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Single-Channel Analysis of the Conductance Fluctuations Induced in Lipid Bilayer Membranes by Complement Proteins C5b-9

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Summary. Single-channel analysis of electrical fluctuations induced in planar bilayer membranes by the purified human complement proteins C5b6, C7, C8, and C9 have been analyzed. Reconstitution experiments with lipid bilayer membranes showed that the C5b-9 proteins formed pores only if all proteins were present at one side of the membrane. The complement pores had an average single-channel conductance of 3.1 nS at 0.15 M KCl. The histogram of the complement pores suggested a substantial variation of the size of the single channel. The linear relationship between single-channel conductance at fixed ionic strength and the aqueous mobility of the ions in the bulk aqueous phase indicated that the ions move inside the complement pore in a manner similar to the way they move in the aqueous phase. The minimum diameter of the pores as judged from the conductance data is approximately 3 nm. The complement channels showed no apparent voltage control or regulation up to transmembrane potentials of 100 mV. At neutral pH the pore is three to four times more permeable for alkali ions than for chloride, which may be explained by the existence of fixed negatively charged groups in or near the pore. The significance of these observations to current molecular models of the membrane lesion formed by these cytolytic serum proteins is considered.

Key Words complement · lipid bilayer · membrane · membrane pore · membrane conductance

Introduction

The cytolytic activity of human serum resides in the capacity of complement proteins C5b-9 to increase the permeability of lipid bilayers to aqueous solute, thereby undermining the solute barrier function of the plasma membrane (reviewed by Esser, 1982; Bhakdi & Tranum-Jensen, 1983; Muller-Eberhard, 1984). Membrane insertion of hydrophobic peptides exposed during assembly of the C5b-9 complex can result in increased ion conductance across both artificial (Wobschall & McKeon, 1975; Michaels, Abramovitz, Hammer & Mayer, 1978) and biological membranes, (Green & Goldberg, 1960; Mayer, 1972; Lauf, 1975, 1978), which in the case of the erythrocyte results in the collapse of electrochemical gradients and consequent colloid-osmotic membrane rupture. In other metabolically-active cells these conductance changes in the plasma membrane initiated by the C5b-9 proteins can be actively reversed, resulting in a restoration of the cell's homeostatic electrochemical steady state (Jackson, Stephens & LeCar, 1981; Campbell & Morgan, 1985; Wiedmer & Sims, 1985).

The molecular mechanisms by which these five serum proteins alter membrane permeability and induce cell lysis remains unresolved. Based primarily upon the ultrastructural appearance of the assembled C5b-9 complex as depicted by negative-stain electron microscopy, it has been proposed that hollow tubules of polymerized C9—which have been shown to form spontaneously upon interaction of this protein with membrane-bound C5b-8—serve to span the lipid bilayer, providing an aqueous channel for ion diffusion (Tschopp, Müller-Eberhard & Podack, 1982). Nevertheless, the known capacity of these proteins to disrupt bilayer structure, and recent evidence suggesting that C5b-9 pore-forming and cytolytic activities can be expressed without tubular polymerization of C9, suggest that increased permeability after C9 binding may also relate to changes in membrane structure *per se* (Esser, Kolb, Podack & Müller-Eberhard, 1979; Dankert & Esser, 1985). Finally, recent analysis of the functional properties of the C5b-9 channel as derived from the measured rate of the solute permeation or by measurement of membrane electrical conductance has provided evidence that the pores formed by these proteins are of heterogeneous function, estimates of pore radii ranging from <0.4 to >5 nm, depending upon the stoichiometry of C5b-9 binding as well as the membrane density of bound complexes (Boyle, Gee & Borsos, 1979; Sims & Lauf, 1980; Jackson et al., 1981; Sims, 1981; Ramm, Whitlow & Mayer, 1983, 1985; Bhakdi & Tranum-Jensen, 1984). This apparent structural and func-

