# Formation of ion-conducting channels by the membrane attack complex proteins of complement

John W. Shiver, John R. Dankert, and Alfred F. Esser

Laboratory for Structural Biology, Department of Comparative and Experimental Pathology, University of Florida, Gainesville, Florida 32610

ABSTRACT The effects of sequential additions of purified human complement proteins C5b-6, C7, C8, and C9 to assemble the C5b-9 membrane attack complex (MAC) of complement on electrical properties of planar lipid bilayers have been analyzed. The high resistance state of such membranes was impaired after assembly of large numbers of C5b-8 complexes as indicated by the appearance of rapidly fluctuating membrane currents. The C5b-8 induced conductance was voltage dependent and rectifying at higher voltages. Addition of C9 to membranes with very few C5b-8 complexes caused appearance of few discrete single channels of low conductance (5–25 pS) but after some time very large (>0.5 nS) jumps in conductance could be monitored. This high macroscopic conductance state was dominated by 125-pS channels having a lifetime of ~1 s. The high conductance state was not stable and declined again after a period of 1–3 h. Incorporation of MAC extracted from complement-lysed erythrocytes into liposomes and subsequent transformation of such complexes into planar bilayers via an intermediate monolayer state resulted in channels with characteristics similar to the ones produced by sequential assembly of C5b-9. Comparison of the high-conductance C5b-9 channel characteristics (lifetime, ion preference, ionic-strength dependence) with those produced by poly(C9) (the circular or tubular aggregation product of C9) as published by Young, J. D.-E., Z. A. Cohn, and E. R. Podack. (1986. *Science* [*Wash. DC*]. 233:184–190.) indicates that the two are significantly different.

## INTRODUCTION

The hemolytic and cytotoxic activities of complement result from the fusion of five plasma proteins (complement proteins are named in accordance with recommendations in *Bull. WHO* [1968, 2]) C5, C6, C7, C8, and C9 into large complex of $M_r$ 1–2 million referred to as the membrane attack complex (MAC) of complement (1, 2). Assembly of the MAC starts with the cleavage of C5 into two fragments, C5a and C5b. Then, without further enzymatic action, a complex is produced with a proposed composition of $(C5b, 6, 7, 8)_1(C9)_n$, where $n$ usually varies between one and six when the complex is formed on natural membranes. However, $n$ could be as high as 15 when C9 is allowed to polymerize and provided that sufficient C9 molecules are available (2). The following scheme summarizes the known reactions:

$$C5b + C6 \rightarrow C5b\text{-}6 \quad (1)$$

$$C5b\text{-}6 + C7 \rightarrow C5b\text{-}7 \xrightarrow{+M} MC5b\text{-}7 \xrightarrow{+C8} \cdot MC5b\text{-}8 \xrightarrow{+n\,C9} MC5b\text{-}9_n \quad (2)$$

$$C5b\text{-}7 + S \rightarrow SC5b\text{-}8 \xrightarrow{+C8} SC5b\text{-}8 \xrightarrow{+C9} SC5b\text{-}9 \quad (3)$$

Reaction 1 can be carried out in vitro by activation of C7-deficient serum. This allows isolation of active C5b-6 (3) which then can be used to study reactions 2 or 3 under precise conditions chosen by the experimenter (4). When reaction 2 is carried out in the presence of target cells it causes lysis of such cells and this process is known as "reactive lysis" (3). When the assembly process is carried out in serum in the absence of a membrane (reaction 3), then the $S$ protein substitutes for a membrane and inactive SC5b-7, SC5b-8 and SC5b-9 complexes are formed. In addition, it is also known that C9 can aggregate to form a tubular polymerization product referred to as poly(C9) (reaction 4); this reaction is catalyzed by metal ions such as $Zn^{2+}$ and favored at low ionic strength but can also proceed through proteolysis with trypsin (2, 5, 6).

$$n\,C9 \xrightarrow[\text{trypsin}]{Zn^{2+}\text{ or}} \text{poly(C9)}. \quad (4)$$

Poly(C9) and MC5b-9 display similar ringlike structures in the electron microscope after negative staining (2, 7–10).

The MC5b-9 complex can be introduced into bilayer lipid membranes (BLM), and Benz et al. (11), Menestrina and Pasquali (12), and Young and Young (13) are among the more recent in a long list of investigators who examined the single channel characteristics of MC5b-9

Address correspondence to Dr. Alfred F. Esser.

Dr. Shiver's current address is National Cancer Institute, National Institutes of Health, Building 10, Room 4B17, Bethesda, MD 20892.



