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The Membrane Attack Complex of Complement: Lipid Insertion of Tubular and Nontubular Polymerized C9†

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ABSTRACT: The membrane-restricted photoactivatable carbene generator 3-(trifluoromethyl)-3-(*m*-[^{125}I]-iodophenyl)diazirine [Brunner, J., & Semenza, G. (1981) *Biochemistry 20*, 7174-7182] was used to label the subunits of the membrane attack complex of complement (C5b-9). C5b-9 complexes either were assembled from serum on erythrocyte membranes or were reconstituted from purified components on liposomes. After irradiation, most of the probe is bound to C9 independent of the membrane system used, indicating that the wall of the transmembrane channel is predominantly composed of C9. No difference was observed whether polymerized C9 was in the tubular or nontubular form [Podack, E. R., & Tschopp, J. (1983) *J. Biol. Chem. 257*, 15204-15212], showing that tubule closure is not essential for successful lipid insertion. The same label distribution between the two forms of polymerized C9 was obtained by analyzing zinc-polymerized C9 in the absence of C5b-8. Since the photoreactive probe reacted with at least two distinct polypeptide segments within C9, lipid interaction does not occur via a single segment of hydrophobic amino acids.

Association of the hydrophilic complement proteins C5b, C6, C7, C8, and C9 leads to a multimolecular structure termed the membrane attack complex (MAC)[1] (Podack & Tschopp, 1984). During the assembly process, the proteins expose lipid binding sites and insert into membranes (Bhakdi & Tranum-Jensen, 1983). Lipid insertion occurs during the formation of the intermediate complex C5b-7 (Mayer, 1982). Once associated with the membrane, C5b-7 binds C8. Several copies of C9 then interact with the membrane-bound C5b-8 forming the so-called membrane attack complex (MAC). Bound C9 then undergoes a conformational change allowing the formation of tubular polymers [poly(C9)] of different sizes

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[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid disodium salt; ER, rabbit erythrocytes; MAC, complex of the complement proteins C5b, C6, C7, C8, and C9; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TAN, 20 mM Tris, 90 mM NaCl, 0.2 mM EDTA, and 0.02% NaN_3, adjusted to pH 8.1 with acetic acid; TBS, 10 mM Tris and 150 mM NaCl, adjusted to pH 7.5 with HCl; [^{125}I]TID, 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine; Tris, (hydroxymethyl)aminomethane.

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(containing up to 18 protomers) (Podack et al., 1982). If 12 or more C9 protomers have assembled, tubule closure occurs. Tubular poly(C9), i.e., poly(C9) in the form of a closed tubule, is extremely stable and is not dissociated by 1% SDS (Podack & Tschopp, 1983). Tubular poly(C9) migrates on SDS–PAGE with an apparent M_r of 1.2 million. In contrast, poly(C9) with a protomer number smaller than 12 fails to form a closed tubule. This nontubular poly(C9) can be dissociated by SDS.

Assembly of both forms of poly(C9) occurs also in the absence of C5b-8. Zinc ions mediate C9 polymerization (Tschopp, 1984b), forming polymers indistinguishable from those described for MAC-associated poly(C9).

Direct evidence that the MAC inserts into the lipid bilayer during its assembly came from labeling studies with photoactivatable, membrane-restricted reagents containing either the 4-azido-2-nitrophenyl (Hu et al., 1981; Steckel et al., 1983) or the alkylazido group (Podack et al., 1981). Although these reagents differ considerably in molecular shape and photochemical properties, the results of all studies led to the same conclusion, namely, that in the fully assembled MAC C9 is in direct contact with the lipid phase. There were, however, quantitative differences in the relative extent to which individual MAC constituents were labeled.

The goal of the present study is to determine whether hydrophobic labeling of C9 can demonstrate differences between nontubular poly(C9) and the tubular form. In addition to the biochemical differences already mentioned for the two forms of C9, it seems likely that they also differ with respect to the degree of contact with the lipid bilayer. The extent to which the different forms of poly(C9) are in contact with the hydrophobic portion of the lipid bilayer may be detected by labeling with an appropriate reagent. In principle, one can envision three alternatives: (i) Nontubular poly(C9) is in more intimate contact with lipids than tubular poly(C9). Therefore, in the nontubular form, lipid would interact with both the outside and inside of the pore. (ii) Nontubular poly(C9) shows less lipid contact than tubular poly(C9). Thus, because of the open-ring structure of poly(C9), insertion into the lipid bilayer might be less efficient than in the case of fully closed tubular C9. (iii) The extent of lipid contact is similar or identical for both tubular and nontubular poly(C9).