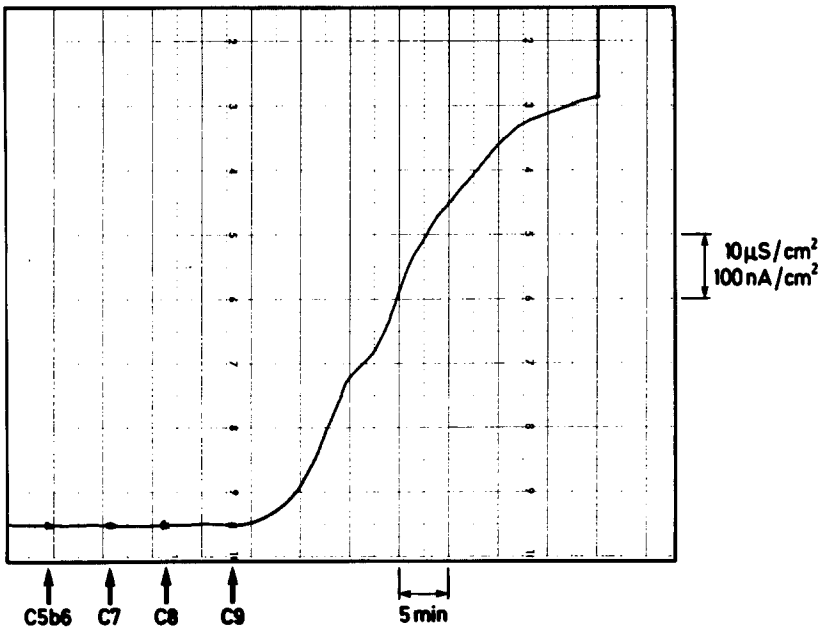

Fig. 1. Specific membrane conductance as a function of time after the addition of complement proteins C5b6, C7, C8 and C9 (concentration 1 μg/ml) to a black lipid bilayer membrane of diphytanoylphosphatidylcholine/phosphatidylserine (molar ratio 4 : 1). The arrows indicate the time of addition. The aqueous phase contained 0.15 M KCl, 5 mM HEPES, pH 7.5; T = 25°C. The applied membrane potential was 10 mV. The membrane broke at the upper end of the trace

tional heterogeneity underscores the need to apply methodologies suited to resolving unit conductance changes which occur during C5b-9 binding, in order to identify the ion flux mediated by each discrete pore site.

Accordingly, we now describe an analysis of the unitary (single-channel) conductance changes which occur in lipid bilayer membranes (BLM) exposed to the purified human C5b-9 proteins. This method represents a powerful tool for the characterization of single conductive events (Benz, 1985). We demonstrate that the C5b-9 complement proteins form defined channels in lipid bilayer membranes with an average single-channel conductance of 3.1 nS in 0.15 M KCl. A broad histogram of the single-channel conductance was observed, indicative of variability to the stoichiometry of the channel-forming unit. The complement channels displayed a small discrimination for different ions of a magnitude suggesting that the ions move inside the pore in a manner similar to the way they move in the aqueous phase. A small cation selectivity was also observed, suggesting an excess of negatively charged groups in or near the pore.

Materials and Methods

Complement Proteins

Human complement proteins C5b6, C7, C8 and C9 were isolated and assayed for functional activity as previously described (Sims, 1983, 1984; Sims & Wiedmer, 1984*a,b*). The purified proteins were stored at −20°C as 1 mg/ml stock solutions in 150 mM NaCl, 10 mM TES, 0.02% NaN_3, pH 7.5, containing 40% (wt/vol) glycerol. When prepared and stored under these conditions, the individual proteins were found to have no detectable effect on bilayer conductance at the concentrations employed in these experiments (*see below*).

Black Lipid Bilayer Experiments

Optically black lipid bilayer membranes were formed as described previously (Benz, Janko, Boos & Läuger, 1978). The instrumentation consisted of a Teflon chamber with two aqueous compartments connected by a small circular hole. The hole had a surface area of either 1 mm² (for the microscopic conductance measurements) or 0.05 mm² (in the case of the single-channel experiments). Membranes were formed across the hole by painting on a 1% solution of diphytanoylphosphatidylcholine and phosphatidylserine (molar ratio 4 : 1, Avanti Biochemicals, Birmingham, AL) dissolved in *n*-decane (Fluka, Buchs, Switzerland) or oxidized cholesterol/*n*-decane (Benz et al., 1978). The aqueous salt solutions (Merck, Darmstadt, FRG) were buffered with 5 mM HEPES, pH 7.5. The complement proteins were added from concentrated stock solutions either to the aqueous phase bathing a membrane in the black state or immediately prior to membrane formation, to prevent protein inactivation. The temperature was kept at 25°C throughout.