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on BLM. The only electrophysiological measurements with complement on natural membranes were performed by Stephens and Henkart (14) and Jackson et al. (15). The conclusion reached from these studies was that C9 was responsible for channel formation across BLM or natural membranes. Native C9 alone has no affinity for membranes. However, as we demonstrated previously (16), the isolated carboxy-terminal thrombic fragment of C9 (C9b) is capable of producing single channels in BLM.

The MC5b-9 complex can be incorporated into BLM or liposomes by sequential assembly, as shown in reactions 1 and 2, or the final complex can be extracted first from complement-lysed erythrocytes (17, 18) and reincorporated into proteoliposomes (18) which then can be used to prepare BLM by apposition of two monolayers (19). The aims of our studies were to directly compare the channel characteristics of MC5b-9 prepared by these different methods, and also with the poly(C9) channel as reported by Young et al. (20). To facilitate distinction between the assembled and the reconstituted complex in a target membrane we will refer in this paper to the former as the C5b-9 complex and the latter as MAC.

## EXPERIMENTAL PROCEDURES

### Chemicals

NaCl, KCl, and Tris were purchased from Fisher Scientific (Orlando, Fl), and sodium deoxycholate and N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (SB 3-12) from Calbiochem (San Diego, CA). 6-Carboxy fluorescein (6-CF) was supplied by Molecular Probes, Inc., (Eugene, OR), and Mops was from Research Organics (Cleveland, OH).

### Solutions

All buffers and solutions were prepared from deionized water (Continental Water Systems, El Paso, TX) which was further purified for planar lipid bilayer experiments by ion exchange chromatography and filtration through activated charcoal ("WATER-I", Gelman Sciences, Ann Arbor, MI).

### Lipids

Egg and soybean phosphatidylcholine was purchased from Avanti Polar Lipids (Pelham, AL). Asolectin was prepared from soybean lecithin (Sigman Chemical Co., St. Louis, MO) by the method of Kagawa and Racker (21).

### Proteins

Human complement proteins C5b-6, C7, C8, and C9 were isolated as described elswhere (4). The membrane-associated C5b-9 complex (MAC) was extracted with SB 3-12 from complement lysed rabbit erythrocyte ghosts as described by Ware et al. (17). Protein concentrations were determined by using the following coefficients ($\epsilon^{1\%}_{1cm}$) at 280 nm: C5b-6, 10.8; C7, 9.9; C8, 14.9; and C9, 9.6 (4).

### Target structures

Large, mostly unilamellar liposomes (LUV) were prepared by sonication and freeze-thawing. In brief, 4 mg of egg lecithin dissolved in hexane/$CH_3OH$ (9:1) were dried in a test tube under a stream of nitrogen and then lyophilized for 12 h. Buffer (0.5 ml of 100 mM KCl, 10 mM Mops, 100 mM 6-CF, pH 7.2) was added and the suspension was sonicated with a probe sonicator (Heat Systems-Ultrasonics, Inc., Plainview, NY) under nitrogen for 1 h until translucent at ~ 10°C above the phase transition temperature of the lipid. The resulting vesicles were frozen (in dry ice/ethanol) and thawed twice and then passed over a Sephadex G-100 column equilibrated with KCl-Mops buffer to remove unencapsulated 6-CF.

Incorporation of MAC into LUV was achieved in two different ways. In one case complexes were assembled on LUV by sequential addition of 10 μg each of purified C5b-6, C7, C8 and 30 μg C9 to 10 ml LUV (0.5 mg total lipid) and the vesicles were separated from unincorporated proteins by pelleting in an air driven ultracentrifuge (Beckman Instruments, Inc., Fullerton, CA). Otherwise, extracted MAC in SB 3-12 (2 mg total protein) was thoroughly mixed with 40 mg of egg lecithin and buffer containing 300 mg of deoxycholate. The solution was chromatographed on Sephadex G-100 and the vesicle-containing void fractions were collected. Electron microscopic examination revealed that most LUV carried one or more MAC complexes (data not shown).

Planar lipid bilayers were made by the apposition of two asolectin monolayers (16, 22). Two adjacent compartments in a Teflon chamber were separated by a thin (12.5 μm) Teflon sheet into which a 250-μm diameter aperture had been punched. The aperture was treated with a 1%-solution of squalene (Sigma Chemical Co., St Louis, MO) in *n*-pentane. Then, buffer solution (typically 10 or 100 mM NaCl, 10 mM Tris, pH 7.0) was introduced into the compartments such that they were filled below the level of the aperture. A phospholipid monolayer was formed by spreading 10 μl of a 0.5%-solution of phospholipid in pentane on the buffer in each compartment and waiting for the solvent to evaporate.

