: spectroscopic characterization. Biochemistry 16: 813–819

Sklar LA, Hudson BS, Simoni RD (1977b) Conjugated polyene fatty acids as fluorescent probes: synthetic phospholipid membranes studies. Biochemistry 16: 819–828

Tecoma ES, Sklar LA, Simoni RD, Hudson BS (1977) Conjugated polyene fatty acids as fluorescent probes: Biosynthetic incorporation of parinaric acid by Escherichia coli and studies of phase transitions. Biochemistry 16: 829–835

Trewehella MA, Collins FD (1973) Nature of the fatty acyl chains in different phospholipids. Biochim Biophys Acta 296: 51–61

Unanue ER, Ungewickell E, Branton D (1981) Clathrin membrane interaction. Cell 26: 439–446

Vanderkooi JM, Collis JB (1974) Pyrene: a probe of lateral diffusion in the hydrophobic region of membranes. Biochemistry 13: 4000–4006

Vincent M, de Foresta B, Gallay J, Alfsen A (1982) Nanosecond fluorescence anisotropy decays of n-(9-anthroyloxy)fatty acids in dipalmitoylcholine vesicles with regard to isotropic solvents. Biochemistry 21: 708–716

Wolber PK, Hudson BS (1981) Fluorescence lifetime and time resolved polarization anisotropy studies of acyl chains order and dynamics in lipid bilayers. Biochemistry 20: 2800–2810

Woodward MP, Roth RF (1978) Coated vesicles characterization, selective dissociation and reassembly. Proc Natl Acad Sci USA 75: 4394–4398