Biochimica et Biophysica Acta, 776 (1984) 279–287
Elsevier

BBA 72211

ROLE OF GANGLIOSIDES IN ADHESION AND CONDUCTANCE CHANGES IN LARGE SPHERICAL MODEL MEMBRANES

GREGORY J. BREWER * and P.D. THOMAS

Department of Medical Microbiology and Immunology, Southern Illinois University School of Medicine, P.O. Box 3926, Springfield, IL 62708 (U.S.A.)

(Received February 14th, 1984)

Key words: Ganglioside; Membrane adhesion; Membrane conductance; Glycolipid; Model membrane

The formation of two spherical model membranes at the tips of two syringes has allowed us to study the role of gangliosides in membrane adhesion and look for changes in conductance between two such membranes during the process of adhesion. Membranes were formed in aqueous 100 mM NaCl, 10 mM KCl, 1 mM $CaCl_2$ from 1% (w/v) egg phosphatidylcholine in *n*-decane, with or without mixed bovine brain gangliosides. After thinning to the 'black' bilayer state, two membranes were moved into contact. With gangliosides, the contact area and conductance increased colinearly with time over a 5 to 20 min period of adhesion. The role of electrostatic bridging by calcium was investigated. In the absence of calcium or in the presence of 2 mM EDTA, adhesion proceeded after a longer lag time at about one-half the normal rate. As the ganglioside concentration was increased from 0 to 15 mol%, the electrical conductance of individual membranes decreased 3-fold from 48 ± 30 nS/cm^2 to 17 ± 13 nS/cm^2. The conductance was pH dependent with a minimum at neutral values. At neutral pH, when two membranes containing 4.1 mol% gangliosides adhered, the region of adhesion had a specific conductance three times that of the nonadhering regions of membranes. Without gangliosides, the specific conductance of the contact region was the same as that of non-adhering regions of the membrane. These data suggest that mixed gangliosides can mediate an adhesion-dependent increase in conductance.

Introduction

Cell-cell adhesion is responsible for the integrity of organs. The loss of cell-cell adhesion is an early step of metastasis of tumor cells. An understanding of control of adhesion requires the identification of the molecules on the cell surface that are mediating the interaction. Types of molecules which have been implicated for roles in adhesion involve carbohydrate: glycoproteins [1–6], glycosyltransferase/glycosidase-carbohydrate acceptors [7,8], and glycolipids [9,10]. Associated with the tumorigenic transformation of chick embryo fibroblasts [11] and other but not all cultured cells [12] is a decline in adhesiveness and in the quantity and complexity of the sialic acid-bearing glycolipids, gangliosides. The development of a simple model membrane system has allowed us to test the hypothesis that gangliosides can mediate membrane-membrane adhesion [13].

Large spherical model membranes are three-dimensional versions of the traditional Mueller-Rudin [14] planar black lipid membranes. Pagano and Thompson [15] were the first to report the formation of these lipid-bilayer membranes with diameters in the millimeter range. Later, Yguera-

Abbreviations: PC, phosphatidylcholine; NBD-PE, *N*-(7-nitrobenzo-2-oxa-1,3-diazol-4-yl)phosphatidylethanolamine; Mes, 2-(*N*-morpholino) ethanesulfonic acid.

bide and Stryer [16] described the elegant use of these membranes for structural measurements of oriented bilayers using fluorescent probes. They also suggested their advantages for simultaneous measurements of structural and functional (electrical) properties. Neher [17] made 'inflated bilayers' to study their apposition but did not ascertain whether bulk membrane forming solution was involved in the 'fusion'. Breisblatt and Ohki [18,19] used large spherical model membranes to study true fusion at elevated temperatures. Shagina et al. [20] used a single large spherical membrane to monitor transmembrane ion fluxes. We have adopted these concepts with a modification which allows electrical access to the interior of the spherule; the membrane is allowed to remain attached to the tip of the syringe. Thus, we have combined the electrical/functional advantages of planar bilayer membranes with the structural advantages of spherical membranes in order to study membrane adhesion.

Several characteristics are predicted that distinguish functional adhesion from nonfunctional contact. (1) After the initial contact of two membranes, the force of adhesion will increase the area of contact. Membranes which contact but do not adhere do not exert an adhesive force. Contacting membranes may be separated to the extent that the surface molecules do not interact. (2) A signal of contact or adhesion may be evident as a change in conductance between the two membranes. If there are structural changes caused by the interaction of surface molecules, the ionic conductance may be altered. (3) Molecules involved in adhesion in the contact region will be restricted in mobility. Membranes made without gangliosides can serve as a control for specificity. In this report, we describe experiments with large spherical model membranes containing gangliosides which demonstrate the first two characteristics above.

