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Proteolytic modification of human complement protein C9: Loss of poly(C9) and circular lesion formation without impairment of function

(hemolysis/membrane protein)

JOHN R. DANKERT AND ALFRED F. ESSER*

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

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ABSTRACT **We have compared the ability of thrombin-cleaved C9 ($C9^n$) with that of native C9 to produce tubular or ring-like poly(C9) and to express the classical complement lesion on target membranes. Three procedures were used to produce poly(C9): (*i*) limited proteolysis with trypsin, (*ii*) interaction with small unilamellar lipid vesicles, and (*iii*) incubation with a 2- to 4-fold molar excess of $ZnCl_2$. In contrast to C9, which could be converted to *tubular* poly(C9), $C9^n$ was converted to smaller peptides by the first procedure and was aggregated into *string-like* poly(C9) by the other two methods. C9-depleted human serum (R−9 serum) was reconstituted with either C9 or $C9^n$ and these sera were then used to lyse sensitized sheep erythrocytes. Numerous classical complement lesions could be detected on ghost membranes obtained from cells lysed by C9-reconstituted R−9 serum but only a few on ghost membranes produced by $C9^n$-reconstituted R−9 serum. $C9^n$ was shown to be hemolytically as active as C9 even when tested under "single-hit" conditions and it was about twice as efficient when compared with C9 in releasing sucrose and inulin from resealed ghosts. These results are interpreted to indicate that formation of the classical complement lesion is only incidental to lysis and not an obligatory event and that enlargement of the "functional pore size" of the complement lesion is not linked to formation of a circular membrane attack complex.**

One hallmark of serum complement is its ability to lyse erythrocytes and bacteria and it is now axiomatic that lysis results from the formation of lesions on the target membrane. The structural entity associated with the complement lesion was first visualized in the electron microscope by Dourmashkin and co-workers (1–3) as a ring-like structure of ≈20 nm outer and ≈10 nm inner diameter. Those researchers also showed that formation of the morphological lesion requires participation of all five terminal complement proteins, including C9, although lysis of sheep erythrocytes proceeds in the absence of C9 (4). Based in part on the physical appearance of the lesion in the electron microscope and in part on the properties of low molecular weight ionophores, Mayer (5) introduced the "doughnut" theory, which describes the lesion as a stable hollow structure composed of proteins C5b, C6, C7, C8, and C9. This structure was considered to provide a water-filled transmembrane pore of ≈10 nm diameter that allows polar substances to equilibrate across the hydrophobic membrane barrier, and cell death results from ensuing colloid-osmotic effects.

An alternative view for the mechanism of immune hemolysis was presented by Kinsky (6) and Lachmann and co-workers (7, 8), who suggested that lysis is caused by a detergent-like action of the terminal complement proteins that assemble on the target to form a "leaky patch." This hypothesis is supported by studies by Esser and co-workers, (9–11), who investigated the effect of the terminal proteins on lipid organization in the target membrane and the mechanism of virolysis by complement. In addition, Lauf (12) has reported that the functional characteristics of immune hemolysis are inconsistent with a 10-nm cylindrical pore as seen in the electron microscope, a view that is reinforced by the later data of Sims and Lauf (13–15).

Although each of the terminal proteins, C5–C9, that combines to form the membrane attack complex (MAC) has been isolated and partially characterized (16), there is no agreement on which one of the two mechanisms described above is more suitable in explaining the function of the MAC. In particular, there is no agreement on the stoichiometry of the complex and its functional aggregation states (16–20). Central to the current debate is the role of poly(C9) to the function of the MAC. This polymer was first described by Podack and Tschopp (21) as a tubular structure composed of 15 (±3) C9 monomers (22) and closely resembles the extracted MAC seen in the electron microscope (23). For this reason, it was concluded that a completely assembled MAC having the *maximal* pore size is composed of $(C5b\text{-}8)_1C9_{15}$ (24–26). However, direct binding studies by Sims (17) and Stewart *et al.* (18) show that at most 4 C9 molecules per C8 molecule can bind to sheep or human erythrocyte membranes.