Membrane conductance measurements were performed using a pair of calomel electrodes with volt bridges switched in series with a voltage source and an electrometer (Keithley, 610C). For single-channel experiments the electrometer was replaced by a current amplifier (Keithley 427). The amplified signal was monitored with a storage oscilloscope and recorded on a tape recorder or on a strip chart recorder. The single-channel instrumentation had a time resolution of 1–10 msec dependent on the magnitude of the single-channel conductance. Zero current membrane potential measurements were performed by establish-

ing a salt gradient across membranes containing complement pores as described earlier (Benz, Janko & Läuger, 1979).

Results

Change in Macroscopic Conductance after C5b-9 Assembly

As shown by the data of Fig. 1, successive addition of complement proteins C5b6, C7, C8 and C9 to BLM formed from a 4:1 mixture of diphytanoylphosphatidylcholine; phosphatidylserine, always resulted in a sharp increase in conductance, which was detected approximately 1 min after addition of C9 to the aqueous phase. The delay in the response to C9 addition is likely to reflect the time for diffusion across the unstirred layer, or to the slow activation rate of C9 at 25°C (*see* Sims & Wiedmer, 1984*b*). Under the conditions of these experiments (proteins each at 1 μg/ml), no change in membrane conductance was observed after addition of each of the individual components (C5b6, C7, C8 or C9) or after incubation of the membrane with C5b6, C7 and C8 in the absence of added C9 (*see* Discussion).

Multiple experiments performed under the conditions of Fig. 1 revealed a large variability in the net increase in membrane conductance recorded after C5b-9 additions (ranging from 10^{-5} S/cm^2 to 5×10^{-4} S/cm^2 under identical experimental conditions—i.e., a concentration of 1 μg/ml for all complement proteins). Furthermore, under these conditions, the membrane was found to be highly unstable, frequently breaking within 5–10 min after C9 additions (to the C5b-8 treated membrane). By contrast, membranes exposed only to the C5b-8 proteins were observed to exhibit normal stability, remaining essentially unchanged over 1–2 hr.

Current-Voltage Relationship

As shown by the data of Fig. 2, the C5b-9 induced current was observed to obey a linear dependence on transmembrane potential (V_m), for V_m less than 80–100 mV, suggesting that the complement channel is not voltage-gated up to a transmembrane potential of 100 mV. Similarly, applied voltages of 10–60 mV during addition of the C5b-9 proteins did not substantially affect the resulting membrane conductance, suggesting that C5b-9 assembly occurs independently of V_m. A similar conclusion was reached previously on the basis of direct measurement of C5b-9 binding to valinomycin-treated erythrocyte membranes (Sims & Wiedmer, 1984*a*). Unfortunately, it was not possible to perform these experiments at $V_m > 100$ mV due to the instability of the membrane at higher voltages after C9 additions.

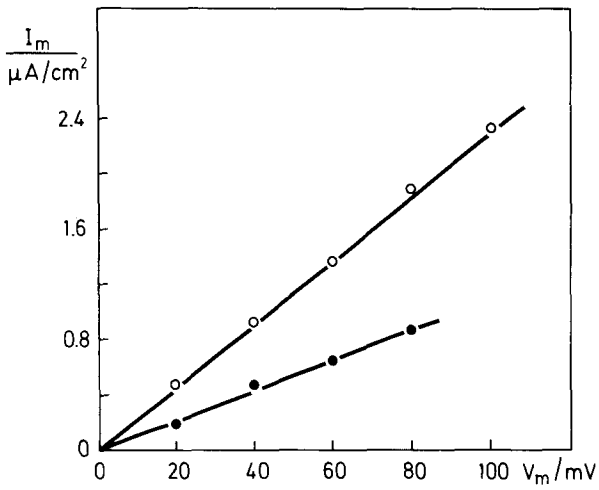

Fig. 2. Current *vs.* voltage characteristics of membranes formed from diphytanoylphosphatidylcholine/phosphatidylserine (molar ratio 4:1) in the presence of 1 μg/ml of each of the complement proteins C5b6, C7, C8 and C9. The aqueous phase contained 0.15 M KCl and 5 mM HEPES, pH 7.5; T = 25°C. The two lines represent data obtained from separate experiments performed under identical conditions