Thereafter, more buffer solution was injected under the monolayers to raise them over the aperture, thus forming a bilayer covering the aperture. Bilayer formation was continuously monitored by the increase in electrical capacitance between the two compartments. All planar bilayers were studied at room temperature.

Incorporation of the C5b-9 complex into planar bilayers was achieved in two different ways. The complex was either assembled from the fliud phase by sequential addition of proteins C5b-6, C7, C8 and C9 into the *cis* compartment ("reactive lysis" procedure) or MAC reconstituted into proteoliposomes (see above) was added slowly to the existing asolectin monolayer in the *cis* compartment as described (19, 23).

## Single channel recordings

Voltages were applied to the compartment to which the proteins were added (the *cis* side), and the opposite compartment (the *trans* side) was defined as being at ground potential. Positive current is movement of positive ions from the protein-containing to the protein-free side of the membrane (*cis* to *trans*). Current was measured using Ag/AgCl electrodes and was converted to voltage with Keithley 427 current amplifier (Keithley Instruments, Inc., Cleveland, OH) set to a rise time of 1 ms. The signal was recorded on either a strip chart recorder (BD-40, Kipp & Zonen, Delft, Holland) or, when current-voltage curves were measured, on a X-Y recorder (BD-90, Kipp & Zonen).

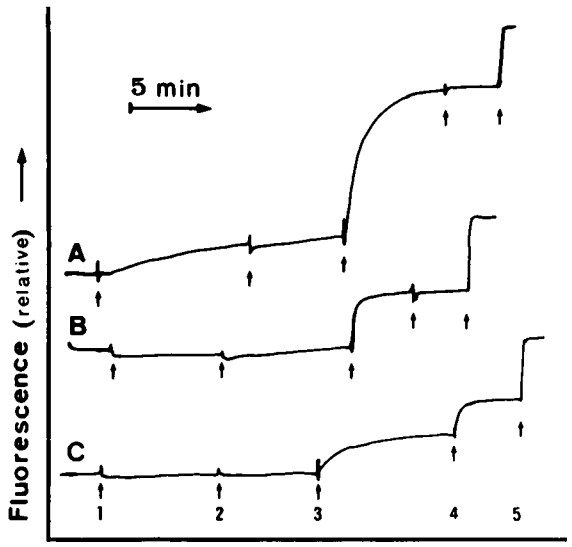


FIGURE 1 Release of carboxyfluorescein (100 mM) from large unilamellar lipid vesicles (LUV) mediated by reactive lysis. LUV (10-μg lipid in 0.3-mL buffer) prepared from either asolectin (trace *A*) or egg lecithin (trace *B*) or egg lecithin/30% cholesterol (mol/mol) (trace *C*) were treated with sequential additions of 1 μg each of C5b-6, C7, C8, and C9 at time points 1, 2, 3 and 4, respectively. Fluorescence was continuously monitored at 520 ± 4 nm (excitation = 480 ± 4 nm) in a stirred cuvette thermostatted to 37°C. Total marker release was achieved by addition of 2 μl of 10% Triton X-100 at time point 5.

# RESULTS

## Reactive lysis of liposomes

Large unilamellar liposomes (LUV) prepared from egg lecithin, with and without cholesterol, or asolectin were tested for release of entrapped 6-carboxy fluorescein (6-CF) after treatment with sequentially added C5b-6, C7, C8, and C9 (Fig. 1). These experiments are similar to those reported by others (24, 25) and demonstrate that "fluid" bilayers are lysed already by C5b-8 whereas cholesterol-containing "solid" bilayers require the complete membrane attack complex (MAC). Asolectin vesicles, which have not been tested before as targets for complement attack, behave similarly to egg lecithin vesicles. These results are included here to serve as a comparison for the BLM experiments described below.

## Membrane conductance changes

When C5b-6 and C7 were added sequentially to a planar bilayer prepared from asolectin no change in conductance was observed (Fig. 2*A*). Addition of C8 to assemble the C5b-8 complex resulted in an immediate increase in conductance which fluctuated rapidly yet single channels could not be resolved even at the fastest time resolution (< 1 ms) of our instrument. Fig. 2*B* shows the voltage dependence of the C5b-8 induced ion conductance and demonstrates some current rectification, i.e., positive currents continued to increase in a hyperbolic (super-ohmic) fashion above +40 mV but plateaued significantly as the potential increased at negative polarity.

