Biochimica et Biophysica Acta 859 (1986) 1–9
Elsevier

BBA 73127

Osmotic swelling of unilamellar vesicles by the stopped-flow light scattering method. Influence of vesicle size, solute, temperature, cholesterol and three α,ω-dihydroxycarotenoids

Alain Milon [a], Tarik Lazrak [a], Anne-Marie Albrecht [b], Geneviève Wolff [a], Gilbert Weill [c], Guy Ourisson [a,*] and Yoichi Nakatani [a]

[a] *Laboratoire de Chimie des Substances Naturelles UA 31 CNRS, Centre de Neurochimie, 5 rue Blaise Pascal 67084 Strasbourg,* [b] *Laboratoire de Chimie Physique et d'Electroanalyse, UA 405 CNRS, ENSCS, Institut de Chimie, 1 rue Blaise Pascal, 67008 Strasbourg and* [c] *Institut Charles Sadron (CRM-EAHP), CNRS et Université Louis Pasteur, 6 rue Boussingault, 67083 Strasbourg (France)*

(Received February 11th, 1986)

Key words α,ω-Dihydroxycarotenoid, Membrane reinforcement, Unilamellar vesicle, Water permeability, Stopped flow, Light scattering

α,ω-Dihydroxycarotenoids are postulated to play the rôle of membrane reinforcers in certain procaryotes, like cholesterol in eucaryotes. In order to evaluate the rigidifying effect of these polyterpenoids on lipid bilayers, osmotic swelling of unilamellar vesicles has been followed by measuring the light scattering intensity changes in a stopped-flow apparatus. A model based upon the Rayleigh-Gans theory was developped. It shows that the variation of light scattering intensity ($\Delta I/I_0$) is proportional to that of vesicle radius ($\Delta R/R_0$) for a given R_0 (initial vesicle radius). An emperical relationship between $-\Delta I/I_0$ and Z (dissymmetry measured by light scattering) was established: $-\Delta I/I_0$ is proportional to $(Z-1)$. Therefore, the value $-\Delta I/(I_0 \cdot (Z-1))$ is independent of vesicle radius and can be used for the evaluation of bilayer rigidity. The water permeability was measured and it was shown that it is the limiting factor of the kinetics of swelling When incorporated into dimyristoylphophatidylcholine vesicles, cholesterol and α,ω-dihydroxycarotenoids lower the water permeability and the value of $-\Delta I/(I_0 \cdot (Z-1))$. So, at least on model systems, α,ω-dihydroxycarotenoids exert on a phospholipid bilayer a reinforcement effect similar to cholesterol.

Introduction

Cholesterol is ubiquitous in eukaryotic cells and is believed to play the rôle of mechanical reinforcer of membranes Its effects on model membranes have been described by a variety of techniques (for a review, see Ref 1) In order to study the effect of various postulated procaryotic cholesterol surrogates like hopanoids and α,ω-dihydroxylated carotenoids [2], we have studied recently the osmotic swelling of vesicles [3] In this paper, we present new developments of this method and we study the influence of three dihydroxycarotenoids on the properties of a dimyristoylphosphatidylcholine bilayer These carotenoids are models of carotenoids postulated to reinforce membrane bilayers of prokaryotic cells in a trans-membranal manner [2] We have proven recently that they can be reincorporated in the

* To whom correspondence should be addressed
Abbreviations DMPC, L-α-dimyristoylphosphatidylcholine, Tris, 2-amino-2-hydroxymethylpropane-1,3-diol, SUV, small unilamellar vesicle, LUV, large unilamellar vesicle

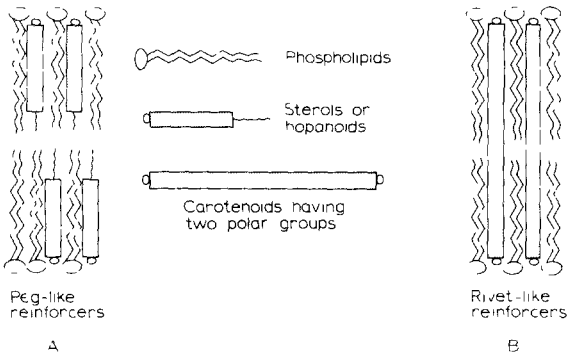

Fig 1 Model for cholesterol and carotenoid insertion in a lipid bilayer

hydrophobic core of lipid bilayers [4] (Fig 1)

