

Polarity Decrease at the Adhesive Junction between Two Model Membranes Containing Gangliosides[†]

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ABSTRACT: The increased electrical conductance previously observed between two model membranes containing gangliosides suggests the creation of a new environment in the adhesive junction [Brewer, G. J., & Thomas, P. D. (1984) *Biochim. Biophys. Acta* 776, 279]. In order to provide a mechanism for this novel finding, we now report an investigation of the micropolarity in the adhesive junction. Emission from the fluorescent probe PRODAN is a sensitive measure of polarity of the probe environment. A bimodal linear relationship correlates the emission wavelength from PRODAN with the inverse of solvent dielectric constant ($1/\epsilon$). A better single linear relationship is obtained using Reichardt's relative polarity measure (RPM). Creation of two macroscopic spherical lipid bilayers from phosphatidylcholine, brain gangliosides, and PRODAN allowed selective excitation and observation of fluorescence from either a single bilayer or the double bilayer in the adhesive junction. The reported PRODAN polarity of -0.57 in a single ganglioside-containing membrane was midway between the polarity of water and *n*-hexane, suggesting PRODAN localization near the lipid carbonyls. The adhesive *junctional* region exhibited two new less polar environments of PRODAN fluorescence, $\text{RPM} = -0.45$ and -0.29 . These measures are consistent with a relatively dehydrated immobilized phase. These changes were not observed in the adhesion zone between two membranes made with phosphatidylcholine without gangliosides. The changes in molecular structure in the junction that could be responsible for the altered PRODAN emission are discussed. A decrease in the hydrocarbon thickness of junctional membranes or a decrease in the aqueous junctional polarity could be responsible for the polarity decrease reported by PRODAN.

When two cells come together, the conjunction of their surface molecules could produce a new surface environment with physical-chemical properties unlike those that existed previously. This simple "new milieu" concept could contribute to an understanding of important biological phenomena such as contact inhibition of cellular motility (Abercrombie, 1979) and the loss of intercellular adhesion in metastasis (Poste & Fidler, 1980). The new milieu is of further interest because it could provide to the cell a signal for contact sensation to modulate enzymatic activity without necessarily requiring high-affinity specific protein interactions or hormonal signals between cells in contact. Consideration of this concept has led to our investigation of a simple membrane system in which membrane-membrane interaction can be studied with defined membrane components in the absence of uncharacterized metabolic events present in whole cells.

Model Membranes. The large spherical membranes used here (Brewer & Thomas, 1984) are spherical analogues of the useful planar "bilayer lipid membranes" originally described by Mueller et al. (1962). They are similar to the spherules created by Pagano and Thompson (1967). In addition to spectroscopic access (Yguerabide & Stryer, 1971), additional advantages accrue from leaving these membranes attached to the tips of the syringes from which they are formed: electrical continuity to the interior and physical manipulation of two membranes into contact. This permits simultaneous assessments of membrane structure and adhesive function.

Gangliosides. The sialic acid-containing glycosphingolipids, gangliosides, are significant but functionally uncharacterized components of the cell surface of vertebrates (Svennerholm et al., 1980). Because their repulsive electrostatic charge density is less at small radii, they are expected to concentrate

with other negatively charged lipids at filipodial extensions and microvilli where cell contact is first expected (Israelachvili, 1973). Because the evidence for ganglioside function at the cell surface in vivo is largely circumstantial, we have begun studies of reconstituted membranes containing only phosphatidylcholine and the desired gangliosides dissolved in decane. Associated with the adhesion of two such membranes, a 3-fold increase in conductance was observed to be ganglioside-dependent (Brewer & Thomas, 1984). This signal for membrane contact could be explained by a number of mechanisms. (1) Since the specific conductance of these membranes made without gangliosides is approximately 3-fold that of membranes with gangliosides (Brewer & Thomas, 1984), a depletion of gangliosides from the junction would leave a region of adhering phospholipid of higher conductance than the rest of the membrane. (2) Since single ganglioside-containing membranes show a 10-fold higher conductance at pH 5.0 than at pH 7.45 (Brewer & Thomas, 1984), acidification of the junctional region by enrichment of the acidic gangliosides would conduct more readily. (3) Since the energy barrier to membrane conductance drops when the thickness of the membrane drops, conductance across two such membranes could be increased by thinning of each membrane in the junctional region. (4) Since the energy barrier to membrane conductance depends on the difference in polarity between the aqueous phase and the membrane interior, conductance across two such barriers could be increased by lowering the polarity between the membranes. Evidence consistent with a change in polarity at the junction of two membranes is reported here. Investigation of the other mechanisms will be the subject of subsequent communications.