The reagent used in this study is [^{125}I]TID, the precursor of a highly reactive carbene (Brunner & Semenza, 1981). This intermediate is capable of reacting with the full range of amino acid side chains including aliphatic residues and therefore differs in a fundamental respect from azido-based reagents, which appear to react with polypeptide segments containing particularly reactive residues. Work over the past years with hydrophobic photoactivatable reagents has revealed that labeling patterns obtained with different reagents from the same membrane may be quite different. Such results have led to some controversy about which regions of a protein are in contact with the lipid bilayer. Examples are the Na^+,K^+-ATPase (Karlish et al., 1977; Jørgensen et al., 1982; Farley et al., 1980, Jørgensen & Brunner, 1983), the F_0 moiety of F_1F_0-ATP synthase from *Escherichia coli* (Hoppe et al., 1983a,b, 1984), and Thy 1, a surface glycoprotein anchored to the lymphocyte membrane solely via a stearic acyl chain (Luescher et al., 1984). In all these cases, the labeling patterns obtained with [^{125}I]TID could be satisfactorily reconciled with topological information derived from other experiments.

A further goal of the present study was to investigate whether or not the type of membrane used to assemble MAC had any measurable effect on the labeling of the individual components. In the three previous studies of MAC–lipid interactions (Hu et al., 1981; Podack et al., 1981; Steckel et al., 1983), different photolabels as well as different target membranes were used. Therefore, this question has not yet been answered.

Materials and Methods

Purification of Proteins. C5b-6, C7, C8, and C9 were isolated from human blood according to procedures previously published (Podack & Müller-Eberhard, 1980; Podack et al., 1979; Kolb & Müller-Eberhard, 1976; Biesecker & Müller-Eberhard, 1980).

C9 Fragments. C9 fragments were obtained by incubation of intact C9 in the presence of thrombin (Sigma, St. Louis, MO) or trypsin (Serva, Heidelberg, Germany) according to Biesecker et al. (1982). Briefly, 200 μg of C9 was incubated with 5–10 μg of trypsin or with 60 μg of thrombin at 37 °C. The reaction was stopped by addition of soybean trypsin inhibitor (Sigma, St. Louis, MO) (trypsin to inhibitor ratio = 1:3) after 10 min or by addition of PMSF (Sigma, St. Louis, MO) (final concentration 2 mM) after 2 h in the case of thrombin.

Preparation of Vesicles. Small unilamellar vesicles were prepared according to Mason & Hang (1978). Briefly, egg or soybean lecithin (Avanti Polar Lipid, Birmingham, AL) and 10–20% (w/w) cholesterol (Sigma, St. Louis, MO) were dried under nitrogen flux, resuspended in TBS (10 mM Tris-HCl, pH 7.4, containing 150 mM NaCl and 0.02% NaN_3), and sonicated 45–60 min at 4 °C until a clear solution was obtained. The vesicles were fractionated by means of a Sepharose CL-4B column (1.6 × 60 cm) and concentrated to approximately 15 mg/mL. The homogeneity of the vesicles was routinely checked by analtical ultracentrifugation (Beckmann, Model E), where the vesicles exhibited an *s* rate of 1.8–2.2 S (Mason & Hang, 1978).

Synthesis of Membrane Probe. 3-(Trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine ([^{125}I]TID) was prepared with a specific radioactivity of 10 Ci mmol^{-1} as described by Brunner & Semenza (1981). The reagent was stored as an ethanolic solution (40 μCi/μL at −20 °C.

Photolabeling Protocols. Vesicles containing [^{125}I]TID were prepared by incubation of the suspended vesicles with [^{125}I]-TID dissolved in ethanol (final ethanol concentration <2%) and by agitating for 5 min at 4 °C.

Spontaneous C9 polymerization was induced by incubation of isolated C9 (1 mg/mL) for 2 h at 37 °C in the presence of vesicles (3 mg of lipid/mL) containing [^{125}I]TID in TBS containing 2×10^{-5} M Zn^{2+} (Tschopp, 1984b). Lipid insertion of $C9_{try}$ or $C9_{thro}$ was carried out by incubating the nicked proteins in the absence of Zn^{2+} for 16 h at 37 °C. For C5b-8-induced C9 polymerization, the following final concentrations of reagents were chosen: vesicles containing [^{125}I]TID, 2.5 mg of lipid/mL; C5b-6, 120 μg/mL; C8, 30 μg/mL; C9, 150 μg/mL. C7 (45 μg/mL) was added after incubation of the mixture at 37 °C for 5 min. Complex formation of C5b-9 was then induced by incubating the proteins for 60 min at 37 °C.