Materials and methods

Materials. Egg phosphatidylcholine (egg PC) was purified by extraction and column chromatography from chicken egg yolk [21]. Purity was determined by thin-layer chromatography with development in chloroform/methanol/water (65 : 25 : 4, v/v) and detection with I_2 vapor. Mixed gangliosides were isolated by extraction from fresh-frozen bovine brains from a local slaughter house [22]. Water used for the extraction of gangliosides contained 2 mM EDTA except the last dialysis which was against water without EDTA. *N*-(7-nitrobenzo-2-oxa-1,3-diazol-4-yl)-phosphatidylethanolamine (NBD-PE) was from Avanti Polar Lipids (No. FPE-22, Birmingham, AL). Reagent grade acetone, diethyl ether and *n*-decane (Matheson, Coleman, and Bell, Norwood, OH) were distilled and dried by passage over aluminum oxide (J.T. Baker, Phillipsburg, NJ) supported by glass wool in a pasteur pipet [23]. All salts were high purity from Alpha Inorganics (Division of Ventron, Danvers, MA) and were roasted before weighing. Ultrapure Tris base was from Schwarz-Mann (Orangeburg, NY). All water used was deionized to a conductivity less than 18 megohms and was carbon-filtered.

Spherical lipid-bilayer membranes. Spherical bilayer membranes [15] were made in an aqueous environment by gently expanding a film of membrane forming solution from the tips of two syringes. Membrane forming solution was prepared in a glass vial (Pierce No. 13200) by mixing $CHCl_3$ solutions containing 250 μg egg PC with the indicated amounts of mixed gangliosides, and 10 μg NBD-PE as a fluorescent probe. The solvent was removed in a rotary evaporator under vacuum with purges of N_2. Further drying was achieved by solution and drying from acetone and twice from ether [23]. Final solution was achieved in 25 μl *n*-decane at 40 °C. While the membrane forming solution was being prepared, two threaded plunger syringes (Hamilton No. TP1750LT) and a 1 × 2 × 4 cm glass cuvette (American Scientific Products No. S7032) were filled with electrolyte containing 100 mM NaCl, 10 mM KCl, 1 mM $CaCl_2$, 1 mM Tris-HCl (pH 7.4) which had been freshly filtered through a 0.45 μm pore membrane (Gelman No. GN-6).

An air gap was created at the tip of each syringe and the glass was coated only on the cross sectioned surface of the tip with membrane forming solution without probe. The solvent was carefully evaporated under a stream of N_2. This step was required for reliable membrane formation, probably providing a more wettable surface than clean glass. While the tips were drying (approx. 10

min), 2 μl membrane forming solution without fluorescent probe was deposited on the surface of the 6 ml of electrolyte in the cuvette and stirred to equilibrate [24]. All but a hemisphere of air was expelled from the tip of the syringe, whereupon it was dipped in the membrane forming solution to pick up about 0.5 μl without contaminating the membrane solution in the vial with electrolyte. The plunger was slightly retracted while transferring the syringe to the cuvette. To form the membrane, the threaded plunger was slowly advanced until a spherical thick film was formed about twice the diameter of the tip of the syringe. The membrane spontaneously thinned to the 'black' bilayer state over a period of 1–2 h. The electrolyte was buffered at pH 7.4 with 1 mM Tris-HCl unless indicated as follows: for pH 5.0 and 5.5, sodium acetate; pH 5.85, unbuffered; pH 5.5, 6.0, and 6.8, Tris-maleate; pH 6.5, Mes; pH 8.0 and 8.4, Tris-HCl (all 1 mM). All experiments were conducted at room temperature, 20–23°C.

Apparatus. The apparatus for simultaneously monitoring structural and electrical properties of the membranes is depicted in Fig. 1. The syringes were mounted on *x-y-z* micromanipulators (Narishige) and the entire apparatus on a massive optical bench mounted on springs to minimize vibration. Electrical connection to the interior of a membrane attached to the tip of a syringe was obtained through a machined hollow Teflon adapter with a side port into which was cemented (5 min Epoxy) a chloridized silver electrode. The tip of the syringe was a glass capillary (Drummond 50 μl Microcap). A chloridized silver reference electrode was immersed in the cuvet. The junctional polarizations of the electrodes were less the 2 mV. A ramp voltage of ±50 mV, 0.02 Hz was applied either across one membrane at a time (*trans* mode) or between the two membranes (*cis* mode) and the resulting current was monitored [25]. The feedback resistor in the current amplifier was variable up to 10^9 Ω in order to measure small currents. Thus, a display of applied voltage on one axis against amplified current (voltage/feedback resistance) produced a simultaneous measure of conductance from the slope of the *I-V* curve and capacitance from the current offset at $V = 0$, $C = \Delta I/(dV/dt)$. dV/dt was typically 4 mV/s.