Polarity Probe. The fluorescent probe 6-propionyl-2-(dimethylamino)naphthalene (PRODAN) was specifically designed by Weber and Farris (1979) with a large dipole moment

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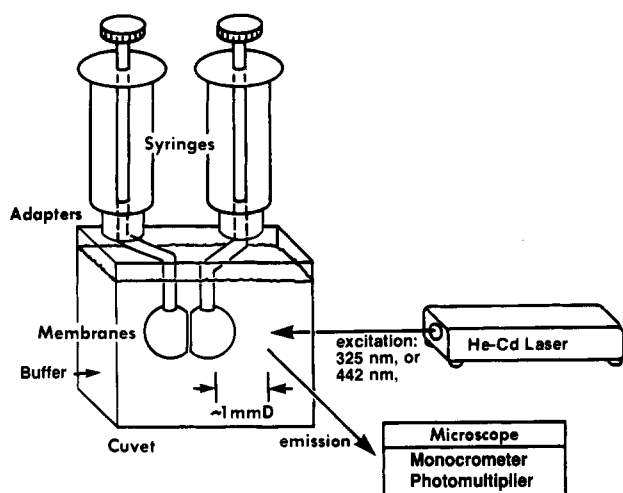


FIGURE 1: Apparatus for microscopic fluorescence spectroscopy of large spherical bilayer membranes. Membranes are formed at the tips of two syringes filled with buffer and immersed in a cuvette containing buffer (not to scale). Micromanipulators not shown attach to the syringes to appose two membranes. Fluorescent probes such as PRODAN in the membrane are excited by the ultraviolet laser line. Junctional and single bilayer regions of the membranes are imaged by a microscope with an attached monochromator and photomultiplier.

so that the emission wavelength would depend on the polarity of the environment, i.e., the dipole moment of surrounding lipids and water. The molecule is also uncharged so that emission would not depend on electric fields in a membrane. The naphthalene moiety favors membrane localization while the keto and tertiary amino groups can be expected to promote localization in the polar headgroup region. The classical Stokes shift reflects an energy difference between absorption and emission ($\Delta\nu$), which is related theoretically by the Lippert equation (Lippert, 1975) to the dielectric constant (ϵ) and index of refraction (n) of the environment:

$$hc\Delta\nu = \frac{2(\mu^* - \mu)^2}{a^3} \left[\frac{(\epsilon - 1)}{(2\epsilon + 1)} - \frac{(n^2 - 1)}{(2n^2 + 1)} \right] + \text{constant} \quad (1)$$

$$hc\Delta\nu = \frac{2(\mu^* - \mu)^2}{a^3} \Delta f + \text{constant} \quad (2)$$

where h is Planck's constant, c is the speed of light, μ^* and μ are the dipole moments of the excited and ground states, and a is the radius of the cavity in which the probe resides. The Δf term is the orientational polarizability. It will be shown that a good correlation exists for a more simple relationship for PRODAN over its range of emission from 400 nm in cyclohexane to 530 nm in water.

MATERIALS AND METHODS

Figure 1 shows the basic apparatus for membrane microspectroscopy. The materials and methods for formation of large spherical bilayer membranes from purified egg yolk phosphatidylcholine (EPC) and beef brain gangliosides have been described (Brewer & Thomas, 1984). Briefly, EPC and gangliosides were mixed in the desired proportions in CH_2Cl_2 - CH_3OH , the solvent was evaporated, and the sample was redissolved in *n*-decane. This membrane-forming solution was deposited on the glass tips of two syringes filled with Tris-buffered saline. With the tips in a cuvette filled with saline, advancing the plunger produced a 1-mm diameter spherical thin film which spontaneously thinned to a lipid bilayer. Two

of these spherical bilayers were brought into contact, from which a zone of adhesion enlarged spontaneously. PRODAN (Molecular Probes, Eugene, OR) was incorporated at 1 mol % relative to other lipids. Fluorescence was excited by the 2 mW 325-nm line from a He-Cd laser (Liconix, Sunnyvale, CA). Fluorescence emission was collected at 90° to the laser beam through a Zeiss 20× LWD objective into a photomultiplier with voltage set to count single photons. For recording spectra, the photomultiplier output was multiplexed with an output from the Instruments SA H10 monochromator into an Apple II+ computer. Pyridine-*N*-oxide, *n*-decane, and 1-butanol were purchased from Aldrich Chemical Co. (Milwaukee, WI). Other chemicals were from Sigma Chemical Co. (St. Louis, MO). Absorption spectra were recorded on a Beckman Acta II dual beam spectrometer (Fullerton, CA). Uncorrected fluorescence spectra were obtained in the instrument described previously (Brewer & Thomas 1984). Dielectric constants were obtained from Dean (1979).

RESULTS

Calibration to Physical Parameters. The fluorescent probe PRODAN was used in order to determine the polarity of different environments in a membrane. Calibration of the orientational polarizability for PRODAN in a number of pure solvent environments has been reported by Weber and Farris (1979). Although the dipole interaction theory of Lippert (1975) predicts a linear relation of Stokes shift to orientational polarizability, a linear correlation coefficient (r^2) of only 0.89 is found, with a particularly poor fit for the more polar solvents. An equally mediocre fit is obtained when the orientational polarizability, Δf , is simplified by ignoring the electronic polarizability term involving n .