The same protocol was used for labeling the proteins with TID in the absence of lipids, with the exception that the addition of vesicles was omitted. Photoactivation of the different preparations was effected by exposure of the sample to a 350-W high-pressure mercury lamp for 4 min at 4 °C (Brunner & Semenza, 1981).

Lipid-bound proteins were separated from free proteins by floatation. Briefly, the sample was mixed with an equal volume of 70% Ficoll 70 (Pharmacia, Uppsala, Sweden) in TBS and

overlayed with 25% Ficoll 70 and then TBS. The separation was achieved after a centrifugation of 30 min at 40 000 rpm at 4 °C (rotor SW55TI, Beckman). Lipid-bound proteins were recovered at the interface of TBS and 25% Ficoll. Free proteins remained in the layer containing 35% Ficoll. The fractions containing proteins were precipitated by addition of acetone (90% final) at −24 °C for 16 h. After centrifugation, the pellet was dissolved in 20% SDS and subjected to SDS–PAGE.

Isolation of MAC from Rabbit Erythrocytes. A total of 10 mL of packed and washed rabbit erythrocytes was incubated with frequent agitation for 1 h at 37 °C with 180 mL of fresh total human serum; 10 mL of 2 mM EDTA, pH 8.0, was added prior to centrifugation at 27000*g* at 4 °C for 30 min (Sorvall, SS34). The pellet was resuspended in 10 mL of 5 mM PO_4, pH 8.0, [^{125}I]TID was added, and then the mixture was photoactivated. After one wash, the pellet was dissolved in 5 mL of TAN containing 10% DOC and 2 mM PMSF by incubation for 1 h at 37 °C and 1 h at 22 °C. The solution was centrifuged 10 min at 15 000 rpm, and after addition of one drop of bromophenol blue, the supernatant was loaded onto a Sepharose CL-4B column (2 × 100 cm). The column was eluted at room temperature with TAN containing 1% DOC at 15 mL/h. The fractions were assayed for ^{125}I radioactivity, and certain positive samples were analyzed by SDS–PAGE. Fractions corresponding to the MAC were pooled, dialyzed for 4 days against several changes of water containing 0.1% SDS at pH 7.0, and then lyophilized.

Polyacrylamide Gel Electrophoresis. All samples were subjected to electrophoresis utilizing the Laemmli system in 2.5–10% acrylamide gradient gels at 150 V for 3 h under nonreducing conditions. When two-dimensional gels were run, the samples were first loaded under nonreducing conditions on a 2.5–10% acrylamide gel. The bands were excised and incubated for 40 min at 22 °C in 140 mM 2-mercaptoethanol and 10 mM EDTA in sample buffer prior to loading on a 10% acrylamide gel. The gels were photographed, dried, and autoradiographed (Kodak X-Omat AR films). The relative degree of photolabeling of each (R) was calculated from the normalized (in percent) peak areas (A) obtained by scanning (Zeiss, KM II scanner densitometer) both the autoradiogram (a) and the Coomassie Blue (cb) stained gel with