Single-Channel Conductances

To resolve single-channel conductances, the C5b-9 proteins (final concentrations 1–100 ng/ml) were added to the aqueous phase of membranes formed across a 0.05-mm^2 orifice under voltage-clamping conditions (*see* Fig. 3). Sequential addition of C5b6 + C7 + C8 + C9 resulted in a stepwise increase in membrane conductance (at clamped V_m), which was observed only after C9 addition. No conductance increase was observed upon addition of the individual C5b-9 proteins, or upon mixing of the proteins in the absence of any one component. Under the conditions of these experiments, we were also unable to observe any increase in conductance when the additions of the C5b-9 proteins were distributed between the aqueous phases *cis* and *trans* to the membrane, suggesting that pore formation occurs only when all components interact at the same surface of the bilayer.

In Fig. 3 is shown a typical record obtained after addition of the C5b-9 proteins to a BLM formed from a mixture of diphytanoylphosphatidylcholine and phosphatidylserine (molar ratio 4:1). Both aqueous compartments contained 0.15 M KCl, pH 7.5. As illustrated by the figure, conductance steps were mostly directed upward (i.e., channel formation), with downward conductance

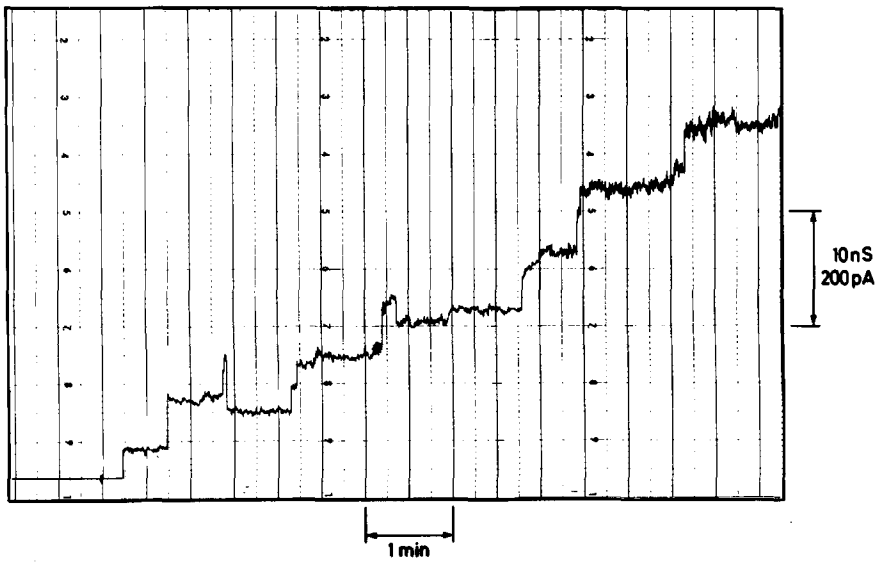

Fig. 3. Stepwise increase of the membrane current after the addition of C5b6, C7, C8, and C9 to one side of a membrane from diphytanoylphosphatidylcholine/phosphatidylserine (molar ratio 4 : 1). The aqueous phase contained 50 ng/ml of each component in 0.15 M KCl, 5 mM HEPES, pH 7.5; T = 25°C. The applied voltage was 20 mV

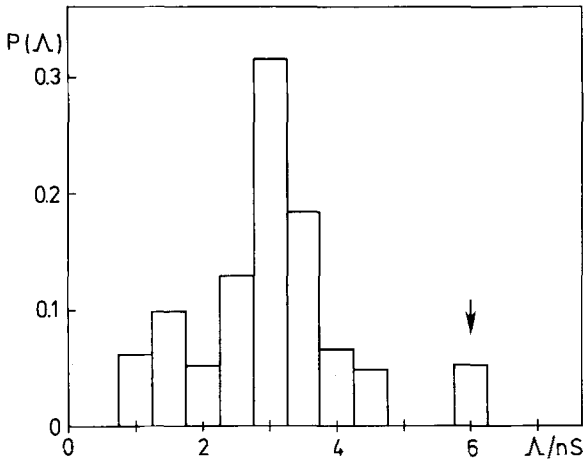

Fig. 4. Histogram of the conductance fluctuations observed with membranes from diphytanoylphosphatidylcholine/phosphatidylserine in the presence of C5b6, C7, C8, and C9. The aqueous phase contained 0.5 M KCl, and 5 mM HEPES, pH 7.5. The applied membrane potential was either 10 or 20 mV. The average single-channel conductance of 173 single-channel events was 3.1 nS. Arrow denotes double-channel event