While the polar anomalies can be relegated to "specific solvent effects" (Lakowicz, 1983), their presence as a group suggests an interaction with PRODAN different from that of apolar solvents. In fact, if the apolar solvents are considered separately, a more simple linear relationship of emission maximum (nm) and solvent dielectric constant ($1/\epsilon$) produces a better correlation coefficient (Figure 2A; $r^2 = 0.957$).¹ A similar subcategorization for the polar solvents also produces a good linear correlation coefficient ($r^2 = 0.985$). This simplifies experimental analysis of PRODAN environments when the Stokes shift cannot be measured because of excitation with a fixed wavelength laser. It suggests that there are two very different modes of interaction of solvent with PRODAN and that the most significant component of the interaction energy is the molecular reorientation of apolar solvents with the excited state of PRODAN. For polar solvents, which exhibit very large wavelength shifts for small changes in ϵ , the interaction energy with PRODAN is very sensitive to the measure of solvent polarity ϵ , suggesting a dipole-dipole interaction. For apolar solvents, the interaction is fairly insensitive to large changes in ϵ , suggesting interaction of induced dipoles or dispersion forces (Israelachvili, 1985).

A possible problem in these relationships is that the orientational polarizability is a bulk phase parameter composed of macroscopic measures of solvent molecular orientation to an external electric field (dielectric constant, ϵ) and electronic orientation to the electric field of visible light (index of refraction, n). Ultimately, a probe-solvent interaction is molecular, so that a measure of solvent interaction with a dipolar probe could provide an empirical scale of the strength of in-

¹ In general, when the rate of solvent dipole reorientation is greater than the rate of emission, the emission spectrum will be independent of excitation wavelength (Macgregor & Weber, 1986).

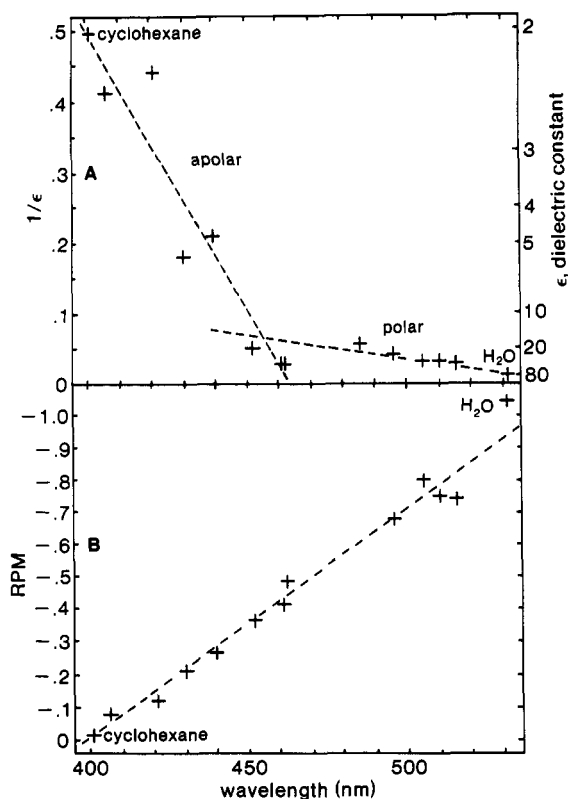


FIGURE 2: (A) Relationship of PRODAN emission maximum (λ) to dielectric constant, $1/\epsilon$. Lines were fit by linear regression to the polar solvents ($1/\epsilon = -7.77 \times 10^{-4}\lambda + 0.43$, $r^2 = 0.985$) and to the apolar solvents ($1/\epsilon = -7.87 \times 10^{-3}\lambda + 3.65$, $r^2 = 0.957$). Technically, PRODAN emission energy should be expressed as the inverse of wavelength (wavenumber), but since this energy range is small, the more familiar wavelength measure is used without significant distortion. Solvents from left to right are cyclohexane, triethylamine, benzene, chlorobenzene, chloroform, acetone, dimethylformamide, acetonitrile, butanol, ethanol, methanol, propylene glycol, ethylene glycol, and water [Weber and Farris (1979) except butanol]. (B) Relationship of PRODAN emission wavelength (λ) to relative polarity measure, RPM. The RPM ranges from 0 for *n*-hexane to near -1 for water. The solvent RPM values were obtained from the absorption transition energies for pyridiniophenolate (Reichardt, 1979) and correlated with the above 13 solvents for which emission maxima of PRODAN were reported. The line represents $\text{RPM} = -0.00727(\lambda) + 2.91$ with a correlation coefficient of 0.99.

teraction and the amount of relaxation that contributes to the fluorescence emission. Kosower (1958) and Reichardt (1979) have devised such a polarity scale, by measuring the shift in ultraviolet absorption transition energies of pyridinium molecules with fixed dipoles. Therefore, we correlated the PRODAN emission maxima in the same 13 common solvents with the Reichardt relative polarity measures (RPM) for the interaction of solvents with pyridiniophenolate (Figure 2B). The RPM is scaled from 0 for apolar *n*-hexane to near -1 for polar water. The excellent correlation obtained ($r^2 = 0.99$) demonstrates that this method inherently spans the transition from dipole-dipole to dipole-induced dipole interactions. The Kosower polarity scale has been successfully applied to fluorescence of 9-methylanthroate in membranes (Werner & Hoffman, 1973).