We have employed the stopped-flow light scattering method to follow the osmotic swelling of unilamellar vesicles This method has been often used previously several authors have been interested in the initial swelling rate taken to be a measure of water permeability [5], others have studied the permeation of the solute responsible for the osmotic pressure [6,7] However, the amplitude of the light scattering change ($\Delta I/I_0$) has never been considered We have developped a model relating the amplitude of swelling to the elastic constants of the bilayer (unpublished data)

It was shown that the relative radius change ($\Delta R/R_0$) depends on the vesicle radius We now show the influence of the vesicle radius on the amplitude change ($\Delta I/I_0$) and on the kinetics of swelling We establish that the kinetics is indeed controlled by the water permeability Finally, applying the Rayleigh-Gans theory, we discuss the relationship between $\Delta I/I_0$ and $\Delta R/R_0$

Experimental

Materials (3*S*,3′*S*)-Decaprenoastaxanthin was prepared by the method described in Ref 4 (3*S*,3′*S*)-Astaxanthin and (3*R*,3′*R*)-zeaxanthin were kindly provided by F Hoffman-La Roche (Basel) Cholesterol from Fluka AG (Buchs) L-α-Dimyristoylphosphatidylcholine (DMPC) from Avanti Polar Lipids Inc (Birmingham), was kept at −20°C in chloroform solutions in sealed tubes The purity of the lipids was checked by thin-layer chromatography on F254 silica gel plates (0 25 mm thick) from Merck, Darmstadt (eluent $CHCl_3/CH_3OH$/conc ammonia, 65 25 4, v/v)

Preparation of vesicles Small unilamellar vesicles (SUVs) were prepared by sonication [8] (Branson Sonifier B-30, power setting 5) A solution of the lipid in chloroform was evaporated to dryness in vacuo without heating The desired amount of buffer (350 mM NaCl/1 mM EDTA-Na_2/5 mM NaN_3/10 mM Tris-HCl (pH 8), 'Ultrapure water' from Millipore) was added and argon was bubbled through for 15 min The sample was sonicated slightly above 23°C (phase transition temperature of DMPC) for 2 h It was then centrifuged (10000 × *g*, 30 min, 4°C) and the supernatant was collected and filtered through polycarbonate filters Large unilamellar vesicles (LUVs) were prepared by a modified procedure of the ether injection method [9,10] An ether-ethanol solution of the lipid was injected (injection rate 0 2 ml/min), into 5 ml of buffer maintained at 60°C The volume ratio of ether and ethanol, and the DMPC concentration in the injected solution (1 mg/ml to 20 mg/ml) are varied to vary the vesicle radii The vesicle suspension was dialyzed against the buffer twice in 1 l of buffer to remove the remaining solvent (3 h, then overnight at 4°C, dialysis tubing Spectrapor 2, Spectrum Medical Industries, Los Angeles) The samples was then filtered through polycarbonate filters (Nucleopore, DMF, 0 8 μm, 0 4 μm and 0 1 μm filters, twice through each) This procedure gives vesicles of reasonable homogeneity as shown previously by electron microscopy [4] The dissymmetry of the vesicles ($Z = I(45°)/I(135°)$) is determined by light scattering using a photogoniodiffusiometer Fica 42000 equipped with a 5 mW He-Ne Laser vertically polarized (SA Optilas, France)

Unilamellar vesicles were prepared from DMPC, or DMPC + 30% molar additive, with radii varying from 45 nm to 150 nm The smallest ones were obtained by sonication Their gel filtration on a Sepharose 4BCL column (Pharmacia France SA, Bois d'Arcy) showed only one peak corresponding to unilamellar vesicles free of multilamellar structures [8] In the case of DMPC alone, the dissymmetry of scattered light was 1 35, corresponding to a radius of 45 nm When prepared by the same procedure, vesicles containing cholesterol or carotenoids were always larger than those with DMPC alone

To get larger vesicles we have combined two known methods the ether injection method [9] and its modification by Kremer et al [10] In this method the vesicle distribution is affected by two parameters the solvent in which lipids are dissolved (ethanol gives smaller vesicles than ether), and the lipid concentration in the injected solution (higher concentrations give larger vesicles [11]) Kremer et al have studied the second parameter in detail but they used only very low concentrations of lipid which makes their method inconvenient By varying these two parameters we got vesicles with dissymmetries between 4 and 20 (corresponding to a radius of 85 to 110 nm) in reasonable concentration