steps only rarely observed. Similar recordings obtained over extended observation times, suggest that the lifetime of the C5b-9 pore on these membranes is at least 5 min. As is evident from the trace in Fig. 3, the formation of increased numbers of C5b-9 channels resulted in increased current noise of the single-channel record, suggesting that the C5b-9 pore is not a stably-fixed conducting pathway, but undergoes intermolecular fluctuations ("channel breathing"; *see* Sigworth, 1985). The likelihood for fluctuations in the conducting state of the C5b-9 pore has been suggested previously, based on the variance between the apparent solute exclusion radius versus solute permeability of the C5b-9 pore (Jackson et al., 1981; Sims, 1981).

As is also evident from the data of Fig. 3, the single-step conductance increments observed after C9 addition (to C5b-8) were not uniform in size, varying over a range of conductances. A histogram of the step conductances measured in 0.15 M KCl is plotted in Fig. 4. The step current fluctuations were found to distribute over an approximately sixfold range of conductances. A similar variability of the unitary conductances was observed in experiments performed in the presence of LiCl and NaCl (substituted for KCl; *see below*). Occasionally, dimers of single channels were observed (*see* Fig. 4). The broad distribution of the size of the step conductances observed after C9 addition may reflect substates of a single pore or molecular heterogeneity between different pores (e.g. due to variable C5b-9 stoichiometry; *see* Discussion).

Influence of Ionic Strength on C56-9 Pore Conductance

Estimates of the average conductance increment ($\bar{\Lambda}$) under single-channel conditions were obtained by averaging the size of the step increments observed after C9 addition. Experiments were performed in the presence of either KCl, NaCl, or LiCl at concentrations ranging from 0.05 to 1 M. In all cases, $\bar{\Lambda}$ was derived on the basis of ≥50 observed current fluctuations recorded in single-channel experiments. Comparison of $\bar{\Lambda}$ obtained for the different salts at the same ionic strength suggests that the average single-channel conductance generally follows the specific conductance (σ) of the aqueous

Table 1. Average single-channel conductance $\bar{\Lambda}$ of the complement channel in different salt solutions[a]

Salt	Conc. (M)	$\bar{\Lambda}$ (nS)	σ (mS cm^{-1})	$\bar{\Lambda}/\sigma$ (cm × 10^8)
KCl	0.05	2.5	7.0	36
	0.15	3.1	21.0	15
	0.5	6.7	55	12
	1.0	11.2	112	10
NaCl	0.15	2.6	14	19
LiCl	0.15	1.8	11	16
RbCl	0.15	3.2	22	15
TrisCl	0.15	1.3	8.5	15
KCH_3COO	0.15	1.6	10	16

[a] The membranes were formed from diphytanoylphosphatidylcholine/phosphatidylserine (molar ratio 4 : 1) dissolved in *n*-decane. The aqueous solutions were buffered with 5 mM HEPES at pH 7.5; T = 25°C; V_m = 10 mV.

Table 2. Average single-channel conductance $\bar{\Lambda}$ of the complement channel for different lipids used for membrane formation[a]

Lipid	$\bar{\Lambda}$ (nS)
Diphytanoyl PC	3.5
Diphytanoyl PC/PS (molar ratio 4 : 1)	3.1
Diphytanoyl PC/cholesterol (molar ratio 1 : 1)	3.4
Oxidized cholesterol	3.3

[a] The membranes were formed from decane solutions. The aqueous phase contained 0.15 M KCl, 5 mM HEPES at pH 7.5; T = 25°C. The applied voltage was 10 mV.