$$R = A_a/A_{cb}$$

Results

Photolabeling of MAC Reconstituted on Vesicles. The photolabel [^{125}I]TID was incorporated into vesicles composed of egg lecithin and cholesterol (molar ratio 9:1), and the purified proteins C5b-6, C7, C8, and C9 were added. The molar ratio of C9 to C8 was kept at 12:1 in order to favor the formation of tubular, SDS-resistant, poly(C9) (Tschopp et al., 1985a,b). After photoactivation, the protein–vesicle conjugates were floated. Figure 1 shows their analysis by SDS–polyacrylamide gel electrophoresis under nonreducing conditions, stained with Coomassie Blue, and its corresponding autoradiogram. Mostly C9/C8β and to a smaller extent C5b were labeled by the membrane-restricted probe. C9 as part of tubular (M_r 1.100K) and nontubular (migrating as monomeric C9, M_r 75K) poly(C9) were labeled, indicating lipid insertion of both forms of poly(C9). In order to exclude that the dominant labeling of C9/C8β was due to a preferential labeling of these proteins by the probe, solutions containing the probe and proteins were irradiated in the absence of vesicles. C9 assembled either in the MAC or in its monomeric form did not exhibit a preferential reactivity (Table I), since the labeling intensities varied at most 2–3 times. Thus, TID seems to display little intrinsic affinity for any of these proteins. The addition of purified precursor proteins to lipid vesicles does not result in complete insertion of the assembled MAC into the lipid bilayer. A substantial amount of MAC assembles in the fluid phase and aggregates upon exposure of the hydrophobic domains. These complexes, which upon flotation stay at the bottom of the centrifuge tube, are not labeled by TID (Figure 1), indicating that complete insertion of the label into the vesicles has occurred under our experimental conditions. This observation was confirmed in the experimental shown in Table I. C5b-9 was assembled first and then added to TID-containing vesicles, conditions under which the C5b-9 fails to insert into the lipid bilayer (Tschopp et al., 1982). No labeling of the proteins was observed, indicating that TID, once incorporated in the membrane, does not interact with proteins in the fluid phase. Thus, separation of proteins inserted in the membrane from unbound proteins seems not to be required. However, in order to exclude completely the possibility of nonspecific labeling, all protein–vesicle conjugates were floated before SDS–PAGE analysis.

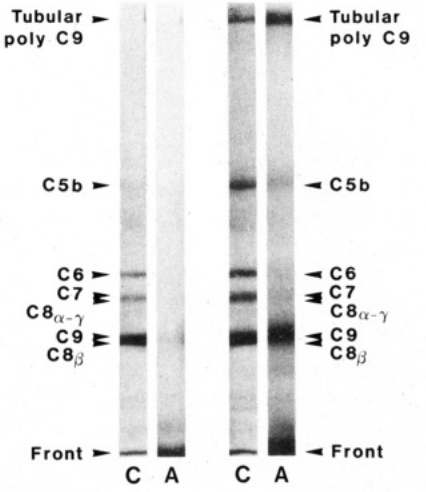

FIGURE 1: Photolabeling of reconstituted MAC on lipid vesicles. The MAC was assembled on [^{125}I]TID-containing lipid vesicles, and the probe was then photoactivated. Unbound proteins (left panel) were separated from vesicle-bound proteins (right panel) by floatation and subjected to SDS–PAGE analysis: (C) Coomassie blue staining; (A) autoradiogram.

Table I: Comparison of TID Reactivities with Individual Complement Components[a]

protein	complex	relative reactivity[b]
C5b	C5b-6	1.11
	C5b-9	1.06
C6	C5b-6	0.52
	C5b-9	0.41
C7 + C8α-γ	C5b-9	0.42
C8β + C9	C5b-9	0.65
C9		1
poly(C9)	C5b-9	2.2
C5b[c]	C5b-9	0
C6[c]	C5b-9	0
C7[c]	C5b-9	0
C8[c]	C5b-9	0
C9[c]	C5b-9	0

[a] Individual proteins or complexes were incubated with TID exactly like in the experiments shown in Figure 1, except that the vesicles were omitted. Thus, TID reacted with hydrophobic pockets of the proteins. [b] Samples were analyzed by SDS–PAGE, the amount of proteins was determined by Coomassie Blue staining, and TID labeling was calculated from the autoradiogram, as described under Materials and Methods. [c] In this set of experiments, the C5b-9 was assembled first and then added to TID-containing vesicles.