Stopped-flow measurements The optical part of a Durrum-Gibson stopped-flow spectrophotometer was modified (Fig 2) to measure the light-scattering intensity at right angle The light beam is a 6 mW He-Ne vertically polarized laser of high amplitude stability (Model 120S SA, Spectra Physics, Les Ulis, France) beam amplitude noise (1–100 kHz) < 0 5% rms, beam amplitude ripple (1–120 Hz) < 0 2% rms, wavelength $\lambda = 632\ 8$ nm Before each experiment the photomultiplier voltage is adjusted to get 200 mV output voltage with a toluene solution With the buffer alone the output tension is then 150 mV The scattering intensity with vesicle suspensions is typically between 2 and 5 V Toluene is filtered through 0 5 μm Teflon Filters (Millex SR, Millipore SA, France), vesicle suspensions are kept at 40°C, degased under vacuum, and filtered through 0 2 μm polycarbonate filters just before the experiment to limit noise due to dust and bubbles The noise of the measured signal is about 1% of the signal in the worst conditions (fast kinetics for which the use of an electronic filter is not allowed) Typical phospholipid concentration is 10^{-4} M (0 07 mg/ml of DMPC) When temperature is not specified, the experiment is run at 33°C

Results and Discussion

Light scattering stopped-flow experiments and theoretical simulation

Stopped-flow experiment Fig 3 shows the proportionality between scattered light and vesicle concentration in the range typically used (0 2 mg/ml to 0 01 mg/ml of phospholipid) It shows that in this range the measured intensity corresponds only to light scattered by the vesicles, and that there is no multiple scattering After rapid mixing of a suspension of vesicles (in 350 mM NaCl buffer) with the same volume of 50 mM NaCl buffer, there is a decrease in scattered light which corresponds to the swelling of vesicles and to inflow of water into the vesicles (Fig 4) we have shown by dynamic light scattering that under these conditions, the vesicle radius increases of about 1% to 5% depending on the initial radius (unpublished data) Furthermore, a decrease in

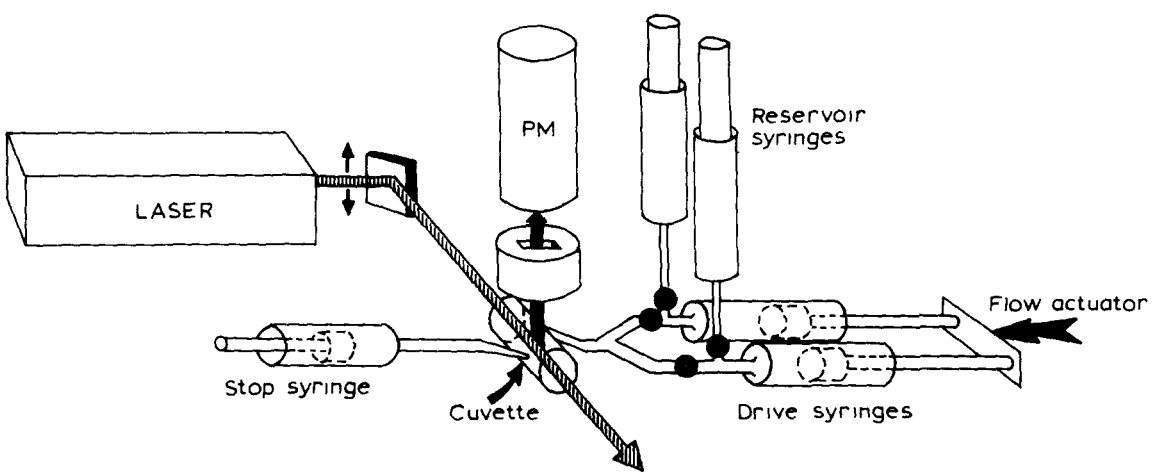

Fig 2 Light scattering stopped-flow apparatus The photomultiplier (PM) detects scattered light at 90° from the incident beam The beam is a 6 mW He-Ne laser vertically polarized

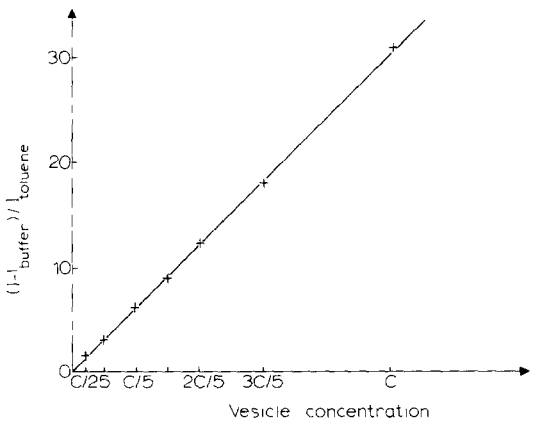

Fig 3 Intensity of scattered light versus concentration of DMPC vesicles $Z = 4\,1$, DMPC concentration $c = 2$ mg/ml, I_{buffer} scattered light by the buffer alone, $I_{toluene}$ scattered light by toluene (as reference), I scattered light by the vesicle solution

scattered light when the vesicles swell can be anticipated by the Rayleigh-Gans theory of light scattering (see below)