Observations of the membranes were possible at the front face of the cuvet through a horizontally oriented Zeiss microscope fitted with long working distance objectives 1.25, 3.2 and 20× with 8× oculars. The 'epi' body tube added an additional 1.6× magnification. The membranes were illuminated from the side (90° to the microscope) with either white light from a fiber optics source (No. 170D, Dolan-Jenner) or blue light (442 nm) from a 10 mW He-Cd laser (No. 4110, Liconix). The image was passed through the microscope phototube to a light amplifying device (No. 211, Javelin Electronics), a black and white TV camera (No. WV1450, Panasonic) and a VHS recorder (No. NV8350, Panasonic) and monitor (not shown). Membrane sizes were measured directly from the TV monitor and calibrated from the diameter of the syringe tip measured with a micrometer. Membrane areas were calculated by $A = \pi dh$, where d is the diameter (width) and h is the height from the bottom of the membrane to its junction with the bulk membrane forming solution.

Data Analysis. An Apple II + microcomputer with a Ada-Lab analog to digital interface and curve fitting software (Interactive-Microware, State College, PA) were used to analyze data. From the equivalent circuit in Fig. 1B, conductance between the two membranes before contact ($G_{cis\ \text{before}}$) can be compared to the theoretical conductance of direct current,

$$G_{cis} = (R + L)^{-1}$$

After contact, $G_{cis\ \text{after}}$ is

$$G_{cis} = (2B)^{-1} + (R + L)^{-1}$$

Similarly,

$$G_{\text{L}} = L^{-1} + (2B + R)^{-1}$$

$$G_{\text{R}} = R^{-1} + (2B + L)^{-1}$$

These three equations in three unknowns were reduced to two quadratics and solved numerically using a program written in BASIC. The capacitance current at $V = 0$ was calculated similarly from the following equations.