Fluorescence of PRODAN in Membranes. With these calibrations, relative membrane polarity (RPM) can be determined from measurements of the PRODAN emission maximum in single bilayer membranes. The results in Figure 3A show the complex emission pattern expected for PRODAN in multiple environments in a membrane containing 4 mol % gangliosides in egg phosphatidylcholine. PRODAN partitions between the aqueous and membrane phases. A scan outside

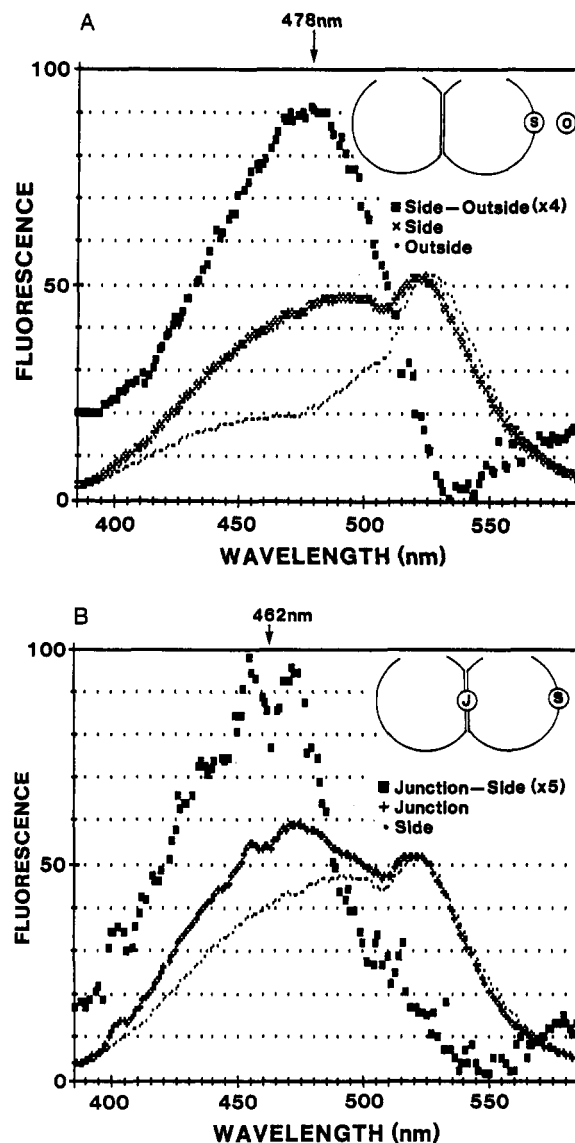


FIGURE 3: PRODAN emission from large spherical model membranes containing 4 mol % gangliosides in egg phosphatidylcholine. (A) Before adhesion, photons emitted at indicated wavelengths were counted from a region of the electrolyte outside (O) the membranes (---). A similar spectrum was obtained from the intersection of the laser beam with the side (S) of the membrane (xxx). A difference spectrum (■) was computed and expanded $\times 4$. A membrane-specific peak is observed at 478 nm. (B) After adhesion, similar spectra were recorded from the side (S, ---) and the junctional (J, ++++) regions of the membranes. The difference spectrum (■) expanded $\times 5$ exhibits a new maximum centered at 462 nm.

the membrane reveals emission from a primarily aqueous phase at high wavelength (maximum 527 nm), the same maximum observed with PRODAN in pure water in our optical system.² With this background, emission collected from the intersection of the exciting laser beam and the side of the membrane can be corrected for the large portion of the signal resulting from emission in water. This produces a unique peak at 478 nm. When two such membranes are brought into contact, the

² The shoulder in the region of 500 nm is not observed in pure water. This suggests its origin from decane-lipid-ganglioside micelles which are present in the aqueous phase at equilibrium. The rise in the difference spectrum above 550 nm is not considered significant since the trough at 535–545 nm is not reproducible. The difference in optima for water in our optical system (527 nm) and that previously reported (530 nm) (Weber & Farris, 1979) is due most likely to our lack of correction for spectral sensitivity. The uncorrected emission maximum of PRODAN in water was 521 nm on a new Perkin-Elmer LS-5 spectrofluorimeter.