Because the structural appearance of poly(C9) reinforced the concepts of the original doughnut theory and also suggested a close relationship between poly(C9) formation and the nature of the lytic pore, we have investigated the formation of this polymer and its relation to the lytic function of complement in more detail. As reported elsewhere (27), we have identified the experimental conditions required for circular polymerization of C9. At room temperature C9 will aggregate to poly(C9) when allowed to interact with small unilamellar lipid vesicles (SUVs) or after limited proteolysis with trypsin. At higher temperatures (46°C), poly(C9) formation can be catalyzed with metal ions, the best of which appears to be Zn^{2+} (28). However, as mentioned before (27), we have also noticed that C9 proteolysed by α-thrombin has lost its ability to form poly(C9) under the conditions described above and will not support formation of the classical complement lesion. The purpose of the present work was to investigate this phenomenon in more detail and to test whether the suggested relationship between poly(C9) formation and lesion formation remains tenable.

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Abbreviations: C9, human complement component 9; $C9^n$, thrombin-cleaved C9; MAC, membrane attack complex; SUV, small unilamellar vesicle.

*To whom reprint requests should be addressed at: University of Florida, Box J-145/JHMHC, Gainesville, FL 32610.

MATERIALS AND METHODS

Chemicals. EDTA, $CaCl_2$, NaCl, KCl, Tris, and sodium barbital were purchased from Fisher, and Mops was from Research Organics (Cleveland, OH). Acrylamide, bisacrylamide, urea, and $NaDodSO_4$ were obtained from Serva, and diphenylhexatriene was supplied by Molecular Probes (Junction City, OR). $Na^{125}I$ was purchased from New England Nuclear and the iodination reagent (Iodo-Beads) was from Pierce. All other reagents and chemicals were purchased from Sigma. All buffers and solutions were prepared from ultrapure water (Continental Water Systems, El Paso, TX).

Plasma and Sera. Fresh frozen human ACD-plasma was obtained from Civitan Regional Blood Center (Gainesville, FL) and serum was prepared by addition of $CaCl_2$ to a final concentration of 20 mM and incubation for 2 hr at 37°C. Sera deficient in human C8 or C9, R−8 and R−9 serum, respectively, were prepared by immunoaffinity methods as outlined (27).

Proteins. Human C5b-6, C7, C8, and C9 were purified as described (27, 29). Human α-thrombin was a gift of John Fenton (Albany, NY), L-1-tosylamido-2-phenylethyl chloro-