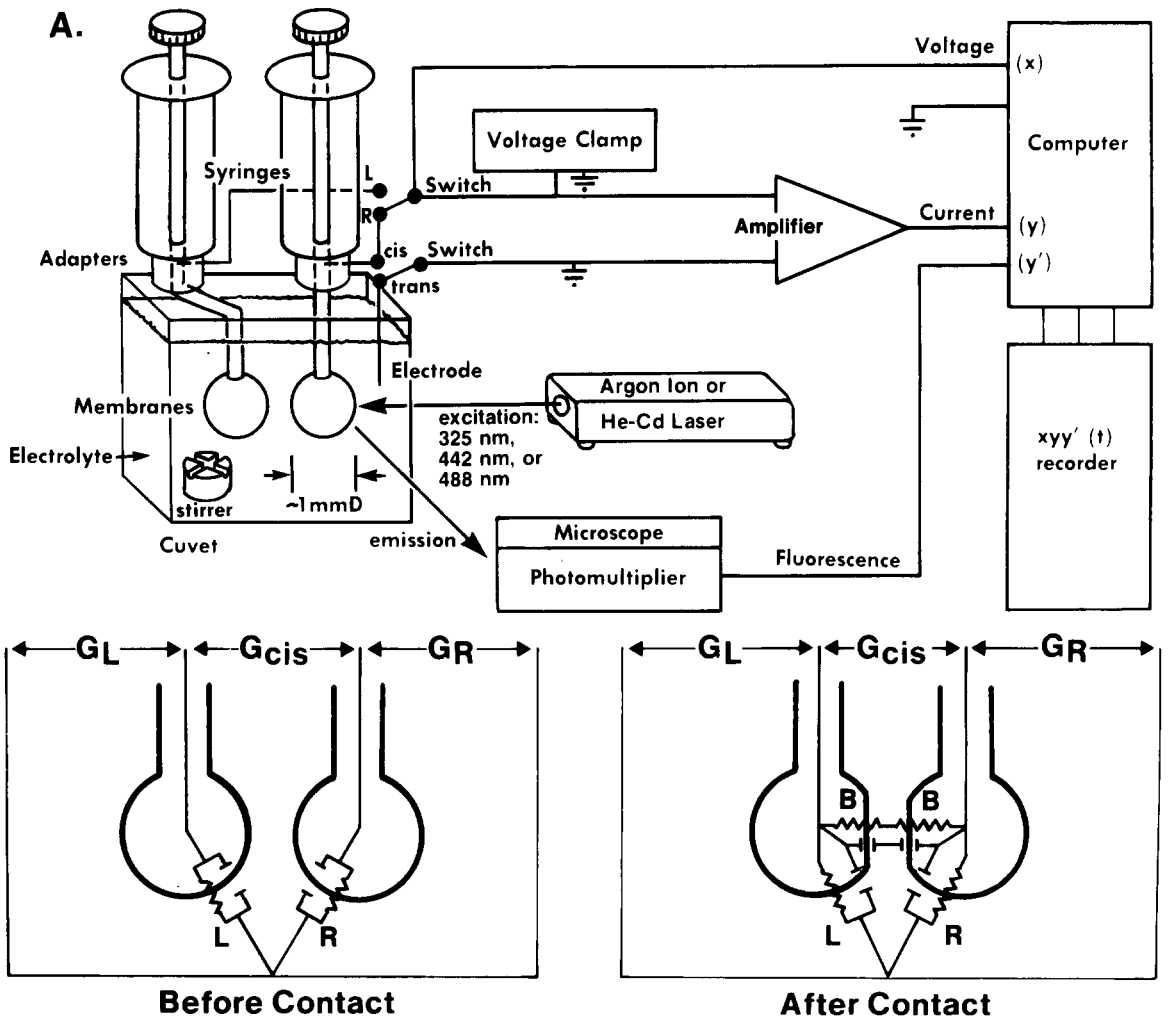

Fig. 1. (A) Apparatus for monitoring large spherical model membranes. Membranes are formed at the tips of two syringes. Functional electrical measurements are possible simultaneously with structural observations of adhesion. See Methods for details. (B) Equivalent circuit before and after adhesion. Note the possibility of a specific conductance (resistance) or capacitance in the contact region different from that of the rest of the membrane.

Before contact, $C_{cis} = (R^{-1} + L^{-1})^{-1}$

After contact, $C_{cis} = (L^{-1} + R^{-1})^{-1} + B/2$

$C_L = (R^{-1} + 2/B)^{-1} + L$

$C_R = (L^{-1} + 2/B)^{-1} + R$

Results

Spherical model membranes were formed at the tips of two syringes from 1% egg phosphatidylcholine in *n*-decane. When two of these membranes were mechanically brought into contact, the area of the contact region increased with time, over a period of 30 min thereby demonstrating the adhesion of the membranes [13]. If the syringes were slightly separated by mechanical displacement, adhesion was also indicated by the distortion of the membranes on their supports while the adhesion zone remained intact. Usually, an equilibrium was reached between the adhesion force and the elastic deformability before the membranes had to be moved apart in order to prevent the spread of the

zone of adhesion into the torus of bulk membrane forming solution at the tips of the syringes. The adhesion was found to be reversible over a 30 min period after moving the syringes apart. The adhesion zone was composed of two lipid bilayers as shown by lack of mixing of two fluorescent probes across the junction.

Measurement of the specific conductance between the two membranes could also provide evidence of true adhesion. Two semi-fused membranes with a single bilayer in the junction should have a higher conductance than two bilayers. We found the specific conductance of individual egg PC membranes to be 48 ± 30 nS/cm^2. In measuring the conductance between two membranes before adhesion, we found a specific conductance close to that calculated for the two measured conductors in series. If this value was called 100%, the change in conductance between the two membranes after adhesion was found to be $+2 \pm 5\%$. Thus, conductance measurements also suggest the apposition of two bilayers during adhesion.

Measurement of the membrane electrical capacitance could also provide information about the junction. Capacitance is related to the thickness, d, of the membrane dielectric by $C = \epsilon\epsilon_0/d$, where ϵ is the dielectric constant of the membrane interior and ϵ_0 is the permittivity of the free space. Total capacitance was measured from the offset in membrane current at V_0 about one hour after thinning and one hour after adhesion. By dividing the measured total capacitance of individual membranes by their areas, a specific capacitance of 0.34 ± 0.04 μF/cm^2 was determined. After adhesion, the capacitance of the non-junctional portions of the two membranes was computed to be 0.46 ± 0.06 μF/cm^2. This increase can be attributed to further thinning with time as more decane is excluded from the bilayer [26]. From the proportion of membrane area involved in adhesion at this time, we calculated the specific capacitance of each membrane in the contact region to be 1.3 ± 0.4 μF/cm^2, or 2.8 ± 0.6 times that of the non-junctional portions of the membranes. Thus, the junctional increase in specific capacitance could be due to either a rise in the dielectric constant from 2.2 [27] to 6.2 or a drop in thickness of each membrane from about 50 Å [27] to 18 Å. Since the latter is not consistent with a lipid bilayer, the capacitance measurements suggest a change in the dielectric constant of the junctional region.