Table I: PRODAN Emission and Environmental Polarity^a

environment	measured emission max (nm)	polarity, inferred RPM	dielectric constant	
			apolar scale	polar scale
ganglioside-membrane side	478	-0.57		17
ganglioside-membrane junction	462	-0.45		14
	440 (shoulder)	-0.29	5	11
EPC-membrane side	486	-0.62		19
EPC-membrane junction	495	-0.69		22
environment	measured emission max (nm)	measured RPM	RPM, Reichardt (1979)	ϵ , Dean (1979)
<i>n</i> -decane, 100%	421	-0.07		(2)
tricaproin, 100%	438	ns		
1-pentanol				(14)
<i>n</i> -propyl acetate			(-0.21)	(6)
1-butanol, 100%	496	-0.62	(-0.62)	(17.5)
1-dodecanol			(-0.51)	(5)
glycerol triacetate			(-0.35)	
glycerol, 100%	ns	-0.75		(42.5)
glycerol, 50% in water	ns	-0.61		
glycerol phosphate, 1 M, pH 7	454	-0.17		
potassium phosphate, 0.5 M, pH 7	487	-0.61		
sodium chloride, 5 M	455	-0.88		(50) (Werner & Hoffman, 1973)
phosphoryl choline, 0.5 M, pH 7	493	-0.95		
choline chloride, 4.2 M, pH 7	500	-0.95		
water	527	-1.02	(-1.04)	

^a All emission maxima are corrected for the corresponding aqueous or membrane control. Solutions are made in water and maxima are corrected for emission in water. ns, probe not soluble. Dielectric constants and RPM are interpolated from Figure 2, panels A and B, respectively, unless indicated otherwise by parentheses (values from the literature). Measured RPM's were obtained by analyzing the UV absorption maximum of pyridine-*N*-oxide in the indicated solvent (Kosower, 1958). The absorption maximum, ν (cm⁻¹), is converted to absorption energy, $E = h\nu$ (kcal/mol) where h is Planck's constant, and to RPM (Reichardt, 1979) by $RPM = (E_{nh} - 6.14 \times 10^{-3}\nu + 181)/E_{nh}$, where E_{nh} is the absorption energy of pyridiniophenolate in *n*-hexane, 30.9 kcal/mol.

contact point grows to become a circular adhesion zone of two bilayers (Brewer & Thomas, 1986). PRODAN emission from this junctional area exhibits a new maximum³ centered at 462 nm and a reproducible shoulder at 440 nm when the emission from a single bilayer is subtracted (Figure 3B). Subtraction is justified by noting that the two outer lamellae in the junction of two bilayers are equivalent to a single nonadhesive bilayer. Similar results were obtained from the adhesive junction of membranes initially containing 12 mol % gangliosides. Larger proportions of gangliosides are nonadhesive, probably due to electrostatic repulsion (Brewer & Thomas, 1986).

Analogous measurements of PRODAN emission from membranes made without gangliosides (EPC alone) exhibited an emission maximum of 486 nm on the side (Figure 4A). When the junction was similarly examined, the difference spectrum was much smaller with an ill-defined maximum around 495 nm (Figure 4B). These higher wavelengths and a small junctional change in the opposite direction are remarkably different from membranes containing gangliosides.

Membrane Polarity before Adhesion. Correlations between PRODAN emission and environmental polarity are collected in Table I. Comparisons are also included for several model "solvents" for portions of the membrane polar headgroup region which were examined for specific interactions with PRODAN. From the controls, PRODAN in a membrane is in an environment more polar than the pure hydrocarbon decane or the triglyceride tricaproin. The environment appears similar in polarity and dielectric to 1-butanol but similar only in polarity to dodecanol, combining both dipolar and induced-dipolar properties. If one assumes a cross-sectional area of PC to be 70 Å² and a headgroup thickness of 7 Å, then the

headgroup concentration amounts to 3 M. Therefore, aqueous solutions of headgroup moieties were formulated near this concentration or near their solubility limits. PRODAN in a membrane exhibits fluorescence emission characteristic of an environment less polar than that of the penultimate choline and dissimilar to glycerol phosphate and phosphorylcholine. The observed polarity is similar to that of 0.5 M potassium phosphate or 50% glycerol. Anisotropic molecular orientation in a membrane is likely to cause changes from dilute solutions. The importance of orientational factors or specific binding is suggested by the low PRODAN emission wavelengths and low RPM observed in 5 M NaCl and 1 M glycerol phosphate. A bound state of water has been demonstrated in high concentrations of NaCl that depress the measured dielectric constant from 78 down to 50 (Hasted et al., 1948; Wang & Bruner, 1978). In membranes, because the amount of polarized water increases with the surface charge density, the local dielectric constant theoretically decreases (Zuckermann et al., 1985). Pyrene (Glushko et al., 1981) or merocyanine 540 (Montich et al., 1985) appear to be other fluorescence probes suitable for estimating dielectric constants and polarity in different regions of membranes. Kimura and Ikegami (1985) have similarly used a different probe, dansylphosphatidylethanolamine, to detect changes in local dielectric through the bilayer phase transition of liposomes.