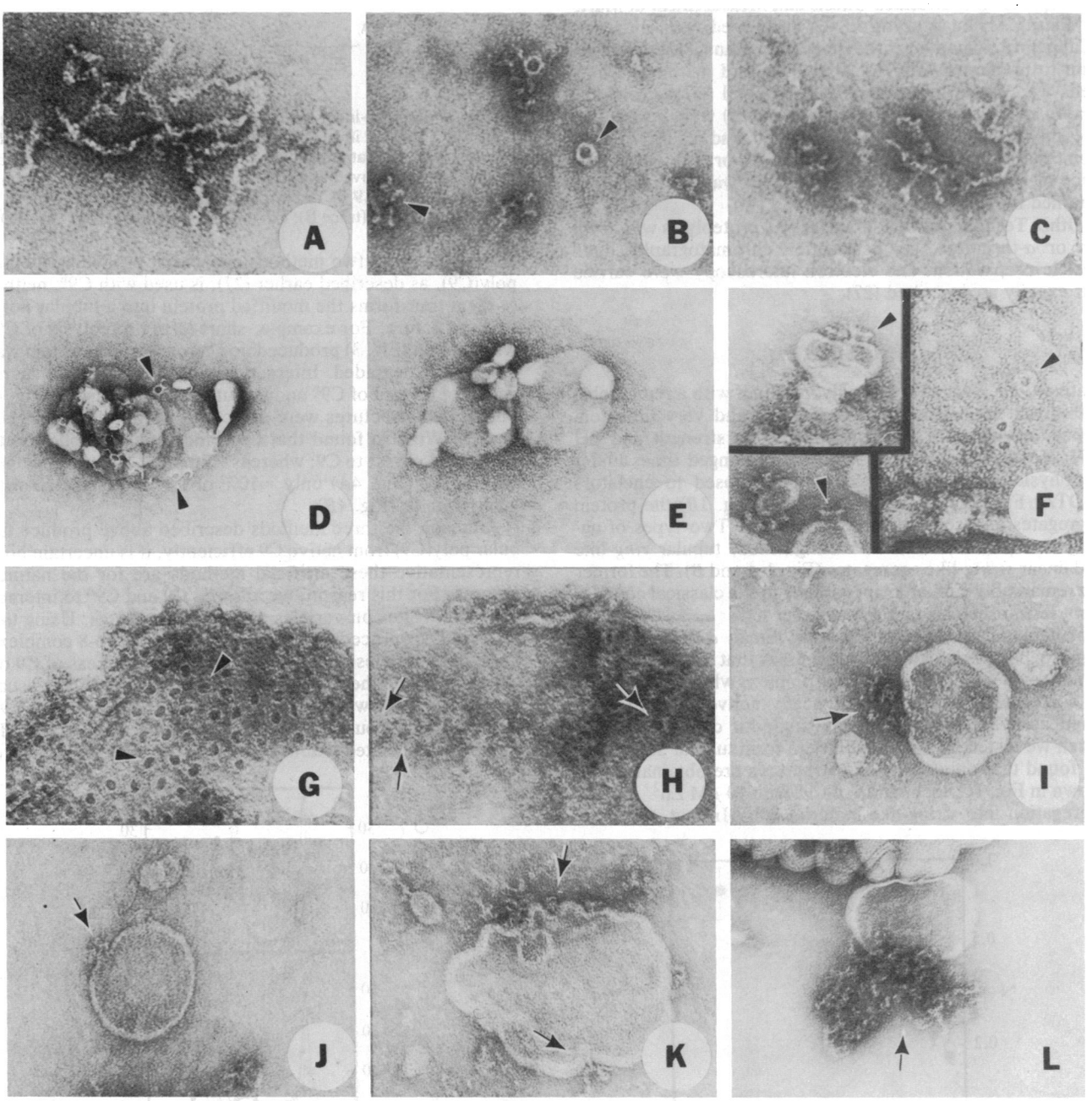

FIG. 1. Variability of the ultrastructure of C9 and $C9^n$ polymers. (*A–C*) Ultrastructure of $C9^n$ (*C*) or C9 (*B*) after incubation at 1 mg/ml with 10 μM $ZnCl_2$ in 10 mM Tris·HCl/150 mM NaCl/0.02% NaN_3, pH 7.2, for 72 hr at 37°C or of C9 (*A*) after incubation with 5 mM EDTA/10 mM Tris·HCl/150 mM NaCl/0.02% NaN_3, pH 7.2, for 72 hr at 37°C. (*D* and *E*) C9–dipalmitoyllecithin SUV aggregates present in fraction 12 of the chromatogram shown in Fig. 4*A* and $C9^n$–SUV aggregates present in fraction 12 of the chromatogram shown in Fig. 4*B*, respectively. (*G* and *H*) Surface structure of erythrocyte membranes after lysis with R−9 serum reconstituted with C9 at 180 μg/ml (*G*) or with $C9^n$ at 180 μg/ml (*H*); classical complement lesions are seen as mostly circular light rings surrounding a central well of deposited stain in *G* but not in *H*. (*F* and *I–L*) Ultrastructure of C5b-9 complexes (*F*) or C5b-9^n complexes (*I–L*) assembled on egg lecithin vesicles by the reactive lysis procedure. The C5b-9^n complexes appear string-like (arrows) in contrast with the tubular C5b-9 complexes (arrowheads). (Scale: the diameter of the white circles surrounding the locants corresponds to 80 nm for *A–C* and *F–L* and to 160 nm for *D* and *E*.)

methyl ketone-treated trypsin was bought from Worthington, and soybean trypsin inhibitor was from Sigma.

C9 Hemolytic Assay. The hemolytic activity of C9 was assayed under single-hit conditions as outlined by Boyle *et al.* (30). EAC1-7 cells for this assay were prepared as described (27).

C9 Polymerization. The different methods to polymerize C9 have been described (27, 28). In brief, monomeric C9 was either trypsinized as described, allowed to interact with SUVs, incubated with 10 μM $ZnCl_2$, or added to EAC1-7 cells in the presence of C8.