Addition of C9 to bilayers with assembled C5b-8 under conditions described above caused a rapid jump in conductance and membrane rupture. Thus, the amounts of precursor proteins added to the *cis* compartment were reduced to 0.25 μg/ml each. Under these conditions, no C5b-8 induced conductance change could be recorded and, initially, no changes could be measured after addition of small amounts of C9. However, within a few minutes of C9 addition transbilayer currents could be measured which had different characteristics than those seen with high concentrations of C5b-8 alone. First, a few sporadic discrete single channels with different conductance states, ranging from 5 to 25 pS, could be resolved (Fig. 3*A* and *B*), but the most com-

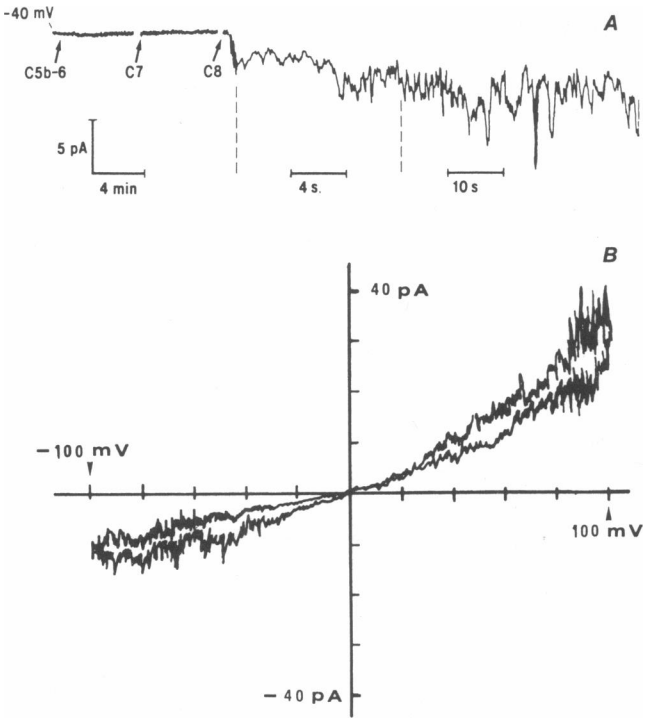


FIGURE 2 Voltage-dependent conductance changes of an asolectin planar lipid bilayer after assembly of the C5b-8 comples. (*A*) The voltage was clamped at −40 mV and both compartments contained 100 mM NaCl, 10 mM phosphate, pH 7.2. 10 μg each of C5b-6 and C7, and 14 μg of C8 were added sequentially to the *cis* side at the indicated time points (*B*) Current-voltage curves for C5b-8 mediated membrane conductance produced as described above.

mon conductance observed was 11 ± 2 pS (determined from four separate experiments and analysis of > 50 channels in each experiment). They were open usually for less than one min, and during this time the baseline conductance remained the same. After some time (15 min or more) the conductance suddenly increased in a series of very rapid (≈ 1 s) steps of 100–500 pS (Fig. 3, transition from trace *A* to trace *C*) of which the majority remained open over a period of minutes. At this point, net conductances were sometimes as large as 2 nS and bilayers would break frequently. At these large membrane currents the baseline would fluctuate rapidly and these fluctuations could be resolved into single channels of ~ 125 ± 10 pS which had a lifetime of ~ 1 sec (Fig. 3 *C*). The size for these channels was derived from three different experiments and more than 40 single channels were analyzed. Unlike the small conductances described above, these channels were very uniform in size. A membrane that broke at high conductances could usually be reformed, sometimes still displaying the large channels seen in Fig. 3 *C*, but also sometimes with only the very small ones shown in Fig. 3 *B*.