DISCUSSION

In comparison to membranes without gangliosides, the environment for PRODAN in ganglioside-containing membranes appears considerably less polar. It may reflect a structural difference that accounts for the mean conductance differences for these membranes (Brewer & Thomas, 1984): 48 nS/cm² for EPC and 17 nS/cm² for EPC + gangliosides. Massey et al. (1985) report a similar difference for PRODAN emission maxima in small bilayer vesicles in the liquid-crystalline state made from dimyristoylphosphatidylcholine (492 nm) and sphingomyelin (485 nm). This may reflect a partial dis-

³ The narrow peaks at 455 and 472 nm most likely arise from non-systematic error in recording the original spectra. The differences are then amplified and provide a measure of the uncertainty in the data, ± 10 fluorescence units. The 50-nm breadth at half-height of the main peak attests to its reality.

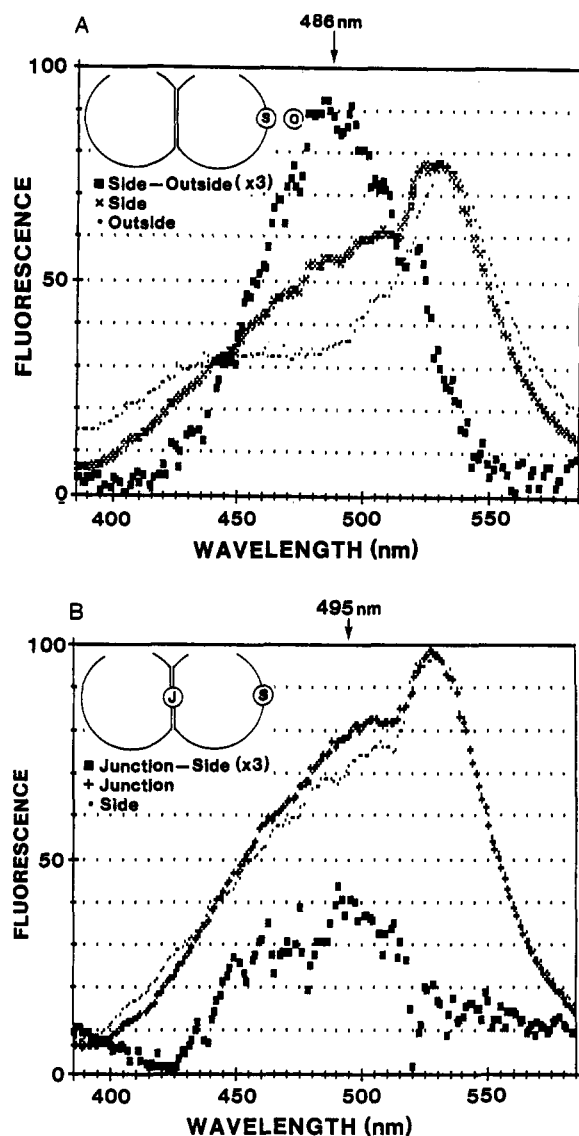


FIGURE 4: PRODAN emission from large spherical model membranes made with egg phosphatidylcholine without gangliosides. (A) Before adhesion. (B) After adhesion. The symbols are the same as in the legend to Figure 3. The spectrum to be subtracted was multiplied by either 2.33 (A) or 1.01 (B) to produce equal intensity of the water peak at 527 nm. The difference spectra were then expanded $\times 3$.

placement of waters of hydration from the carbonyl region or the weaker dipoles themselves in the sphingosine base moieties of the gangliosides (see below). The negligible change in PRODAN emission at the junction of two membranes without gangliosides supports the absence of a structural change in the region of the probe and the absence of a change in conductance across these two adhesive bilayers (Brewer & Thomas, 1984).

Membrane Polarity after Adhesion. The most notable finding occurs for PRODAN emission from membrane junctions containing gangliosides. Since PRODAN itself spans 13 Å of the membrane, its mean environment reflects the net effects of several regions of the bilayer. Further interpretation requires caution about possible inaccuracies of extrapolation of polarities from PRODAN emission maxima in homogeneous solvents to complex lipid bilayers. Before adhesion, the PRODAN emission suggests a mean environment similar to 1-butanol or 1-pentanol with an interpolated RPM of -0.57 to -0.62 . After adhesion, PRODAN appears to sense a less polar environment. Interpolation suggests a new environment with an RPM of -0.45 . A reproducible emission shoulder suggests another environment as low as -0.29 . These values