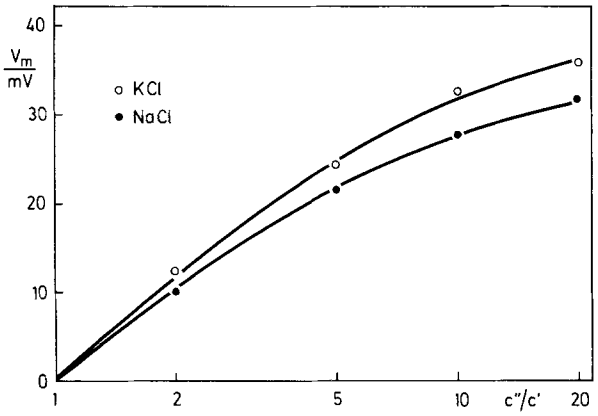

Fig. 5. Zero-current membrane potentials V_m as a function of the salt gradient C''/C' on both sides of membranes from diphytanoylphosphatidylcholine/cholesterol (molar ratio 1 : 1). C' was fixed to 10 mM KCl, 5 mM HEPES, pH 7.5, and C'' was varied between 10^{-2} and 10^{-1} M KCl, 5 mM HEPES, pH 7.5. V_m was positive at the more dilute side. The lines were drawn according to the Goldman-Hodgkin Katz equation (Benz et al., 1979) with the indicated values for P_K/P_{Cl} and P_{Na}/P_{Cl}. The data of separate experiments performed under each set of experimental conditions are shown

phase (Table 1), indicating that the relative mobility of each ion within the pore is similar to that within the bulk aqueous phase [*cf.* estimates for the diffusive permeabilities of nonelectrolytes within the C5b-9 pore reported in Sims (1981)]. Nevertheless, under conditions of changing ionic strength, the ratio $\bar{\Lambda}/\sigma$ was observed to vary inversely with ionic strength, suggesting that the functional state of the membrane pore is altered by ionic strength (*see below*).

It is interesting to note that under the conditions of these experiments, changes in the ionic strength of the aqueous phase affected the average single-channel conductance to a larger extent than was observed when the concentration of the individual C5b-9 proteins was varied. Variations in concentrations of the C5b-8 complements had no influence on the mean size of the complement pore. Increasing concentrations of complement C9 resulted in an increase of the pore-forming probability but had virtually no influence on the average single-channel conductance ($\bar{\Lambda}$ increased by 20% at 1 μg/ml C9 versus 50 ng/ml). The addition of even larger concentrations of complement C9 led to such a rapid pore formation that the single events could not be separated within the time resolution of our experimental instrumentation (*see* Materials and Methods).

Influence of Membrane Composition

As summarized by the data in Table 2, the average single-channel conductance measured for membranes exposed to the C5b-9 proteins was found to be largely unaffected by the inclusion of either phosphatidylserine or cholesterol. Furthermore, the single-channel conductance measured for membranes formed exclusively of oxidized cholesterol closely approximated results obtained for phosphatidylcholine membranes, suggesting that the lipid composition of the bilayer has little influence on the unit conductance of the C5b-9 pore *per se*.

Ion Selectivity of the C5b-9 Pore

The ion selectivity of the C5b-9 pore was investigated by measuring the membrane potential under zero current conditions (Fig. 5). Because of the short lifetime of pure phospholipid membranes in

Table 3. Diameters of the complement pores at different ionic strengths[a]

Ionic strength	$\bar{\Lambda}$ (nS)	Mean pore diameter (nm)		
		(l = 15 nm)	(l = 10 nm)	(l = 5 nm)
0.05	2.5	8.3	6.7	4.8
0.15	3.1	5.3	4.3	3.1
0.5	6.7	4.8	3.9	2.8
1.0	11.2	4.4	3.6	2.5

[a] The pore diameter (d) was calculated according to Eq. (1) using the data given in Table 1 and assuming pore lengths (l) of 15, 10, and 5 nm, respectively.

the presence of C5b-9 proteins, in these experiments membranes of diphytanoylphosphatidylcholine/cholesterol (molar ratio 1 : 1) were employed. The membranes were formed in 10 mM solutions of either NaCl or KCl, and the C5b-9 proteins were added in concentrations sufficient to increase the specific conductances of the BLM by 10^2 above the bare bilayer conductance (10–100 nS/cm^2). After the membrane conductance stabilized (approximately 20 min after C9 addition), the salt concentration on one side of the membrane was raised by addition of the concentrated salt. In all cases, the diluted side (*trans* to salt addition) became positive, reflecting preferential transmembrane movement of the cation (Fig. 5). In these experiments, V_m reached its plateau value 5–10 min after the salt gradient was established.

A fit of the observed values of the zero current membrane potential to the Goldman-Hodgkin-Katz equation (Benz et al., 1979) revealed a ratio of cation/anion permeabilities (P_c/P_a) of 5.2 for KCl and 4.1 for NaCl (Fig. 5). This apparent selectivity of the C5b-9 channel towards cations suggests the presence of an excess of negatively charged groups in the vicinity of the pore, due either to charged residues on the proteins or adjacent phospholipid (*see below*).