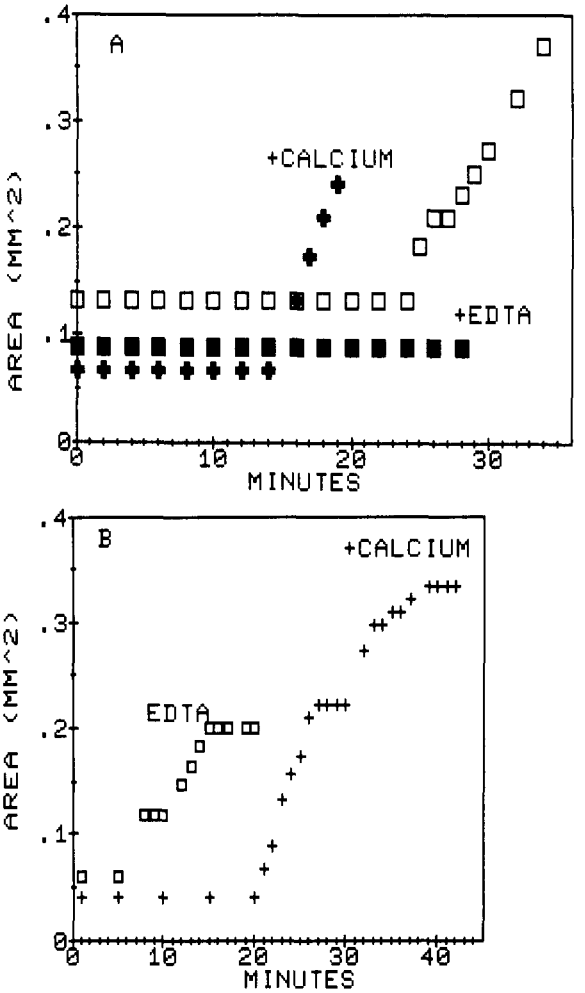

Fig. 2. Effect of chelation of Ca^{2+} on the adhesion of membranes containing 4.1 mol% gangliosides. (A) Membranes were formed in electrolyte containing 1 mM $CaCl_2$. They were moved into contact and monitored in the contact region (+). After 14 min, adhesion proceeded at a rate of 0.037 mm^2/min. These membranes were manually separated. EDTA was added to 2 mM and the membranes were again contacted (■). After 28 min, no adhesion was observed. An additional manual force was applied (□). After 24 min, adhesion proceeded at a rate of 0.022 mm^2/min. (B) Membranes were formed in electrolyte containing 1 mM EDTA. The area of the contact region was computed as a function of time (□). The rate of adhesion was 0.014 mm^2/min. The two membranes were manually separated. $CaCl_2$ was added to 2 mM. The membranes were again brought into contact and monitored (+). The initial rate of adhesion was 0.027 mm^2/min.

Membranes containing gangliosides

In order to more closely model the cell surface, membranes were made containing egg PC and mixed gangliosides extracted from brain. These membranes were also found to adhere. We found the rate of increase in diameter of the adhesium zone (rate of adhesion) to remain constant as the ganglioside concentration in the membrane forming solution ranged from 2 to 10 mol% [13]. Gangliosides in the range of 11 to 15 mol% blocked adhesion. These experiments were conducted in 1 mM Ca^{2+}. We have examined the role of electrostatic bridging in the adhesion of membranes containing gangliosides by chelation of Ca^{2+} with EDTA. Fig. 2A shows that a pair of membranes in the presence of Ca^{2+} adhered at an initial rate of 0.04 mm^2/min. After separation and the addition of EDTA, recontact resulted in adhesion at a 60% slower rate. The converse experiment in Fig. 2B showed that in comparison to membranes made initially in EDTA, the addition of excess Ca^{2+} nearly doubled the rate of adhesion (0.014 to 0.027 mm^2/min). These results suggest that Ca^{2+} facilitated but was not required for adhesion.

The effect of gangliosides on membrane conductance was examined. In individual membranes, 1 to 15 mol% gangliosides reduced the specific conductance 3-fold from 48 ± 30 nS/cm^2 to 17 ± 13 nS/cm^2 (Fig. 3). We were unable to form stable membranes at 16 or 18 mol% gangliosides. When two membranes containing 4.1 mol% gangliosides were allowed to adhere, the conductance between the membranes colinearly increased with the increase in adhesion (Fig. 4). These parameters could be extrapolated back to starting values at 4.7 and 5.0 min, respectively. After adhesion was well established, a manual separation resulted in simultaneous arrest of the increases in area and conductance. It was possible to determine whether the observed increase in conductance was due to a localized increase in the zone of adhesion by measuring the conductance of each adhering membrane individually and the conductance between the two membranes (see Methods and region B of Fig. 1B). In a typical experiment of two membranes containing 4.1 mol% gangliosides, analysis before adhesion indicated an average specific conductance of 7.2 nS/cm^2 (Table IA). The measured conductance between these two membranes was

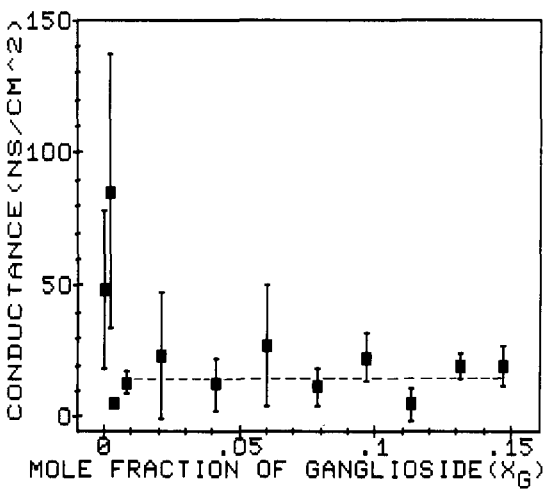

Fig. 3. Effect of ganglioside concentration on the membrane electrical conductance. The conductance of individual membranes was determined and divided by the membrane area to yield specific conductance. The ordinate is the specific conductance (nS/cm^2). The dashed line is the mean for $0.008 < X_G < 0.15$, 17 ± 13 nS/cm^2.