Preparation of Resealed Ghosts and Measurement of Marker Release. Human erythrocytes were used with antiserum (Cappel) to human erythrocytes and human C8-deficient serum to prepare EAC1-7 cells by standard methods. These cells were lysed hypotonically at 0°C and [^{3}H]sucrose and [*methoxy*-^{14}C]inulin (ICN-Radiochemicals) were sealed into the ghosts at 0°C as described by Sims and Lauf (14). End point marker release after addition of C9 or C9^n in the presence of saturating concentrations of C8 was determined as outlined by Ramm *et al.* (31).

Other Techniques. C9 iodination and proteolysis with trypsin or α-thrombin, gel permeation chromatography, lipid vesicle preparation, and electron microscopy were carried out exactly as described (27).

RESULTS

Native C9 is a water-soluble glycoprotein with a remarkable stability at physiological temperatures and very little tendency to aggregate at physiologic ionic strength and pH (27). However, when incubated for prolonged times under nonphysiologic conditions or when exposed to chelators (EDTA; Fig. 1*A*) or metal ions (Zn^{2+}; Fig. 1*B*) the protein aggregates and consequently inactivates. Two types of aggregation products can be distinguished, tubular *ring*-like and linear *string*-like aggregates (Fig. 1 *A* and *B*). The former are remarkably similar in appearance to the classical complement lesion seen on target membranes.

Proteolytic cleavage of C9 with human α-thrombin at a single peptide bond yields two peptides that are held together noncovalently. The resulting complex, which we call C9^n or nicked C9, remains hemolytically active (Fig. 2) even when assayed under stringent single-hit conditions (30). When we tested C9^n for its ability to form tubular structures we found that only string-like structures are obtainable. As shown in Fig. 1*C*, incubation of C9^n with 10 μM Zn^{2+} causes aggregation into string-like material only. In fact, when either of the other two methods capable of producing tubular poly(C9), as described earlier (27), is used with C9^n, neither of them transforms the modified protein into a tubular poly(C9^n) structure. For example, short-term proteolysis of C9^n with trypsin (Fig. 3) produced no aggregates; the protein was completely degraded. Interaction with SUVs caused aggregation of C9 and of C9^n and agglutination of vesicles (Fig. 4) but tubular structures were detectable only for C9 (Fig. 1 *D* and *E*). We also found that C9^n binds considerably less to SUVs in contrast to C9; whereas ≈30% of C9 elutes together with SUVs (Fig. 4*A*) only ≈10% of C9^n is associated with such vesicles (Fig. 4*B*).

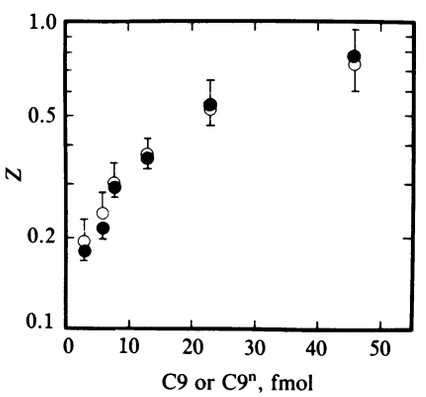

FIG. 2. Hemolytic activity of C9 and C9^n. *Z* plot of the hemolytic activity of C9 (●) and C9^n (○) as measured under single-hit conditions. The indicated amounts of protein were added to 5 × 10^7 EAC1-8 cells per assay mixture. Vertical bars indicate the range of *Z* values obtained with six different C9 or C9^n preparations.

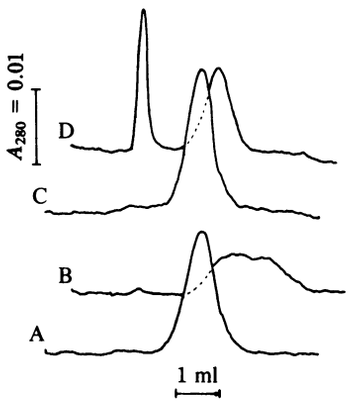

FIG. 3. Trypsin-induced aggregation of C9 and C9^n. Monomeric C9 or C9^n (1 mg/ml in 10 mM Tris·HCl/150 mM NaCl/0.02% NaN_3, pH 7.2) was incubated at 37°C with 0.2% ketone-treated trypsin. Aliquots were removed at different time points, applied to a Sepharose 6B column (0.9 × 18 cm), and eluted at 7 ml/hr. C9^n: t = 0 (trace A), t = 12 hr (trace B); C9: t = 0 (trace C), t = 12 hr (trace D).