The scattered light decreases exponentially with time (Fig 5), the kinetics can thus be characterized by the reaction half-time $t_{1/2}$ This can be affected by the polydispersity of vesicles and an aggregation phenomenon The aggregation of vesicles gives rise to an increase of scattered light and could be observed in the case of high concentrations of salt (> 350 mM) and of phospholipid (> 0 2 mg/ml) It has a characteristic time of several seconds

The relative amplitude change $\Delta I/I_0 = (I_\infty -$

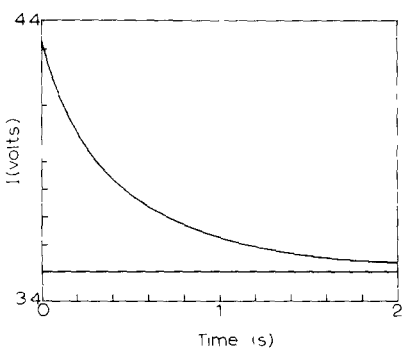

Fig 4 Recording of scattered light versus time during vesicle swelling upon osmotic pressure, NaCl inside 0 35 M, NaCl outside 0 2 M, vesicles made of DMPC + 30% molar cholesterol, $Z = 17$

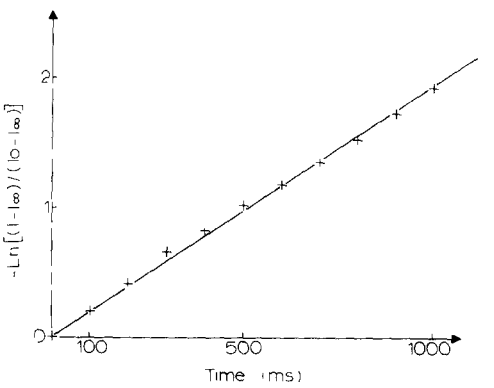

Fig 5 $-\mathrm{Ln}((I - I_\infty)/(I_0 - I_\infty))$ versus time for the document of Fig 4

$I_0)/I_0$ is independent of vesicle concentration, it is proportional to Δc (inside minus outside salt concentration), and therefore to the applied osmotic pressure

LiCl and NaCl give the same $\Delta I/I_0$ We have tried also molecular solutes (glucose, glycerol, ethylene glycol), all of which give rise to vesicle swelling

In the case of ethylene glycol, we observed a two step kinetics first a decrease of scattered light ($t_{1/2} = 300$ ms for a DMPC + 30% molar cholesterol membrane) followed by an exponential increase, back to the initial intensity ($t_{1/2} = 30$ s), corresponding to the diffusion of ethylene glycol through the membrane

Theoretical simulation of vesicle swelling It is important to known the relationship between the

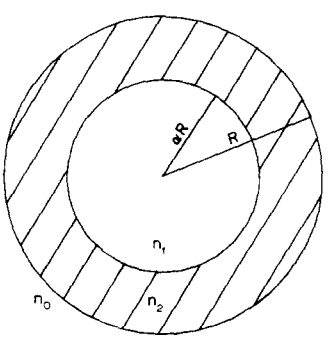

Fig 6 Model of lipid vesicle structure, n_0 is the refractive index of the solution outside of the vesicle, n_1 is the refractive index of the solution inside of the vesicle, n_2 is the refractive index of the lipid bilayer, R is outer radius of the vesicle, αR is inner radius of the vesicle

intensity change and the radius change in the experiment Several authors [5,6,11], following Bangham et al [12] have assumed that the turbidity τ is reciprocally proportional to the volume of the liposomes Yoshikawa et al [13], however, have applied to theory of Mie to large multilamellar vesicles and have shown that τ is reciprocally proportional to $V^{2/3}$, V being the total volume of the vesicles