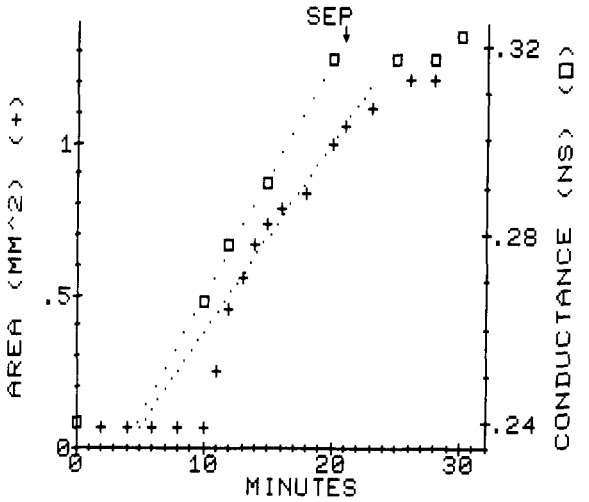

Fig. 4.Temporal relationship between adhesion and conductance of two membranes containing 4.1% gangliosides. The diameter of the contact area was monitored along with the current (I) flowing between the two membranes in response to a ramp voltage (V) clamp, ±50 mV. From the diameter, the area of contact was computed (±). Conductance (nS) was calculated from $(dI/dt)/(dV/dt)$, (□). The slopes of the rates of increase in area and conductance (· · · · · ·) were calculated to intercept the baseline at 5.0 min and 4.7 min, respectively. At the arrow, the membranes were manually separated but remained adhered to each other.

TABLE I

CONDUCTANCE MEASUREMENTS FOR TWO MEMBRANES CONTAINING 4.1 mol% GANGLIOSIDES

	Measured resistance (GΩ)	Measured conductance (nS)	Calculated resistance (GΩ)	Calculated conductance (nS)	Area (cm^2)	Specific conductance (nS/cm^2)
(A) Before adhesion						
Left membrane	1.54	0.65			0.105	6.2
Right membrane	1.11	0.91			0.111	8.2
(theoretical *cis*)	(2.65)	(0.38)				
cis (between)	2.58	0.39				
(B) After adhesion						
Left membrane (L)	1.35	0.74	1.59	0.63	0.094	6.7
Right membrane (R)	0.97	1.03	1.08	0.93	0.100	9.3
(theoretical *cis*)	(2.32)	(0.43)				
cis (between)	2.00	0.50				
Adhesion zone (B)			4.0	0.25	0.0106	23.7

102% of the theoretical series conductances of the two membranes. However, after adhesion (Table IB) the measured conductance between the two membranes increased 28%, and was 16% more than the theoretical series conductances of the two membranes after adhesion. These changes could be analyzed by solving for the component of the change due to the adhesion region. From this analysis, the zone of adhesion was found to have a specific conductance of 23.7 nS/cm^2, three times that of non-adhering membranes.

These experiments were conducted at pH 7.4. For membranes containing 4.1 mol% gangliosides, examination of the rate of adhesion showed an increase in rate from pH 5 to pH 8 but no adhesion at pH 8.4 (data not shown). The conductance of individual membranes declined about 10-fold as the pH was raised from 5.0 to 7.4 (Table II). However, the change in conductance associated with adhesion showed a maximum around neutral pH (Table II). For these membranes made in buffers between pH 6.0 and 7.4, the change in conductance (ΔG) after adhesion was significantly increased above that of membranes made without gangliosides. For membranes made in this pH range, the computed solution for the conductance of the non-adhering region was 6 ± 2 nS/cm^2; that for the adhering region was 20 ± 10 nS/cm^2. The ratio of these conductances indicated a 3.2 ± 1.4 fold increase in the specific conductance of the junctional area.

TABLE II

EFFECT OF pH ON THE CHANGE IN CONDUCTANCE BETWEEN TWO ADHERING MEMBRANES CONTAINING 4.1 MOL% GANGLIOSIDES

Two membranes were formed in electrolyte at the indicated pH. Before adhesion, the conductance of each membrane was determined during a ±50 mV ramp voltage clamp (G, G_L, G_R). The conductance was also measured between the two membranes (G_{cis}). G_{cis} was compared to the theoretical series conductance $G_{theoret}$ $(G_L^{-1} + G_R^{-1})^{-1}$. The membranes were moved into contact. 60 min after adhesion, these values were again recorded. The conductance change (ΔG) was determined:

$$\Delta G = \frac{G_{cis\ after} \times 100}{G_{theoret\ after}} - \frac{G_{cis\ before} \times 100}{G_{theoret\ before}}$$