Although the three methods described above produce tubular poly(C9) from native C9 efficiently, it is uncertain how representative these artificial methods are for the natural process. For this reason, we allowed C9 and C9^n to interact with C5b-8 sites on vesicles and on erythrocytes. Using the reactive lysis procedure (32), we assembled C5b-8 complexes on lipid vesicles and then added a 15-fold excess of C9 or C9^n to complete the reaction. As expected, with C9 the usual lesion structures were observed (Fig. 1*F*) whereas with C9^n one sees foliaceous or tree-like structures (Fig. 1 *I–L*). It should also be noted that in both cases the vesicles appear to

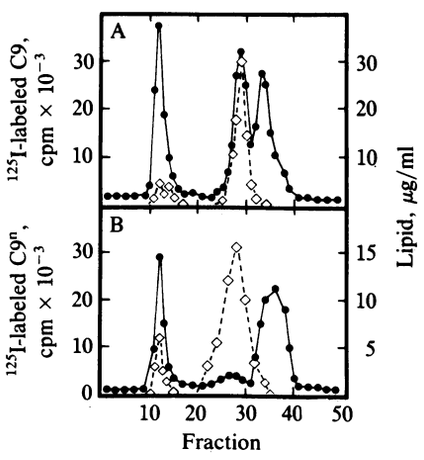

FIG. 4. Interaction of monomeric C9 or C9^n with SUVs. Monomeric ^{125}I-trace-labeled C9 (*A*) or C9^n (*B*) was incubated at room temperature for 5 min with SUVs prepared from dipalmitoyllecithin and the mixtures were chromatographed on Sepharose 2B. Fractions of 1.6 ml were analyzed for radioactivity (●) and vesicle content (◇).

Table 1. Efficiency of $C9^n$ for production of tubular poly(C9)

Procedure used*	Conc.† % C9	Conc.† % $C9^n$	Amount of structure per area examined,‡ %
I	>99	<1	100
	5–15	85–95	<1
II	>99	<1	100
	5–15	85–95	5–15

Various procedures to produce poly(C9) were tested to determine amounts of tubular or ring structures formed.
*Procedure I: incubation with 10 μM $ZnCl_2$ for 72 hr at 37°C. Procedure II: incubation with EAC1-8.
†Determined by densitometry of $NaDodSO_4$/PAGE gels; six different $C9^n$ preparations were tested.
‡Numbers of tubular or ring structures seen per unit micrograph area in C9 preparations were normalized to 100 and numbers of equivalent structures seen in companion preparations with $C9^n$ are expressed as %.

be filled with stain. A similar situation was encountered when R−9 human serum was reconstituted with C9 or with $C9^n$ and these sera were used to lyse sensitized sheep erythrocytes. The familiar classical complement lesions are found on the ghost membrane when C9 is used to reconstitute the C9-deficient serum in contrast to $C9^n$, in which one observes many fewer lesions (Fig. 1 *G* and *H*). Quantitation of the number of lesions observed on vesicles or erythrocytes with either C9 or $C9^n$ indicates that the latter produces 5–15% of the former (Table 1). The effectiveness of the $C9^n$ preparations in producing ring-like lesion structures agrees quite well with the concentration of uncleaved C9 present in $C9^n$ (Table 1). We avoided the practice of proteolyzing ghost membranes before staining, which has been reported (23, 33) to enhance the appearance of the lesion in electron micrographs. Trypsinization of C9 causes aggregation (Fig. 3*C*) into tubular poly(C9) as reported previously (27) and, as we will describe elsewhere, such a treatment can artificially produce "classical lesions" on ghosts and on lipid bilayer membranes without a required lysis step.