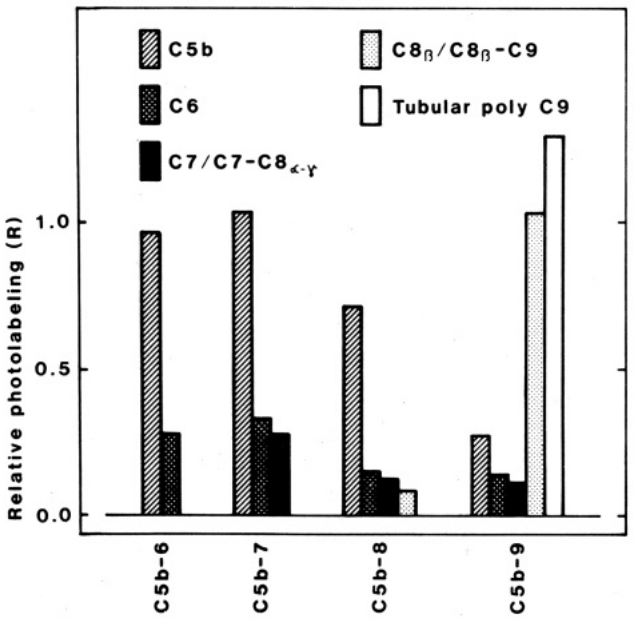

FIGURE 2: Histogram of the relative photolabeling of each component during different stages of MAC assembly. Relative photolabeling intensities were obtained as described under Materials and Methods. The extent of photolabeling of C7/C8α–γ and C8β/C9 are shown together due to the poor resolution of these proteins analyzed by SDS–PAGE.

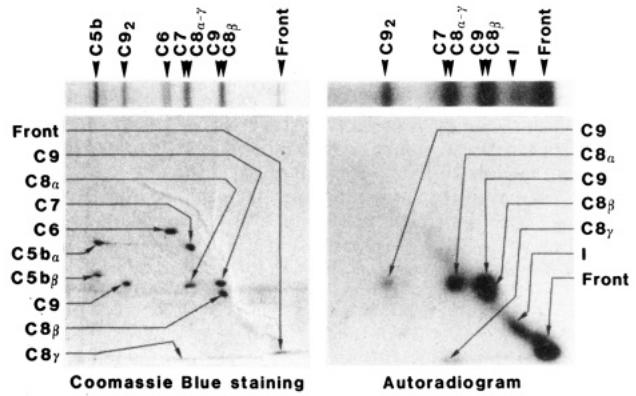

FIGURE 3: SDS–PAGE analysis of MAC purified from red blood cell membranes. First dimension under nonreducing conditions is shown on top of the second dimension, which was run under reducing conditions. An unidentified protein (I) is labeled with [^{125}I]TID.

We then investigated membrane interaction of the individual protein components of the MAC precursors (Figure 2). C5b-6 was added to vesicles containing [^{125}I]TID, and the C5b-6 vesicle conjugates were separated from unbound proteins by floatation in a Ficoll gradient. As already found in a previous study (Hu et al., 1981), C5b-6 was bound to the vesicles; C5b contained 75% of the C5b-6-associated [^{125}I]TID. After the formation of C5b-7, C5b contained 65% of the total label, whereas C6 and C7 shared the remaining 35%. Within the C5b-8 complex, C5b was still the most heavily labeled protein. Only after C9 had been added did the labeling pattern change: nontubular and tubular poly(C9) then became heavily labeled in parallel with a decrease in C5b labeling.

Labeling of the MAC Inserted into Natural Lipid Membranes. To examine possible effects of the type of membrane and its lipid composition on the distribution of radiolabel among MAC constituents, [^{125}I]TID labeling was performed on MAC assembled on rabbit erythrocytes. Following isolation of the red blood cell membranes and their labeling with [^{125}I]TID, the MAC was isolated and subjected to SDS–PAGE (Figure 3). The second dimension of the SDS–PAGE

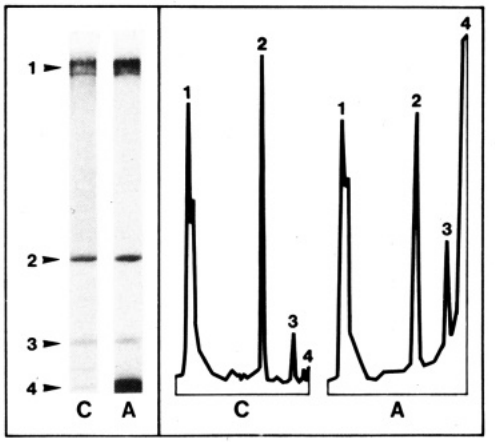

FIGURE 4: Photolabeling of poly(C9). C9 was polymerized in the presence of 10^{-5} M Zn^{2+} and the [^{125}I]TID labeling analyzed by SDS–PAGE (2.5–10% gradient gel): (C) Coomassie Blue stain; (A) autoradiogram; (I) impurity of the C9 lot. This protein is very well labeled by TID, due to its probable identity with HDL (apo-A) (data not shown).