The channels produced by C5b-9 could not sense voltage polarity because membrane current flowing through these channels was symmetrical regardless of voltage polarity at transmembrane potentials up to ±60 mV. Fig. 4 (trace *A*) shows an increasing voltage sweep starting at +45 mV and reversing at +100 mV. At this potential, the net conductance was 2.4 nS. After reaching a transmembrane potential of −80 mV in this experiment, the conductance suddenly decreased in large steps of ~ 0.5 nS to reach a final net conductance of ~ 150 pS at the end of the second sweep (trace *B*). Upon continuing the voltage sweeps, no further sudden changes in net conductances were observed and the current-voltage curves showed largely ohmic behavior (trace *C*). The single-channel conductances that could be observed during this phase of the experiment ranged from 10 to 100 pS. With time these channels would close and not reopen and finally no net conductance above bare membrane levels could be detected. The current-voltage curve shown in Fig. 4 was typical of those seen in several different experiments although it was often difficult to record complete curves from +100 to −100 mV because of the sudden change in net conductance described above. The decay from the high macroscopic conductance state (trace *A*) to the low conductance state (trace *C*) could also be observed with time at a constant transmembrane potential of 40 mV and thus did not require application of high potentials although the decay time was shorter at higher voltages. Because of these sudden changes in conductance state, we did not attempt to collect a large number of channel recordings under fixed conditions for the construction of channel size histograms. Histograms of C5b-9 channels, however, have been published by others (11, 12).

Successful formation of C5b-9 channels was dependent upon addition of all precursor proteins to the same compartment. For example, no channels could be detected if C5b-6, C7 and C9 were added to the *cis* and C8 to the *trans* site, or when C5b-8 was assembled *cis* and C9 was added *trans*.

## MAC-induced conductance changes

Because the MAC can be extracted from lysed target membranes it was of interest to determine whether or not such complexes would behave differently from sequentially assembled C5b-9 complexes upon reconstitution into a lipid bilayer. Thus, two sets of experiments were performed. In one set, detergent solubilized MAC was inserted first into LUV prepared from egg lecithin and the vesicles were added slowly to an existing asolectin monolayer. Or in a second set, C5b-9 com-

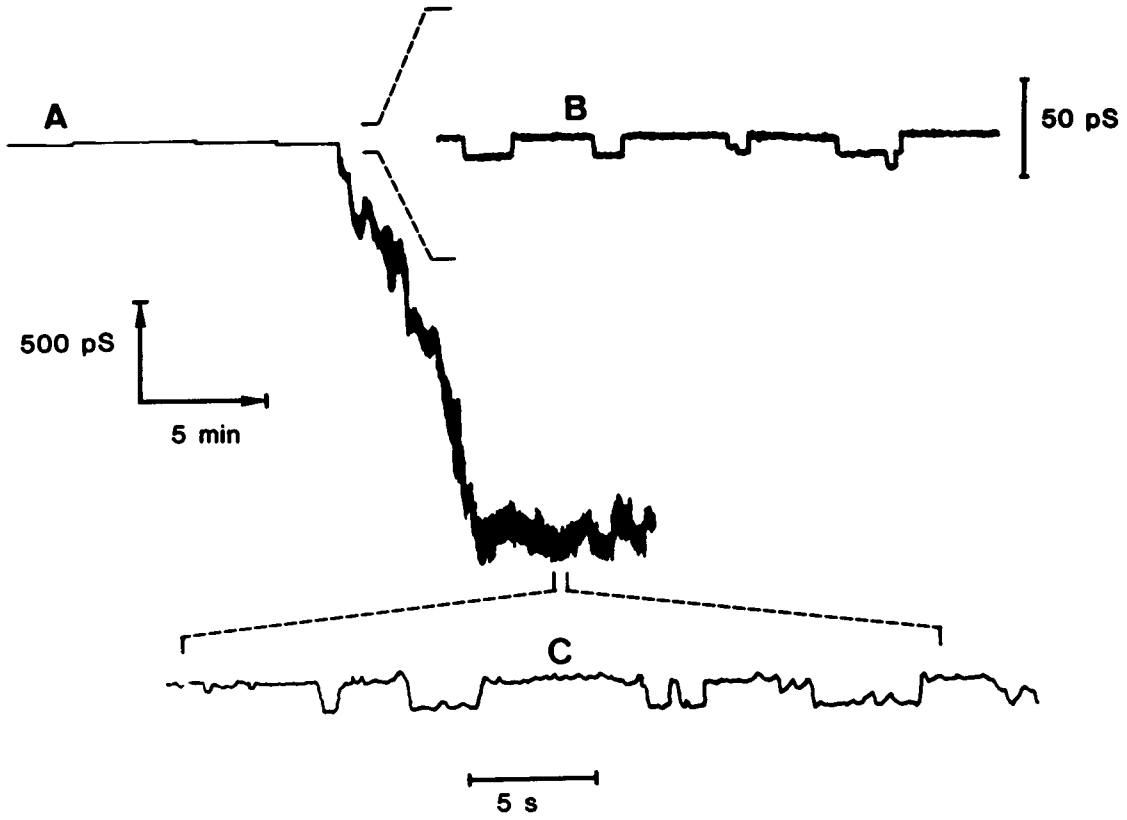


FIGURE 3 Single channel conductances of an asolectin planar lipid bilayer mediated by sequential assembly of C5b-9. The voltage was clamped at −40 mV and both compartments contained 10 mM NaCl, 10 mM phosphate, pH 7.0. Traces *A* and *B* show the presence of single channels of 11 ± 2 pS and the sudden increase in conductance leading to trace *C*. The higher time resolution of trace *C* indicates the presence of single channels with different conductance levels.