suggest a less polar environment around PRODAN and/or closer proximity of PRODAN to the acyl chains possibly due to lipid spreading. They are consistent with dielectric values of 10–30 in the acetyl region of PC derived by Ashcroft et al. (1983) from capacitance measurements but lower than the dielectric of 26 inferred from reaction rates of α -tocopherol (Bellemare & Fragata, 1980). They are understandably less polar than the value of 30 suggested by the location of the charged fluorescent probe ANS (Sachmann & Trauble, 1972). Similar fluorometric studies with merocyanine 540 indicated a dielectric value of 8 in the acetyl region (Lelkes & Miller, 1980); and another study with 9-(dicyanovinyl)julolidine indicated a value of 18 in dipalmitoylphosphatidylcholine (Kung & Reed, 1986). In comparing gangliosides to phospholipids, the single amino ester of the sphingosine base is a weaker dipole and hence less polar than the two carboxylic ester linkages of a phospholipid. However, these differences by themselves are of insufficient magnitude to explain the large changes in PRODAN emission observed at the junction (Flewelling & Hubbell, 1986). Massey et al. (1985) report a large drop in the PRODAN emission maximum from small bilayer vesicles of sphingomyelin or dimyristoylphosphatidylcholine in transition from the liquid-crystalline state (485 and 492 nm, respectively) to the gel state (433 and 437 nm). Lackowicz et al. (1983) reported a similar shift from 470 to 425 nm through the bilayer transition temperature for a PRODAN derivative in phospholipid vesicles. If the shift in emission were due entirely to a viscosity change in the PRODAN environment, then the observed change in maxima from 478 to 462 nm would correspond to a 14 K temperature drop for emission in propylene glycol (Macgregor & Weber, 1981). Therefore, PRODAN emission from the adhesive junction of membranes containing gangliosides may reflect a relatively immobilized state of the lipids in the junction and/or a lower degree of hydration in the carbonyl region in this condensed state. An immobilized state is supported by measurements of fluorescence anisotropy of a paranyryl derivative of gangliosides (Brewer & Matinyan, 1992).

Effects of Junctional Polarity on Transjunctional Conductance. If the decrease in reported polarity reflects a true decrease in the polarity of the adhesion zone, then this could contribute to the 3-fold increase in transjunctional membrane conductance previously reported in the following manner. First, consider a single barrier model to ionic conductance (Figure 5A). Membrane conductance or ionic flux, F_o , can be described by the Boltzmann relation for potential energy E_o experienced by an ion crossing the membrane

$$F_o \propto \exp(-E_o/RT) \quad (3)$$

where RT is the gas constant and temperature ($= 0.6$ kcal/mol at 22 °C). The total potential energy profile can be described by four components (Flewelling & Hubbell, 1986): the electrical Born energy for the bulk dielectric, the image energy for the dielectric interface due to the membrane thinness, the intrinsic dipole potential of the bilayer, and all the neutral energy terms (van der Waals and steric factors). An additional electrostatic component should also be included for membranes containing charged gangliosides. Membrane polarity affects the energy barrier for all of these components. Two of them are considered further. The Born-image energy is a significant component of the total energy barrier for ionic conductance (Macgregor & Weber, 1981; MacDonald, 1976) (Table II). Evaluation of the dielectric constants and membrane thickness for a bilayer of egg phosphatidylcholine (Parsegian, 1969) and a hydrated ion of radius 3 Å (MacDonald, 1976) yields a barrier of 24 kcal/mol. Note that a hydrated ion is considered

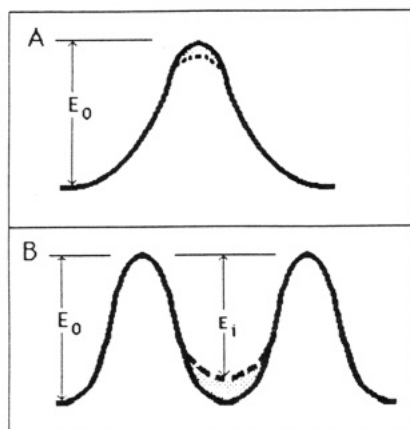


FIGURE 5: (A) Average energy barrier for ionic conductance across two bilayer membranes and the change required for increased conductance across the junction of two membranes containing gangliosides (dashed line). (B) Symmetrical two-barrier one site model (Hille, 1984) in which a change in conductance is achieved by raising the interior trough (dashed lined). A combination of panels A and B might be expected if the gangliosides in adhesion change the dielectric of the membrane interior or alter the dipole potentials in the headgroup region.

Table II: Basic Membrane Parameters of the Born-Image^a Energy for an Ion of Radius r Crossing a Bilayer (Parsegian, 1969) and Possible Changes to Achieve a 3-Fold Increase in Ion Conductance^b

	before contact	after adhesion	change
hydrocarbon dielectric, ϵ_m	2	2.05	+3%
aqueous dielectric, ϵ_{aq}	78	37	-53%
hydrocarbon thickness, d (Å)	36	30	-18%
ionic radius, r (Å)	3	3	assumed constant
conductance (nS/cm ²)	6	20	+320%

^a

$$E_{B-I} = \frac{q^2}{4\pi\epsilon_0} \left[\frac{1}{2r} \left(\frac{1}{\epsilon_m} - \frac{1}{\epsilon_{aq}} \right) - \frac{1}{\epsilon_m d} \ln \frac{2\epsilon_{aq}}{\epsilon_{aq} + \epsilon_m} \right]$$