In this respect, it is interesting to note that most porins of gram-negative bacteria form also slightly-cation selective pores in lipid bilayer membranes (Benz, 1984, 1985). Chemical modification of carboxyl groups of the Omp F-pores of *E. coli* has clearly shown that the cation selectivity of this pore is caused by negatively charged groups in or near the pore (Benz, Tokunaga & Nakae, 1984). Although the data of Fig. 5 suggest a small pore selectivity to K^+ versus Na^+ (P_c/P_a approximating 5.2 versus 4.1), this can largely be accounted for by the increased aqueous mobility of the potassium ion relative to that of sodium (Castellan, 1983).

Discussion

Our data provide direct evidence that the C5b-9 pore exhibits multiple conducting states, with a broad distribution of specific conductances varying over about a fourfold range (*cf.* Fig. 4). The multiple conducting states displayed by the C5b-9-treated BLM are consistent with previous observations relating to an apparent heterogeneity of the complement pore, as suggested on the basis of marker solute exclusion by complement-damaged membranes as well as by electron micrographic study of the ultrastructural features of membrane-inserted C5b-9 complexes (Boyle et al., 1979; Sims & Lauf, 1980; Sims, 1981; Ramm et al., 1983; Bhakdi & Tranum-Jensen, 1984). Since the distribution of conducting states we observe with BLM is largely unaffected by changes in bilayer composition, it is likely that the observed variability in the conducting states of this pore reflects different associative and/or conformational states of the assembled proteins *per se*. Whether this variability derives from changes in the stoichiometry of C9 in the assembled complex, or to higher aggregate states formed by interaction of multiple C5b-9 complexes, remains unresolved by the single-channel measurements, because we were unable to study the pore size at very high C9 concentrations (>1 μg/ml).

As previously noted (*see* Table 1 and Results) the average single-channel conductance of the C5b-9 pore generally followed the specific conductance of the bulk aqueous phase (at constant ionic strength), suggesting that ion mobility within the C5b-9 pore is unimpeded by interactions with the wall of the channel. It is of interest to note that an analysis of the aqueous diffusivities of nonelectrolytes within the C5b-9 pore had previously led one of us to conclude that the mobility of *uncharged* solute within the complement channel also closely approximates that of free diffusion (Sims, 1981). Taken together, these data suggest that transmembrane movement of both charged and uncharged solute across the complement damaged membranes occurs freely, without substantial pore interactions.

Because the specific conductance of an ion across the C5b-9 pore appears directly related to its bulk aqueous conductance (at constant ionic strength), we can use the measured single-channel conductances to estimate the dimensions of the C5b-9 pores, assuming equivalent pore geometries. If we model the C5b-9 channel as a hollow aqueous-

filled cylinder, the average pore radius (r) for a given length (l) of the pore can be calculated from the average single-channel conductance ($\bar{\Lambda}$) according to

$$\bar{\Lambda} = \sigma\pi \cdot r^2/l \quad (1)$$

where the specific conductance of the interior of the pore (σ) is assumed to be that of the bulk aqueous phase. Assuming a pore length of 15 nm as suggested by ultrastructural analysis of the negatively-stained C5b-9 tubule, an average pore diameter of 5.3 nm can be derived from measurements performed in 0.15 M KCl ($\bar{\Lambda}$ = 3.1 nS; σ = 21 mS · cm^{-1}), with diameters ranging from 3.0 nm ($\bar{\Lambda}$ = 1 nS) to 6.0 nm ($\bar{\Lambda}$ = 4 nS) under these conditions. If a shorter length of the complement channel is assumed, estimates of the pore diameters would be correspondingly reduced (*see* Table 3). Assuming the minimum pore length necessary to span the lipid bilayer (approximately 5 nm), an average diameter of 3.1 nm at physiological ionic strength is suggested (experimental range: 1.7 to 4.3 nm).