We have applied the Rayleigh-Gans theory to unilamellar spheres of radius R The model we use is described in Fig 6 The intensity of scattered light is proportional to

$$I(\theta) \sim \left[\int_0^\infty g(r)\ \sinh r/hr\ 4\pi r^2 dr\right]^2 \tag{1}$$

where $h = (4\pi n_o/\lambda)\ \sin(\theta/2)$, λ is the incident wavelength in vacuum, θ is the angle of observation measured from the incident beam, and $g(r)$ is

$$g(r) \sim \left[n^2(r) - n_o^2\right] \tag{2}$$

For the model of Fig 6, we get

$$I(\theta) \sim \left[\left(n_1^2 - n_o^2\right)(\sinh \alpha R - h\alpha R\cosh \alpha R) + \left(n_2^2 - n_o^2\right) \times(\sinh R - hR\cosh R - \sinh \alpha R + h\alpha R\cosh \alpha R)\right]^2 \left[(3/h^3)\right]^2 \tag{3}$$

where n_o is the refractive index of the solution outside the vesicle, n_1 is the refractive index of the solution inside the vesicle, n_2 is the refractive index of the lipid bilayer, R is the outer radius of the vesicle, and αR is the inner radius of the vesicle

The first term in brackets (Eqn 3) corresponds to light scattered by the aqueous interior of the vesicle, when not of the same refractive index as the outside medium, and the second term corresponds to light scattered by the membrane The scattering factor $P(\theta) = I(\theta)/I(0)$ is

$$P(\theta) \sim I(\theta)/\left[\left(n_1^2 - n_o^2\right)\alpha^3 R^3 + \left(n_2^2 - n_o^2\right)(1-\alpha^3)R^3\right]^2 \tag{4}$$

When n_1 is equal to n_o, the result agrees with that of Seufert [14] and of Chong and Colbow [15] In the conditions of the swelling experiments we have shown numerically that both therms are important

TABLE I

DISSYMMETRY ($Z = I(45°)/I(135°)$) vs RADIUS(R) FOR UNILAMELLAR VESICLES OF DMPC

This table was computed using Eqn 3 taking wavelength λ = 632 8 nm, bilayer thickness = 3 nm

R(nm)	Z	R(nm)	Z	R(nm)	Z
10	1 01	70	2 42	97 5	7 93
20	1 06	75	2 84	100	9 34
30	1 15	77 5	3 09	102 5	11 1
35	1 21	80	3 39	105	13 6
40	1 29	82 5	3 74	107 5	16 9
45	1 39	85	4 15	110	21 6
50	1 51	87 5	4 64	112 5	28 5
55	1 67	90	5 23	115	39 3
60	1 86	92 5	5 95	120	91 9
65	2 11	95	6 83	129 7	∞

Starting from Eqn 4 with $n_1 = n_0$, we have computed tables of dissymmetry $Z = I(45°)/I(135°)$ versus radius, for λ = 632 8 nm, taking 3 nm for the bilayer thickness (Table I)

Radii estimated by this method and by the classical method of the Zimm plot are in reasonable agreement as long as the dissymmetry is not too high ($Z < 10$) For example, for Z = 1 57, a radius of 59 nm was found by the Zimm plot and of 52 nm according to Eqn 3 For larger vesicles the polydispersity has a more pronounced effect and cannot be neglected for our preparations

During the osmotic swelling experiment, the value of n_o is determined by the external concentration Just after mixing, n_1 is the value corresponding to the salt concentration c_0 of the preparation (350 mM) Assuming that, during swelling, the bilayer volume remains constant, one can calculate the evolution of R, α and n_1 from the initial values R_0, α_0 and from the relative change in volume $\Delta V/V_0$

$$R^3(1-\alpha^3) = R_0^3(1-\alpha_0^3) \tag{5}$$

$$R^3 = R_0^3(1 + \Delta V/V_0) \tag{6}$$

where R_0 and V_0 are initial values (before swell-

TABLE II

RELATIONSHIP BETWEEN RELATIVE LIGHT SCATTERING INTENSITY CHANGE ($\Delta I/I_0$) AND RELATIVE RADIUS CHANGE ($\Delta R/R_0$)

Swelling of DMPC vesicles (in 350 mM NaCl) after mixing with the same volume of 50 mM NaCl, initial vesicle radius $R_0 = 40$ nm, wavelength $\lambda = 632.8$ nm, scattering angle $\theta = 90°$

$100\,\Delta R/R_0$	0.1	0.2	0.5	1	2	5	10
$-100\,\Delta I/I_0$	0.067	0.133	0.333	0.667	1.34	3.39	6.91

ing) of R and V (V is the total volume of the vesicle)

$$\alpha^3 = \left(\alpha_0^3 + \Delta V/V_0\right)/(1 + \Delta V/V_0) \tag{7}$$

$$n_1^2 - n_0^2 = (n_1 + n_0)(c - c_{ext})\mathrm{d}n/\mathrm{d}c \tag{8}$$

where $\mathrm{d}n/\mathrm{d}c$ is the refractive index increment of sodium chloride ($\mathrm{d}n/\mathrm{d}c = 0.1$ cm^3/g), c_{ext} is the external sodium chloride concentration, and c is the internal sodium chloride concentration (function of time)

$$c - c_{ext} = c_0\ \alpha_0^3/\left(\alpha_0^3 + \Delta V/V_0\right) - c_{ext} \tag{9}$$