P, probability by Student's t-test that the measured mean is not significantly different from the conductance change without gangliosides, 4 ± 6%. n.s., not significant

pH	ΔG (%)	P	G (nS/cm^2)
5.0	−8 ± 4	0.05	117 ± 23
5.5	−1 ± 6	n.s.	46 ± 38
5.85	9 ± 2n7	n.s.	10 ± 5
6.0	12 ± 8	< 0.05	4 ± 1
6.5	16 ± 5	< 0.02	3 ± 1
6.8	11 ± 7	0.05	19 ± 11
7.4	19 ± 10	< 0.05	12 ± 10
8.0	3 ± 8	n.s.	8 ± 2
8.4	no adhesion		

Discussion

In order to test the widely speculated role of gangliosides in mediating cell-cell adhesion, we

designed a model membrane system using defined components. Initially, we chose to include Ca^{2+} in the system because of its frequent requirement in cellular adhesion [28,29]. Indeed, we too find that Ca^{2+} facilitates adhesion in our model system containing gangliosides, but Ca^{2+} is not necessary to observe adhesion. One could argue that Ca^{2+} serves as an electrostatic bridge between the two membranes [30,31]. However, the observation of adhesion in the absence of Ca^{2+}, even though at a diminished rate, suggests that adhesion is not primarily due to divalent electrostatic bridging. In fact, the inhibition of adhesion at mole fractions of gangliosides exceeding 0.10 suggests that increased surface charge density, even in the presence of excess Ca^{2+}, inhibits membrane adhesion, and may even block adhesion electrostatically. Ca^{2+} may facilitate adhesion by causing the gangliosides to aggregate within each individual membrane [32,33].

The remarkable finding of this study was the increase in transmembrane electrical conductance associated with adhesion of membranes containing gangliosides. Their temporal coincidence is consistent with adhesion causing the increase in conductance. The pH profile for individual membranes containing gangliosides shows higher conductance at lower pH, suggesting that protons are carrying the current. At neutral pH, the observation that the presence of gangliosides in egg PC membranes reduced membrane conductance 3 fold is supported by similar findings of Gamble et al. [34] using glucosylceramide. Using similar techniques, adhesion of large spherical bilayer membranes was also studied by Badzhinyan et al. [35]. Their membranes made from bovine brain lipids also produced conductances consistent with the adhesion of two bilayer membranes. Their measurements of membrane capacitance before adhesion of 0.36 $\mu F/cm^2$ [36] are similar to our finding, but after adhesion, they found 0.38 $\mu F/cm^2$ for each membrane in the contact region. Our result of 1.3 $\mu F/cm^2$ could be due to our use of purified egg phosphatidylcholine or our spontaneous induction of adhesion in contrast to their forced adhesion. This high value suggests that the dielectric in the contact region was different from that in the rest of the membrane. Since water has a dielectric constant of 80.2, elevation of the water content of the hydrocarbon region to 5% could increase the bulk dielectric constant to 6.2 while maintaining the thickness of the membrane. Others have noted the sensitivity of the dielectric constant and hence capacitance to polar membrane constituents [37,38].

Although Badzhinyan et al. [39] did not study gangliosides, the inclusion of another charged amphiphile, oleic acid, produced a 2-fold increase in their measurements of specific conductance of the contact region. Because the conductance levels observed in these model systems are several orders of magnitude smaller than observed in vivo, they may not be physiologically significant in themselves. However, our measured 3-fold conductance change must result from a structural change which could have important physiological correlates. For example, without appreciably changing the conductance of the major portion of the membrane, small regions of high conductance may be created by depletion of gangliosides from a region. In considering the apposition of two membranes containing gangliosides, if the contact region were depleted of gangliosides due to adhesion involving egg PC molecules, then a localized, 3-fold, adhesion-specific increase in conductance could be created. Another possible explanation would be a localized decrease of about 2 pH units in the contact region [40]. Discrimination between these mechanisms may be possible with appropriate fluorescent probes. With either mechanism, these findings demonstrate the phenomenon of membrane contact sensation.

The model system described here was designed to simultaneously monitor the structural characteristics of adhesion together with the functional characteristics of membrane conductance at physiological concentrations of calcium [41] and gangliosides [42–45]. In the future, this model system will be utilized to demonstrate specific relationships of adhesion promoting proteins and membrane surface oligosaccharides.

Acknowledgements

We thank Elizabeth Basler and Jeffrey Fassero for early work on this project, Kathy Fritz for assistance in typing and Dr. Richard Pagano for reference to the work of Badzhinyan et al. This

work was supported in part by NIH grant R01 CA34145, NSF grant PCM 77-02927, and the Pearson Family Foundation.