By contrast to results obtained by the substitution of ions (at fixed concentration), the single-channel conductance of the C5b-9 pore is not a linear function of the bulk aqueous conductance when this conductance is varied by changing ionic strength (*cf.* $\bar{\Lambda}/\sigma$, Table 1). Assuming that the specific conductance within the pore obeys the bulk aqueous conductance (*see above*), the average pore diameter appears largest at low ionic strength and remains roughly constant as ionic strength is increased from 0.15 to 1 M (Table 3). Since this nonlinear variation of apparent pore size with ionic strength is unlikely to reflect a specific cation binding site within the pore (*see* effect of cation substitution; Table 1), these data raise the possibility that the conformational and/or associative state of the bound C5b-9 proteins is altered at low ionic strength, resulting in an increase in the average pore diameter.

The dimensions of the C5b-9 pore suggested by the average specific conductance we measure at physiological ionic strength are consistent with the apparent size (5 × 15 nm, diameter × length), of the C5b-9 protein tubule depicted by electron microscopy (Tschopp et al., 1982; Tschopp, 1984; DiScipio & Hugli, 1985). Nevertheless, comparison to data we and others have previously obtained relating to the functional properties of the complement pore in biological membranes suggest that a wide variation in membrane conductance can be observed after C5b-9 assembly, which may relate to specific interactions of the complement proteins with membrane constituents. For example, based on measurements undertaken with BLM exposed to the C5b-9 proteins, Michaels et al. (1978) deduced an average single-channel diameter of 2.5 nm. By contrast, using single-channel conductances recorded for extracellularly patch-clamped muscle cells exposed to antibody + rabbit complement, Jackson et al. (1981) deduced an average pore diameter of 0.8 nm (corresponding to single-channel conductances of 90 pS). Similarly, based on measurements of the permeation of marker solute across the complement-damaged erythrocyte membrane, the exclusion radius of the complement pore has been variously estimated at <0.4 to >5 nm (Boyle et al., 1979; Sims & Lauf, 1980; Ramm et al., 1983, 1985). Although the data of the present study suggest that variable functional states of the complement pore reflect inherent heterogeneity to the assembled protein complex itself, the influence of the target membrane (e.g. planar lipid bilayers versus biological membranes) on apparent pore size must also be considered. For example, one might speculate that intrinsic membrane proteins with the capacity to interact with one or more of the C5b-9 components may restrict their functional activation, resulting in apparent pore radii significantly smaller than that we observe for a pure lipid membrane. In this context it will be of interest to examine how specific C8 and C9 binding proteins recently detected in the human erythrocyte membrane affect ion conductances across the C5b-9 pore inserted into these membranes (Schönermark et al., 1986; Zalman & Müller-Eberhard, 1985).

It is noteworthy that by contrast to the data of Michaels and associates (Michaels et al., 1976, 1978), we failed to detect *transmembrane* interactions between any of the individual components of the C5b-9 complex (i.e., additions made to compartments *trans* to the bilayer), nor did we detect conductance changes if any of the C5b-9 components were omitted (*see* Results). Whether these discrepancies between our findings and those reported by these authors reflect differences in bilayer stability, protein concentration or purity, or effects arising due to diffusion of components through the taurus of nonbilayer lipid and solvent which surrounds the BLM remains to be resolved.

As is evident from the data of Fig. 2, once formed the C5b-9 channel persists for several minutes, suggesting that the activated state of the BLM-inserted proteins is quite stable over time. Nevertheless, we cannot exclude the possibility that the channel undergoes rapid fluctuations between open and closed states on a time scale shorter than the response time of our electronics (≥ 1

msec). Membrane channels formed by rabbit complement in the plasmalemma of skeletal muscle cells have been reported to rapidly open and close, with characteristic times of ~1 msec (Jackson et al., 1981). The apparent stability of the C5b-9 channel in BLM's suggests that the binding interaction between subunits of the pore may be favored in a pure lipid system, over that observed in a biological membrane. The apparent stability of the C5b-9 pore in BLM also stand in contrast to findings with other channel-forming substances where the conducting unit forms through the reversible association of oligomeric subunits (e.g., *see* data for alamethicin in Boheim, 1974).

As noted above (*see* Results), our data suggest that C5b-9 pores exhibit marked selectivity to cations versus anions, raising the possibility that the electrogenic pore conductance might serve to evoke an electrochemically-coupled response from the target cell of immune attack. In this context one might consider whether a cation-selective conductance across the C5b-9 pore underlies recent observations indicating triggered cellular activation (without cell lysis) upon C5b-9 insertion into the plasma membrane (Campbell & Morgan, 1985; Wiedmer & Sims, 1985).

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