^b q is the electronic charge and ϵ_0 is the permittivity of free space. Values after adhesion were obtained by computer iteration of one variable at a time to account for a change in energy of 0.65 kcal/mol.

in order to lower the energy to a biologically relevant range from an unreasonable value in excess of 70 kcal/mol for an unhydrated ion of radius 1 Å. Since the 3-fold higher conductance or ion flux after adhesion, F_a , can also be described by the Boltzmann relation

$$F_a = 3F_o \propto \exp(-E_a/RT) \quad (4)$$

division of eq 4 by eq 3 and solving for E_a yields

$$E_a = E_o - 1.1RT \quad (5)$$

Therefore, a lower energy barrier of 0.65 kcal/mol would account for the 3-fold increase in conductance. This small reduction could be achieved by alterations of polarity of either the membrane or the aqueous phase or membrane thickness as recorded in Table II. Of course, a combination of parameter changes would require less of a change in each individual parameter.

Considering each parameter, sensitivity of the energy barrier to a minuscule change in hydrocarbon polarity (dielectric) is dramatic. Gangliosides present in the junctional region could change the polarity (dielectric constant) by spreading the area per acyl chain due to their bulky headgroup. This may be theoretically equivalent to increasing the number of transient pores. Then PRODAN might reside deeper in the membrane and report a lower polarity than before, even if the polarity at the center rises. The 10-fold higher conductance of planar

bilayers made with chlorodecane ($\epsilon = 4$; Dilger et al., 1979) compared to those made with decane ($\epsilon = 2$), which we have used, substantiates this effect of polarity (membrane dielectric) on conductance. The decrease to an ϵ_{aq} of 37 required to alone account for the conductance increase seems unreasonable since the bulk aqueous phase dielectric can not be changed from 78 in a single barrier model, despite PRODAN reporting a carbonyl region dielectric of 17, down to 14 or 11. The possible thinning of the hydrocarbon down to 30 Å is supported by the measured increase in capacitance in the junction (Brewer & Thomas, 1984) but seems unlikely unless accompanied by interdigitation of acyl chains (McDaniel et al., 1983; McIntosh et al., 1983; Levin et al., 1985). Although McDaniel and McIntosh (1986) have not detected bilayer thinning and interdigitation of acyl chains in membranes containing 30 mol % GM1, our conditions of under 12 mol % gangliosides are more favorable for adhesion and may permit interdigitation and thinning.

The above analysis considered a single rate-limiting averaged barrier to conductance for the apposition of two membranes whose height decreases with adhesion (Figure 5A). To strictly consider conductance across two bilayers, a symmetrical two-barrier one-site model described by Hille (1984) should be discussed (Figure 5B). In this case, two separate energy barriers, each with a Born-image energy would be required: E_o for ion transfer from solution to between the membranes and E_i for ion transfer from between the membranes to solution. Then ionic conductance is dependent on ΔS , the concentration difference of the current-carrying ion between the outside and between the two membranes.

$$F_o \propto \frac{\exp(-E_o/RT) \exp(-E_i/RT)}{2 \exp(-E_i/RT) + \Delta S \exp(-E_o/RT)} \quad (7)$$

Note that if ΔS is small compared to the rate constant related to E_i , then again the flux is dependent on a single energy barrier E_o as in eq 3. If ΔS is not small, e.g., if the gangliosides in the junction concentrate protons, then the second barrier becomes important in controlling the rate of ion flux across the two membranes. Since the height of the second barrier depends on the depth of the middle trough, the observed reduction in polarity could raise the level of this trough in a manner analogous to decreasing the polarity of the aqueous phase. Reduced junctional polarity is also consistent with increased proton binding to sialic acids on the gangliosides. Although the permeant ion is unknown, pH studies suggest H^+/OH^- may be carrying the charge (Brewer & Thomas, 1984). Further development of this model will also require knowledge of the distance between membranes in order to consider a diffusive component to ionic flux (Cooper et al., 1988). Considerations of the effect of junctional changes in surface charge or dipole potential (Simon & McIntosh, 1989), which are considerable for gangliosides (Beitinger et al., 1989), need to be determined.

In conclusion, the polarity-sensitive PRODAN molecule fluoresces at lower wavelengths in the junction of two membranes containing gangliosides. This shift in emission suggests a less polar environment in the junction, which could explain the observed increase in electrical conductance. Thus, the apposition of two model membranes containing gangliosides has created a new surface milieu that could represent a mechanism of altered biological membrane function and contact sensation.

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Registry No. PRODAN, 70504-01-7; *n*-decane, 124-18-5; tri-caproin, 621-70-5; 1-pentanol, 71-41-0; *n*-propyl acetate, 109-60-4; 1-butanol, 71-36-3; 1-dodecanol, 112-53-8; glycerol triacetate, 102-76-1; glycerol, 56-81-5; glycerol phosphate, 12040-65-2; potassium phosphate, 7778-53-2; sodium chloride, 7647-14-5; phosphorylcholine, 107-73-3; choline chloride, 67-48-1.

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