plexes were sequentially assembled on LUV and 20 μl of such vesicles were then added to the monolayer. In either case, it was observed that upon formation of an asymmetric planar bilayer from a pure monolayer in the *trans* compartment and a protein containing monolayer in the *cis* compartment single channels with conductances between 5 and 25 pS could be reported immediately (Fig. 5 *A*). At 40 mV transmembrane potential these channels were very similar to those shown in Fig. 3 *B*, and they lasted from several seconds to minutes. After a period of time, ranging from one to several hours, large conductance steps (200–500 pS) occurred. A voltage sweep (± 100 mV) at this point produced symmetrical currents displaying small (≈ 20 pS; see *arrows* in Fig. 5 *B*) and very large (> 1 nS; *arrowhead* in Fig. 5 *B*) conductance steps. A limited number of experiments were done with liposomes on which only the C5b-8 complex had been assembled. Formation of BLM from such proteoliposomes did not produce any conductances in contrast to the experiments described above where this complex was assembled directly on the BLM. It is conceivable that high concentrations of C5b-8 complexes in a BLM are required to observe membrane currents and it is difficult to achieve such high concentrations using the proteoliposome → BLM conversion technique. Preparations of liposomes not containing proteins never produced any conductance changes in BLM, demonstrating that residual detergents used in the dialysis procedure for the preparation of the MAC-liposomes were not responsible for the observed effects.

## DISCUSSION

The results from our BLM experiments demonstrate that the fully assembled MAC of complement produces single channel in asolectin bilayers that are of variable conductance in magnitude and in time. Whereas the first observation could have been anticipated from earlier results using different techniques, the second observation was not expected. For example, several authors have reported convincing evidence that the complement

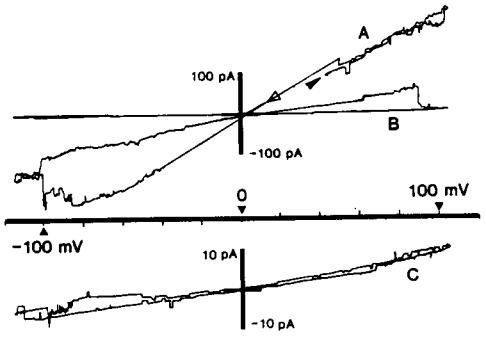


FIGURE 4 Voltage-dependent transmembrane currents produced by sequentially assembled C5b-9 complexes on an asolectin bilayer. Trace *A* was started at 40 mV and at 100 mV the net membrane conductance reaches 2.4 nS. At −80 mV the net conductance starts to decrease in large jumps of ~ 0.5 nS and a conductance of 0.15 nS remains at the end of the sweep (trace *B*) at +95 mV. At this point the current amplification was increased 10-fold the voltage sweep continued (trace *C*). The recordings show the presence of single channels with conductances between 10 and 100 pS that open and close during the sweep time and show a linear, or ohmic, current-voltage relationship. The buffer in both compartments is 10 mM NaCl, 10 mM phosphate, pH 7.0.