References

1 Frazier, W.A., Rosen, S.D., Reitherman, R.W. and Barondes, S.H. (1975) J. Biol. Chem. 250, 7714–7721
2 Pouyssegur, J.M. and Pastan, I. (1976) Proc. Natl. Acad. Sci. USA 73, 544–548
3 Pouyssegur, J.M. and Pastan, I. (1977) J. Biol. Chem. 252, 1639–1646
4 Hausman, R.E. and Moscona, A.A. (1976) Proc. Natl. Acad. Sci. USA 73, 3594-3598
5 Rutishauser, U., Gall, W.E. and Edelman, G.M. (1978) J. Cell Biol. 79, 382–393
6 Geltosky, J.E., Weseman, J., Bakke, A. and Lerner, R.A. (1979) Cell 18, 391–398
7 Roseman, S. (1970) Chem. Phys. Lipids 5, 270–297
8 Shur, B.D. and Roth, S. (1976) Biochim. Biophys. Acta 415, 473–512
9 Huang, R.T. (1978) Nature 276, 624–628
10 Kleinman, H.K., Hewitt, A.T., Murray, J.C., Liotta, L.A., Rennard, S.I., Pennypacker, J.P., McGoodwin, E.B., Martin, G.R. and Fishman, P.H. (1979) J. Supramol. Struct. 11, 69–78
11 Hakomori, S., Wyke, J.A. and Vogt, P.K. (1977) Virology 76, 485–493
12 Fishman, P.H. and Brady, R.O. (1976) Science 194, 906–915
13 Brewer, G.J. and Thomas, P.D. (1984) Science, submitted
14 Mueller, P., Rudin, D.O., Ti Tien, H. and Wescott, W.C. (1962) Nature 194, 979–980
15 Pagano, R. and Thompson, T.E. (1967) Biochim. Biophys. Acta 144, 666–669
16 Yguerabide, J. and Stryer, L. (1971) Proc. Natl. Acad. Sci. USA 68, 1217–1221
17 Neher, E. (1974) Biochim. Biophys. Acta 373, 327–336
18 Breisblatt, W. and Ohki, S. (1975) J. Membrane Biol. 23, 385–401
19 Breisblatt, W. and Ohki, S. (1975) J. Membrane Biol. 29, 127–146
20 Shagina, L.V. Grinfeldt, A.E. and Lev, A.A. (1978) Nature 273, 243–244
21 Papahadjopoulos, D. and Miller, N. (1967) Biochim. Biophys. Acta 135, 624–638
22 Svennerholm, L. and Fredman, P. (1980) Biochim Biophys. Acta 617, 97–109
23 Fettiplace, R., Gordon, L.G.M., Hladky, S.B., Requena, J., Zingsheim, H.P. and Haydon, D.A. (1975) in Methods in Membrane Biology (Korn, E.D., ed.), Vol. 4, pp. 1–75, Plenum Press, New York
24 White, S.H. (1978) Biophys. J. 23, 337–347
25 Huebner, J.S. and Bruner, L.J. (1972) J. Physics E: Scientific Instruments 5, 311–312
26 White, S.H. and Thompson, T.E. (1973) Biochim. Biophys. Acta 323, 7-22
27 Ohki, S. (1970) J. Theor. Biol. 26, 277–287
28 Grinnell, F. (1978) Int. Rev. Cytol. 53, 65–144
29 Hayfil, F., Babinet, C. and Jacob, F. (1981) Cell 26, 447–454
30 Behr, J.P. and Lehn, J. (1973) FEBS Lett. 31, 297–300
31 Cook, W.J. and Bugg, C.E. (1975) Biochim. Biophys. Acta 389, 428–435
32 Sharom, F.J. and Grant, C.W.M. (1978) Biochim. Biophys. Acta 507, 280–293
33 Bertoli, E., Masserini, M., Sonnino, S., Ghidoni, R., Cestaro, B. and Tettamanti, G. (1981) Biochim. Biophys. Acta 468, 196–202
34 Gamble, F., Robello, M., Usai, C. and Marchetti, C. (1982) Biochim. Biophys. Acta 693, 165–172
35 Badzhinyan, S.A., Dunin-Barkovskii, V.L., Kovalev, S.A. and Chailakhyan, L.M. (1971) Biofizika 16, 1019–1024
36 Badzhinyan, S.A. and Chailakhyan, L.M. (1971) Biofizika 16, 1141–1143
37 Hanai, T., Haydon, D.A. and Taylor, J. (1965) J. Theor. Biol. 9, 422–432
38 Dilger, J.P., McLaughlin, S.G.A., McIntosh, T.J. and Simon, S.A. (1979) Science 206, 1196–1198
39 Badzhinyan, S.A., Kovalev, S.A. and Chailakhyan, L.M. (1972) Biofizika 17, 705–708
40 Haines, T.H. (1983) Proc. Natl. Acad. Sci. USA 80, 160–164
41 Scully, R.E. (1980) N. Engl. J. Med. 302, 37–48
42 Svennerholm, L. (1970) in Comprehensive Biochemistry (Florkin, M. and Stotz, E.H., eds.), Vol. 18, p. 206, Elsevier, Amsterdam
43 Breckenridge, W.C., Gombos, G. and Morgan, I.G. (1972) Biochim. Biophys. Acta 266, 695–707
44 Devries, G.H. and Zmachinski, C.J. (1980) J. Neurochem. 34, 424–430
45 Felgner, P.L., Thompson, T.E., Barenholz, Y. and Lichtenberg, D. (1983) Biochemistry 22, 1670–1674