Supposing n_2 is constant during the swelling and equal to 1.46 [14,16], n_0 is also constant for dilute solutions of vesicles

Using Eqns 3 and 7–9 it is possible to get numerically the change of scattered light for a given set of R_0, R, c_{ext}, c_{int} For example, for vesicles of radius $R_0 = 40$ nm, we have computed the relationship between relative radius change ($\Delta R/R_0$) and relative light scattering change ($\Delta I/I_0$) (Table II)

Several conclusions can be drawn from these results

(i) This model predicts correctly the order of magnitude and the sign of the light scattering change for $\Delta R/R_0$ positive, $\Delta I/I_0$ is negative

(ii) It shows that for small changes of R, $\Delta I/I_0$ is proportional to $\Delta R/R_0$, and the value of the proportionality constant k is 0.67 for $R_0 = 40$ nm But the sensitivity k depends on the radius

$$\Delta I/I_0 = -k(R_0)\ \Delta R/R_0 \tag{10}$$

Table III shows the values of k calculated by a similar procedure for each radius

When R_0 is small, k tends to be 0, and this is the origin of the point ($Z = 1$, $\Delta I/I_0 = 0$) in Fig 7

In the case of an exponential change

$$I(t) - I_0 = \Delta I(1 - \exp(-t/\tau)) \tag{11}$$

Eqns 10 and 11 imply that

$$R(t) - R_0 = \Delta R(1 - \exp(-t/\tau)) \tag{12}$$

The functions $I(t)$ and $R(t)$ have the same time constant (τ) Therefore, the measured time constant is the time constant of swelling To our knowledge this is the first demonstration of this frequently used assumption

This simulation clearly shows that the sensitivity of this method depends on vesicle radius It was confirmed by the following experimental results

Influence of the vesicle radius on the amplitude change of light scattering

The amplitude change is related to the elastic properties of the bilayer However, it is not a

TABLE III

RELATIONSHIP BETWEEN INITIAL RADIUS(R_0) AND SENSITIVITY($k(R_0)$) $\Delta I/I_0 = -k(R_0)\ \Delta R/R_0$

Values of $k(R_0)$ were computed from Eqns 3 and 7–9

R_0 (nm)	10	20	30	40	50	60	70	80	90	100	110	120
$k(R_0)$	0.11	0.25	0.43	0.67	0.97	1.33	1.77	2.3	2.9	3.7	4.8	6.3

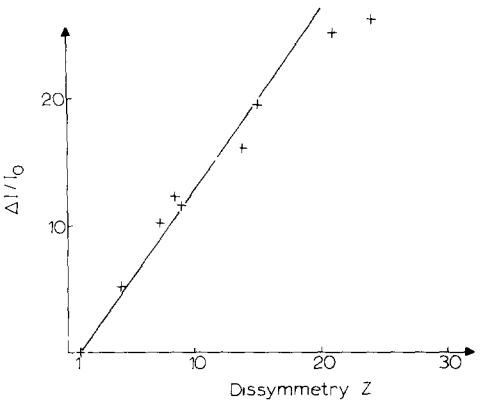

Fig 7 Influence of vesicle radius on the relative light scattering amplitude change ($\Delta I/I_0$) Vesicles made of DMPC + 30% molar cholesterol, prepared in buffer + 350 mM NaCl Rapid mixing with NaCl 50 mM

direct measure of the elastic constants because it depends also on the initial radius We have measured $\Delta I/I_0$ for vesicles (made of DMPC + 30% molar cholesterol) of different sizes The results are presented in Fig 7 It establishes an empirical relationship between $-\Delta I/I_0$ and Z, the dissymmetry measured by light scattering $-\Delta I/I_0$ is proportional to $(Z-1)$ for $Z < 15$

Since we know the relationship between $\Delta I/I_0$ and $\Delta R/R_0$ for a given R_0 (Table III) and the relationship between Z and R (Table I), we can plot the results as ln $\Delta R/R_0$ vs ln R (Fig 8) Despite the scattering of the points, it appears that the radius change could be described by a power law $\Delta R/R_0 \sim R^n$ with n between 3 and 4 More accurate measurements are requested to draw more precise conclusions