Because it had been suggested by several investigators (14, 19, 20, 34, 35) that the concentration of C9 somehow influences the relative "pore size" of the complement lesion on erythrocytes, we tested whether $C9^n$ would behave similarly to C9 in releasing markers of different size from resealed ghosts. We observed that $C9^n$ was very efficient in releasing sucrose from resealed EAC1-7 ghosts in the presence of C8. In agreement with the results of Sims and Lauf (14) and of Ramm *et al.* (31), who used C9, we found that higher amounts of $C9^n$ were required to release inulin as compared with sucrose. However, it is also evident that $C9^n$ is more effective than C9 in releasing both markers (Fig. 5); ≈20 $C9^n$ or 40 C9 molecules per ghost are required to release 65% sucrose and ≈200 $C9^n$ or 400 C9 molecules are necessary for 65% inulin release. If ghosts carrying only a few EAC1-7 sites were used, then inulin efflux was marginal even at high input of C9 relative to C8 although sucrose release was substantial (data not shown).

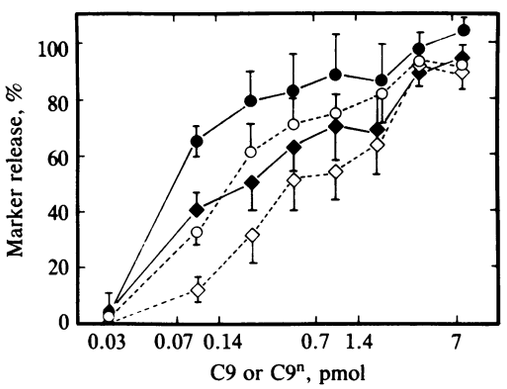

FIG. 5. Dose–response titration of C9 and $C9^n$. Radioactive sucrose (○, ●) and inulin (◇, ◆) were sealed into human EAC1-7 ghosts and marker efflux was effected by addition of 0.23 pmol of human C8 and various amounts of human C9 (dashed line) or human $C9^n$ (solid line) to a total of 3×10^9 ghosts per assay mixture. Each point represents the end point of marker efflux, which was attained after 30 min of incubation at 37°C. Complete release of markers was achieved by three cycles of freezing and thawing and set to 100%; marker release in the absence of C9 or $C9^n$ never exceeded 4% of total release. Vertical bars indicate the range of marker release obtained with six different C9 or $C9^n$ preparations.

DISCUSSION

The similarity between the visual appearance of the classical complement lesion and tubular poly(C9) prompted the hypothesis that the function of the C5b-8 complex on erythrocyte membranes is to catalyze the polymerization of C9 into ring-like structures (24). Our results with thrombin-proteolyzed C9 ($C9^n$) show that the formation of ring-like aggregates on target membranes is only incidental to the hemolytic function of C9 and is not a requirement. At first, the inability of $C9^n$ to form tubular poly(C9) by itself or together with the earlier acting terminal components may appear surprising considering that $C9^n$ is completely active (Fig. 2). However, formation of tubular C9 polymers requires at least two different binding sites on each monomer that must have a precise location with respect to each other to allow ring closure. This situation is shown in Fig. 6 where monomeric C9 is symbolized as a ring with two different binding sites that are offset at a precise angle. For example, for a perfect dodecameric ring the angle θ will be 150°. Any structural change that results in misalignment of these two binding sites obviously will not allow ring closure and only linear or curved aggregates are obtained. Cleavage of a single peptide bond between the a and the b domains in C9 produces a structural change that impairs circular polymerization but it is important to note that the tendency for linear or "random" aggregation is preserved and no loss in hemolytic activity is detected. Very little is known about the events that lead to C9 aggregation or the forces that stabilize the polymers. Other investigators have argued that hydrophobic forces are responsible for polymerization (20). Although such interactions may be initially responsible for contact formation between monomers, we think that formation of new intermolecular disulfide bonds may be of greater importance. This reasoning is in line with recent observations by Ware and Kolb (36), who observed the formation of disulfide-linked C9 dimers, and by Yamamoto *et al.* (37, 38), who demonstrated

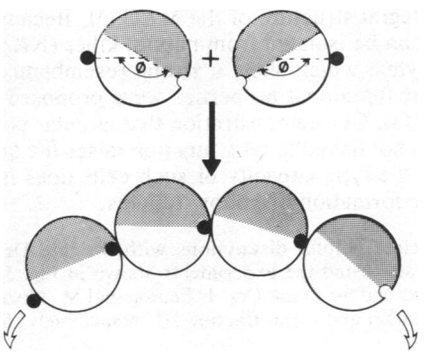

FIG. 6. Schematic representation of C9 aggregation. Monomeric C9 is symbolized by a circle representing a plane through the center of the globular protein with two different binding sites (● and ○) that combine during homopolymerization.

the influence of sulfhydryl reagents on C9 polymer formation. The difficulty in proposing a specific mechanism that explains the aggregation of C9 results from the fact that different treatments cause aggregation—e.g., incubation with metals or chelators at elevated temperatures, interaction with lipids or detergents, and limited proteolysis. It will be a challenge to find a common mechanism that could explain the effect of such different and divergent reactions.