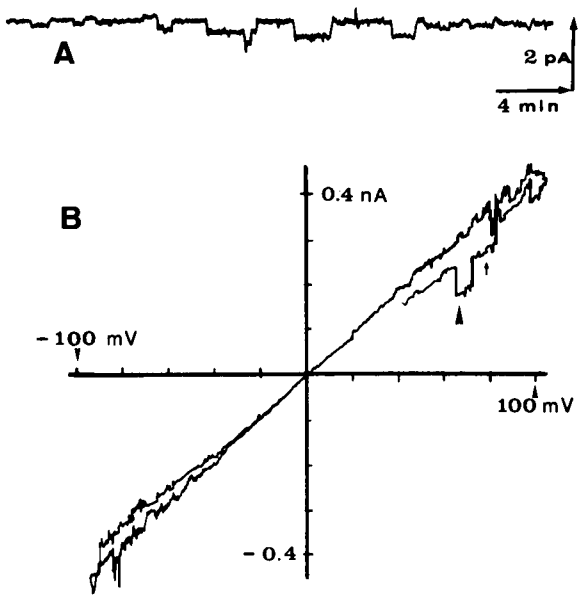


FIGURE 5 Transmembrane currents of C5b-9 complexes assembled on asolectin lipid vesicles which were spread into a monolayer (*cis* compartment) and then transformed into a bilayer. Buffer in both compartments is 100 mM NaCl, 10 mM phosphate, pH 7.2. *A* shows the membrane currents produced at −40 mV. Similar traces were recorded when extracted MAC was present in asolectin planar bilayers and such channels also produced symmetrical currents during a voltage sweep (*B*). Small conductance changes of ~ 20 pS (*arrow*) and very large ones of about 1 nS (*arrowhead*) can be identified.

channel is heterogeneous in size (for a summary see 26). However, it was also reported that, once assembled, the complement lesion was stable and would remain open on erythrocyte target membranes (26, 27). Only on nucleated cells was it possible to demonstrate loss of channels with time (28) and this event was considered to be the result of an active repair mechanism in the target cell (29, 30). Our results demonstrate now that assembly of the MAC on a completely artificial target structure that is metabolically inactive results in channels that display an increase in specific conductance with time followed by a decrease (Figs. 3 and 4), and that finally disappear completely. We do not know the molecular reasons for the disappearance of MAC channels in BLM but suspect that it may be related to the known MAC-mediated release of lipids from membranes and the high stability of MAC-lipid adducts in solution (31, 32).

The appearance of macroscopic conductances after the assembly of the C5b-8 complex is consistent with the results obtained with liposomes (Fig. 1) and also with the known fact that erythrocytes carrying complement proteins 1–8 (EAC1–8) hemolyse slowly (33). The membrane current produced by C5b-8 is nonohmic in nature and high concentrations of terminal proteins are required which may be a manifestation of the multi-hit nature of this lesion as decribed by Gee et al. (34). It should be noted, however, that the membrane currents could not be resolved into single-channel events. This fact clearly separates the C5b-8 induced currents from the C5b-9 currents.

Our results are only partially overlapping with those of Benz et al. (11). These investigators also assembled the C5b-9 complex sequentially as we did and reported that it will produce single channel conductances across bilayers made from *n*-decane and oxidized cholesterol or a mixture of diphytanoylphosphatidylcholine and phosphatidylserine. However, they did not observe conductance changes by the C5b-8 complex alone nor the temporal nature of the C5b-9 induced channels and, more importantly, the single-channel conductances reported by them are about 10-fold higher than ours. Inspection of their data indicates that they chose to work under conditions of low current amplification to observe the very large conductance jumps induced by C5b-9 (see Fig. 3 in their paper). The stepwise increases that they observed are similar to those seen in our Fig. 3 (i.e., the change in net conductance between trace *A* and trace *C*). Under their experimental conditions it is impossible to observe the very small net conductance changes produced by C5b-8 as demonstrated in Fig. 2. C5b-8 induced membrane currents were also recorded by Michaels et al. (35) and their presence is consistent with

the known lytic behavior of C5b-8 (33). The differences in average channel conductance reported by Benz et al. and by us result from our different interpretation of a "single channel event." We decided to include only those events that displayed clear opening and closing features as seen in traces *A* and *C* of Fig. 3 but not the large conductance jumps between these traces (see above) although it would have been justified to consider these jumps as single channels as they did. Additionally, Benz et al. (11) suggested that the increased "current noise" at higher numbers of C5b-9 channels results from intermolecular fluctuations ("channel breathing"). However, it is clear from the tracing in Fig. 3 that the "noise" can be resolved into true single channel events under our experimental conditions. Why these investigators did not observe the decay in membrane current that we have noticed is not clear but may be related to length of observation which sometimes exceeded three hours in our experiments.

Nevertheless, despite these differences, several common features in both sets of data are apparent and are useful in further discussion on the structure of the protein entity that produces these membrane currents. Significantly, like Benz et al. (11) we also observed a decrease of the apparent pore size of the single channels with an increase in the ionic strength of the bathing solution. Provided that a channel furnishes a passageway for ions with the same conductance as bulk water then channel radii ($r$) can be calculated according to the following equation:

$$r = (g \cdot l/\pi \cdot c \cdot \sigma)^{1/2},$$

where $g$ is the single-channel conductance, l is the length of the channel spanning the bilayer (3 nm for an asolectin bilayer), $c$ is the salt concentration of the bulk solution, and $\sigma$ is the equivalent salt conductivity. One also assumes that the membrane is neutral, and although this is not correct for asolectin (which has negatively charged headgroups) it is still illustrative to use this equation for calculating relative pore dimensions. Thus, the calculated diameters for the small channels (10–20 pS) are 0.6–0.8 nm and $\approx$1.9 nm for the larger channels (125 pS). The very large conductance steps of $\approx$0.5 nS (for C5b-9 in 10 nm NaCl) and $\approx$1 nS (for MAC in 100 mM NaCl) correspond to 4.0 and 1.7 nm, respectively, and would increase about two-fold if a pore length of 15 nm is chosen based on the dimensions of the isolated MAC (10, 18). The smaller 10–25 pS C5b-9 channels are reminiscent of the 11-pS C9b channels that we have described earlier (16). As is discussed in more detail elsewhere (19) it is unlikely that trace amounts of C9b in our C9 preparations were responsible for the small C5b-9 channels because we never detected any channel activity from numerous native C9 preparations even when up to 0.3 mg of C9 was added to the bilayer chamber, and because these small channels were completely symmetrical (Fig. 4 *C*), whereas the C9b channels were rectifying (16). In addition, such small C5b-9 channels were confirmed recently also by another group (13).

As detailed above, C5b-8 complexes induce membrane conductances without single-channel formation in a manner reminiscent of some detergents. Addition of small amounts of C9 was sufficient to produce small channels and it can be assumed that the initial C5b-9 complexes that were produced had a C5b-8 to C9 ratio of 1. Thus, it is tempting to spectulate that addition of C9 to C5b-8 complexes converts such complexes into structures with central pores and consequently much higher flux rates than could be achieved by protein complexes that perturb lipid and thereby produce membrane conductances. How the five proteins in a C5b-$8_1C9_1$ complex interact to produce small pores remains speculative, and which peptide segments could form the central channel cannot be deduced, of course, from our data.

Our results together with the earlier ones (11–13) allow, however, a good comparison of the large channel characteristics produced by C5b-9 or MAC with those associated with poly(C9) as reported by Young et al. (20). These investigators have clearly shown that transmembrane currents induced by poly(C9) increased linearly with an increase in ionic strength, an indication that water and ions are freely mobile within the poly(C9) channel. In addition, the poly(C9) channel had a negligible selectivity (1.4-fold) for cations ($Na^+$) over anions ($Cl^-$) and remained open as long as the applied voltage did not exceed 100 mV. In contrast, as demonstrated here, C5b-9 and MAC channels decayed in time and, as observed by Benz et al. (11), C5b-9 complexes exhibited a strong preference (4.1 fold) for cations over anions. It is of interest to note that even the inactive fluid phase SC5b-9 complex when freed of *S* protein by proteolysis (12) produced channels with conductances ($\approx$125–150 pS) similar to those that we observe for the reincorporated MAC. Therefore, channels that contain the other terminal complement proteins in addition to C9, and independent of how they were assembled on BLMs, are quite different from the poly(C9) channel with respect to ion selectivity, ionic strength dependence and lifetime.

The only similarity between poly(C9) and C5b-9 or MAC channels are the large conductance jumps ($>$1 nS) seen in Figs. 3 and 5. However, it cannot be stated with confidence from our results whether or not these are true single channel events because opening and closing events were not frequent enough to allow proper analysis. The magnitude of the net conductance

changes that we have observed is similar to that produced by poly(C9) channels as described by Young et al. (20). However, it should be pointed out that decreasing conductance changes were significantly larger than increasing ones. Thus, it is also entirely possible that the large decrease in conductance does not reflect closing of an individual channel but loss or rejection of a protein complex from the bilayer. Because of our choice to use asolectin lipids for BLM preparation such an event is even more feasible. This mixture contains a high amount of lipids that prefer to assemble in a type II hexagonal phase rather than a bilayer form (36) and since the terminal complement proteins likewise induce bilayer → nonbilayer lipid phase changes (37, 38) it is conceivable that entire C5b-9 complexes together with adjacent nonbilayer lipids separate from the bilayer membrane.

In summary, our results indicate that C5b-8 complexes inserted into BLM formed membrane pores that did not display single-channel characteristics and that addition of less than stoichiometric amounts of C9 to form C5b-$8_1C9_1$ complexes induced small, single ion-conducting channels. C5b-9 complexes with higher C9 multiplicities and independent of how they were assembled or introduced into BLM produced larger 125-pS channels with characteristics quite distinct from those reported for poly(C9) channels.

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