However, as $-\Delta I/(I_0 (Z-1))$ is independent of the vesicle radius, it can be taken as a measure of membrane rigidity For example

for DMPC bilayers

$$-\Delta I/(I_0 (Z-1)) = 3\,7$$

for DMPC + 30% molar cholesterol

$$-\Delta I/(I_0 (Z-1)) = 1\,4$$

Results obtained by this more empirical approach will be discussed in the following paragraphs

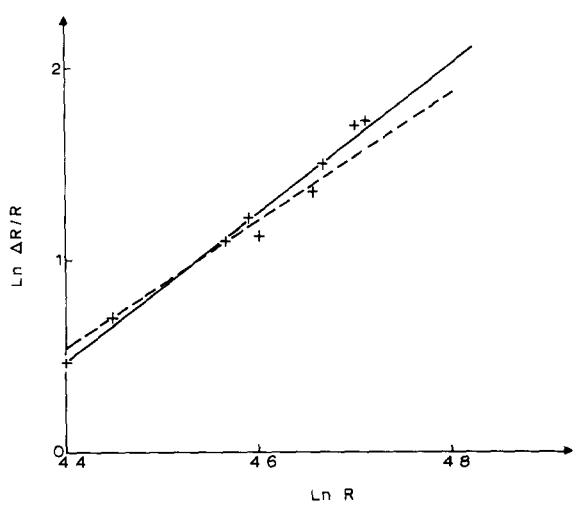

Fig 8 Relationship between relative radius change ($\Delta R/R_0$) and initial vesicle radius (R_0) in the range of radius experimented These data show that $\Delta R/R_0$ is proportional to R_0^n ($\Delta R/R_0 \sim R_0^n$), with n between 3 and 4 ——— linear regression over all points ($n = 3\,9$), — — — linear regression excluding data for large Z (2 points) ($n = 3\,4$)

Water permeability of DMPC-cholesterol bilayers

The permeability coefficient values obtained for planar bilayers by a method using osmotic shocks are different from those obtained with the highly curved bilayers of sonicated vesicles by exchange of isotopes [17] Is this due to curvature effects or to the method employed? Indeed, in the case of the osmotic shock method, the presence of the osmotic pressure could affect the bilayer properties In order to discriminate between these two effects, we have tried two different methods on the same preparation of vesicles The first method is the method developped by Lawaczeck [18] by rapid mixing of vesicles prepared in an H_2O buffer, with a similar isotonic solution prepared in ^2H_2O, one gets an experimental signal (change of scattered light), corresponding to the exchange between H_2O inside and ^2H_2O outside the vesicle. The rate constant is a measure of water permeability through the membrane We have tried this and the osmotic shock method, on DMPC + 30% molar cholesterol vesicles This series of experiments was run only for vesicles of large size ($Z = 14, 15, 18$) as, for smaller sizes, the signal in the ^2H_2O experiment is too small The half-time of the kinetics found by either method is the same $t_{1/2} = 200$ to 250 ms

This shows that, in the case of swelling upon

osmotic pressure the water permeability is the limiting factor

Furthermore, it appears that the water permeability is affected by vesicle size (see Table IV, the results for DMPC + 30% molar cholesterol of various sizes)

Influence of bilayer composition

Table IV summarizes our results in the study of the influence of the bilayer composition The first six lines show how cholesterol influences bilayer rigidity $-\Delta I(I_0\ (Z-1))$ and water permeability ($t_{1/2}$) As expected, cholesterol apparently increases the bilayer rigidity $-\Delta I/(I_0\ (Z-1))$ is 1 4 to 1 7, instead of 3 7 for DMPC alone The water permeability of the DMPC bilayer increases of a factor 50 at the phase transition ($t_{1/2}$ is 40 ms at 33°C and 2000 ms at 15°C with DMPC vesicles having $Z = 10$) as was shown previously by Lawaczeck [19] It is interesting to note that with cholesterol (30%), the water permeability ($t_{1/2}$ = 100 to 200 ms) is intermediate between that of DMPC alone, respectively, above ($t_{1/2} = 20$ ms and below $t_{1/2} = 2000$ ms) the phase transition It fits well with the idea that cholesterol has an ordering effect of DMPC above the phase transition

Finally, the effect of these carotenoids (astaxanthin, zeaxanthin, and decaprenoastaxanthin) on a DMPC bilayer have been compared As was shown in Fig 1, they enter the bilayer in a transmembranal manner and they stabilize both halves of the bilayer Therefore, one should compare 30% molar cholesterol to 15% molar carotenoid However, it is impossible to incorporate more than 8% of the C_{40} carotenoids in a DMPC bilayer [4] They all have an effect similar to cholesterol they lower the water permeability and have an effect on the swelling amplitude corresponding to a rigidification