Although it may be astonishing that $C9^n$ has not lost any hemolytic activity, it should be remembered that the pores required for colloid osmotic lysis of erythrocytes can be very small and, therefore, the absence of circular polymerization may not be noticeable in hemolysis experiments. Release of markers of different sizes from resealed ghosts, however, is a noncolloid osmotic effect that obeys the rules of molecular sieving. As reported here, the release of sucrose (0.9-nm diameter) and inulin (3-nm diameter) is effected equally well by C9 and $C9^n$ and no marked differences in the apparent functional pore size of the lesion could be detected. This result suggests that enlargement of the functional pore size is not linked to formation of tubular poly(C9) because $C9^n$ does not form a tubular structure, nor is the pore size determined by the C8/C9 ratio in the C5b-9 complex because effective inulin release was not observed at a low density of EAC1-7 sites no matter how high the C9/C8 input ratio was. Rather it appears that the functional pore size may be influenced by the total number or the physical size of effective C5b-9 or aggregated C9 complexes on the target membrane as proposed earlier (11, 13–15). This proposal is in contrast to hypotheses introduced by others who relate the functional heterogeneity to the structural heterogeneity by proposing either the simultaneous presence of monomeric and dimeric C5b-9 complexes (24) or the formation of "incompletely" assembled and partially circularized structures (19, 24–26, 34). The latter theory evolved from earlier proposals made by proponents of the single-hit theory of immune lysis (39, 40), who dismissed electron microscopic evidence of excessive lesion formation on lysed erythrocytes as a simple reflection of the formation of "uninserted" or "aborted" complexes. We suggest that multiple C9 molecules are required to aggregate C5b-8 complexes and, for this reason, C9 aggregation is of major importance for lytic function, but circular polymerization is only of incidental nature and may serve to inactivate "activated" C9 molecules (27). Earlier observations provided some evidence to indicate that the MAC is a transmembrane structure (23, 41, 42). It will be interesting to test whether similar results can be obtained with MACs formed from $C9^n$ and whether such a transmembrane configuration is an obligatory requirement for MAC function.

The appearance of poly(C9) in the electron microscope has been taken as evidence that it provides the water-filled transmembrane protein channel (24) that has been postulated to be an integral structure of the MAC (5). Because protein structures can be isolated from natural killer (NK) cells and T lymphocytes, which show a strong resemblance to poly(C9), similar functional properties were proposed for these structures (43). Our demonstration that circular polymerization of C9 is not linked to lytic function raises the question as to whether the lytic capacity of such cells does indeed depend on the formation of hollow tubules.

We appreciate helpful discussions with the late Dr. Manfred Mayer, who suggested the experiments shown in Fig. 5, and with Dr. Peter Sims and we thank Drs. J. Fenton and M. Mozen for supplying α-thrombin and Cohn fraction III, respectively; Sean Ervin for excellent technical assistance; and Jennifer Johns and Kathy Sutton for help in preparing the manuscript. This work was supported by National Institutes of Health Grant R01-AI-19478.