This work shows that the stopped-flow light scattering method is well adapted to the measurement of water permeability and the evaluation of bilayer rigidity The amplitude of the light scattering change, which had never been studied, was interpretated by two distinct approaches

The theoretical simulation of the vesicle swelling makes it possible to compute radius change from the measured intensity change and allows further theoretical studies On the other hand, the calibration curve (Fig 7) allows direct comparison between vesicles of different radius This more empirical approach has been used to compare the reinforcing effect of cholesterol and three carotenoids on model membranes These compounds lower the water permeability and the amplitude of swelling These two parameters are indications of membrane reinforcement So, at least on model systems, incorporated bipolar-carotenoids exert on the bilayer a mechanical role similar to cholesterol [2] However, the solubility of the carotenoids in DMPC bilayers is limited [4], and we are currently

TABLE IV

SUMMARY OF THE EXPERIMENTAL DATA CONCERNING, RELATIVE LIGHT SCATTERING AMPLITUDE CHANGE ($\Delta I/I_0$), WATER PERMEABILITY ($t_{1/2}$) AND BILAYER RIGIDITY ($\Delta I/(I_0\ (Z-1))$), FOR VESICLES OF A GIVEN COMPOSITION AND DISSYMMETRY (Z), AT A GIVEN TEMPERATURE

Composition	Temp (°C)	Z	$-\Delta I/I_0$ (%)	$t_{1/2}$ (ms)	$-\Delta I/(I_0\ (Z-1))$ (%)
DMPC	33	3 7	10	20	3 7
DMPC	15	10	21	2000	2 3
DMPC + 30% molar cholesterol	33	4 2	5 5	70	1 7
	33	7	10 2	90	1 7
	33	9	11 5	115	1 4
	33	15	19 5	260	1 4
DMPC + 6% molar C_{50} astaxanthin	33	4 3	10	25	3
DMPC + 10% molar C_{50} astaxanthin	33	6 5	7	55	1 3
DMPC + 8% molar C_{40} astaxanthin	33	8	16 5	45	2 4
DMPC + 8 5% molar C_{40} zeaxanthin	33	9 3	17	30	2 1

studying the effect of natural bacteriocarotenoids on diphytanyl lecithins bilayers and similar model systems closer to natural bacterial membranes

Acknowledgements

The authors wish to thank CNRS for support of this work and F Hoffmann-La Roche & Co, Basel, who kindly gave us asta- and zeaxanthins

References

1 Demel, R A and De Kruyff, B (1976) Biochim Biophys Acta 457, 109–132
2 Rohmer, M, Bouvier, P and Ourisson, G (1979) Proc Natl Acad Sci USA 76, 847–851
3 Bisseret, P, Wolff, G, Albrecht, A M, Tanaka, T, Nakatani, Y and Ourisson, G (1983) Biochem Biophys Res Commun 110, 320–324
4 Milon, A, Wolff, G, Ourisson, G and Nakatani, Y (1986) Helv Chim Acta 69, 1–13
5 Bittman, R and Blau, L (1972) Biochemistry 11, 4831–4839
6 Kometani, T and Kasai, M (1978) J Membrane Biol 41, 295–308
7 Kannenberg, E, Blume, A, Geckeler, K and Poralla, K (1985) Biochim Biophys Acta 814, 179–185
8 Huang, C H (1969) Biochemistry 8, 344–351
9 Deamer, D and Bangham, A D (1976) Biochim Biophys Acta 443, 629–634
10 Kremer, J M H, Esker, M W J v d, Pathmamanoharan, C and Wiersema, P H (1977) Biochemistry 16, 3932–3935
11 Jain, M K, Toussaint, D G and Cordes, E H (1973) J Membrane Biol 14, 1–14
12 Bangham, A D, De Gier, J and Greville, G D (1967) Chem Phys Lipids 1, 225–246
13 Yoshikawa, W, Akutsu, H and Kyogoku, Y (1983) Biochim Biophys Acta 735, 397–406
14 Seufert, W D (1970) Biophysik 7, 60–73
15 Chong, C S and Colbow, K (1976) Biochim Biophys Acta 436, 260–282
16 Cherry, R J and Chapman, D (1967) J Mol Biol 30, 551–553
17 Disalvo, F A (1985) Chem Phys Lipids 37, 385–397
18 Lawaczeck, R (1984) Biophys J 45, 491–494
19 Lawaczeck, R (1979) J Membrane Biol 51, 229–261