1. Borsos, T., Dourmashkin, R. R. & Humphrey, J. H. (1964) *Nature (London)* **202**, 251–254.
2. Humphrey, J. H. & Dourmashkin, R. R. (1969) *Immunology* **11**, 75–115.
3. Dourmashkin, R. R. (1978) *Immunology* **35**, 205–212.
4. Stolfi, R. L. (1968) *J. Immunol.* **100**, 46–54.
5. Mayer, M. M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2954–2958.
6. Kinsky, S. (1972) *Biochim. Biophys. Acta* **265**, 1–23.
7. Hesketh, T. R., Dourmashkin, R. R., Payne, S. N., Humphrey, J. H. & Lachmann, P. J. (1971) *Nature (London)* **233**, 620–623.
8. Lachmann, P. J., Munn, E. A. & Weissmann, G. (1970) *Immunology* **19**, 983–986.
9. Esser, A. F., Kolb, W. P., Podack, E. R. & Müller-Eberhard, H.-J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1410–1414.
10. Esser, A. F., Bartholomew, R. M., Jensen, F. C. & Müller-Eberhard, H.-J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5843–5847.
11. Esser, A. F. (1982) in *Biological Membranes*, ed. Chapman, D. (Academic, London), Vol. 4, pp. 277–327.
12. Lauf, P. K. (1975) *J. Exp. Med.* **142**, 974–988.
13. Sims, P. J. & Lauf, P. K. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5669–5673.
14. Sims, P. J. & Lauf, P. (1980) *J. Immunol.* **125**, 2617–2625.
15. Sims, P. J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1838–1842.
16. Müller-Eberhard, H.-J. (1984) *Springer Semin. Immunopathol.* **7**, 93–141.
17. Sims, P. J. (1983) *Biochim. Biophys. Acta* **732**, 541–552.
18. Stewart, J. L., Monohan, J. B., Brickner, A. & Sodetz, J. M. (1984) *Biochemistry* **18**, 4016–4022.
19. Bhakdi, S. & Tranum-Jensen, J. (1984) *J. Immunol.* **133**, 1453–1463.
20. Podack, E. R. & Tschopp, J. (1984) *Mol. Immunol.* **21**, 589–603.
21. Podack, E. R. & Tschopp, J. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 574–578.
22. Tschopp, J., Engel, A. & Podack, E. R. (1984) *J. Biol. Chem.* **259**, 1922–1928.
23. Bhakdi, S. (1983) *Biochim. Biophys. Acta* **737**, 305–341.
24. Tschopp, J., Müller-Eberhard, H.-J. & Podack, E. R. (1982) *Nature (London)* **298**, 534–538.
25. Podack, E. R. (1984) *J. Biol. Chem.* **259**, 8641–8647.
26. Tschopp, J. (1984) *J. Biol. Chem.* **259**, 7857–7863.
27. Dankert, J. R., Shiver, J. W. & Esser, A. F. (1984) *Biochemistry*, in press.
28. Tschopp, J. (1985) *J. Biol. Chem.* **259**, 10569–10573.
29. Ishida, B., Wisnieski, B. J., Lavine, C. H. & Esser, A. F. (1982) *J. Biol. Chem.* **257**, 10551–10553.
30. Boyle, M. D. P., Langone, J. J. & Borsos, T. (1978) *J. Immunol.* **120**, 1721–1725.
31. Ramm, L. E., Whitlow, M. B. & Mayer, M. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4751–4755.
32. Lachmann, P. J. & Thompson, R. A. (1970) *J. Exp. Med.* **131**, 643–657.
33. Tranum-Jensen, J., Bhakdi, S., Bhakdi-Lehnen, B., Bjerrum, O. J. & Speth, V. (1978) *Scand. J. Immunol.* **7**, 45–56.
34. Mayer, M. M. (1984) *Complement* **1**, 1–26.
35. Boyle, M. D. P., Gee, A. P. & Borsos, T. (1979) *J. Immunol.* **123**, 77–82.
36. Ware, C. F. & Kolb, W. P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6426–6430.
37. Yamamoto, K., Kawashima, T. & Migita, S. (1982) *J. Biol. Chem.* **257**, 8573–8576.
38. Yamamoto, K. & Migita, S. (1982) *J. Biol. Chem.* **258**, 7887–7889.
39. Mayer, M. M., Michaels, D. W., Ramm, L. E., Whitlow, M. B., Willoughby, J. B. & Shin, M. L. (1981) *CRC Crit. Rev. Immunol.* **2**, 133–165.
40. Boyle, M. D. P. & Borsos, T. (1980) *Mol. Immunol.* **17**, 425–432.
41. Morgan, B. P., Daw, R. A., Siddle, K., Luzio, J. P. & Campbell, A. K. (1983) *J. Immunol. Methods* **64**, 269–281.
42. Whitlow, M. B., Ramm, L. E. & Mayer, M. M. (1985) *J. Biol. Chem.* **260**, 998–1005.
43. Podack, E. R. & Konigsberg, P. J. (1984) *J. Exp. Med.* **160**, 695–710.