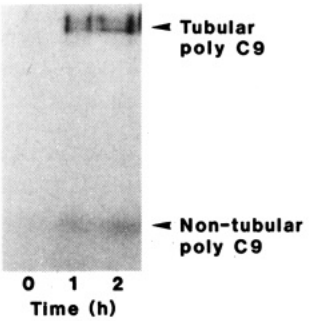

FIGURE 5: Lipid insertion of polymerizing C9. [^{125}I]TID-containing vesicles were incubated with monomeric C9. Polymerization of C9 was induced by the addition of 10^{-5} M Zn^{2+}. After different periods of time, C9–probe interaction was investigated by SDS–PAGE and autoradiography.

run under reducing conditions (Figure 3) shows that C9 was again predominantly labeled. However, there was a distinct difference in intensity depending on whether poly(C9) is dissociated into its monomeric or dimeric form. MAC isolated from erythrocytes often contains a substantial amount of disulfide-linked C9 dimers due to the presence of glutathione (Yamamoto et al., 1982). The probe was found to be bound preferentially to C9 that runs in SDS–PAGE as a monomer. Tubular poly(C9) did not enter the gel in the second dimension. In contrast to the MAC–vesicle system, all three subunits of C8 were labeled, whereas TID was absent from C5, C6, and C7.

Lipid Insertion of Polymerizing C9. In previous paper it was shown that, under conditions favoring circular C9 polymerization, C9 spontaneously inserts into artificial lipid membranes (Tschopp et al., 1982). Two forms of poly(C9) can be distinguished: tubular poly(C9) and nontubular poly(C9) (for the description of the two forms, see the introduction).

Lipid insertion of C9, induced by Zn^{2+} rather than C5b-8, was investigated (Figure 4). Densitometric scans of the gel and of its autoradiogram indicate that 58% of C9 is in its tubular poly(C9) form, having 56% or the TID attached. Nontubular C9 amounts to 42% with 44% of the total amount of TID bound. This result indicates that both forms of poly(C9) interact with the probe to the same degree. Figure 5 shows the kinetic of the insertion of polymerizing C9 into membranes. [^{125}I]TID-containing vesicles were added to

Table II: Comparison of Subunit Labeling of the MAC by Different Membrane-Restricted Photoactivatable Probes

probe	reactive intermediate	target membrane	percent of label in C5b	C6	C7	C8α	C8γ	C8β	C9[a]
[12-(4-azido-2-nitrophenoxy)stearoyl]-[1-^{14}C]glucosamine[b]	nitrene	sonicated vesicles: egg lecithin, cholesterol, dicetylphosphate, tocopherol (4:3:1:0.5)	9	1		3		87	87
[^{3}H]-L-α-1-(palmitoyl/stearyl)-2-(18-azidolinoleoyl)phosphatidylcholine[c]	nitrene	vesicles formed by detergent dialysis: dialeoyllecithin	10	5	4	11		5	65
[^{125}I]hexanoyldiiodo-*N*-(4-azido-2-nitrophenyl)tyramine[d]	nitrene	rabbit erythrocyte membranes	3	2	3	10		1	81
[^{125}I]TID	carbene	rabbit erythrocyte membranes		1	3	17	4	10	65
		sonicated vesicles: egg lecithin, cholesterol (9:1)	10	5		6		79	

[a] Not normalized for 1 molecule of C9. [b] Hu et al., 1981. [c] Podack et al., 1981. [d] Steckel et al., 1983.

monomeric C9, and polymerization of C9 was induced by the addition of 10^{-5} M Zn^{2+}. After different periods of time, C9–vesicle complexes were freed from unbound C9 by floatation, and the degree of lipid insertion was determined by [^{125}I]TID labeling. Immediately after the addition of C9 to the vesicles, only a faint labeling of C9 migrating as monomers C9 was deteceted. After 1 h, C9 lipid insertion became apparent, as evidenced by the increase of TID labeling. In parallel, tubular poly(C9) was detected. After 2 h, no further increase of labeling was observed. Moreover, the hemolytic activity of C9 had completely disappeared (data not shown), indicating that all C9 was polymerized after this period of time (Podack & Tschopp, 1983).

Photolabeling of Hydrohobic Domains of C9. C9 is cleaved by thrombin into two fragments, i.e., C9a (34 kDa) and C9b (37 kDa). Ishida et al. (1982) showed in a careful study that only C9b is in contact with the lipid bilayer. C9 can be cleaved by trypsin (Tschopp et al., 1985b) into two fragments with apparent molecular masses of 53 and 20 kDa. The cleavage occurs within C9b. Thrombin- and trypsin-nicked C9 were incubated with lipid vesicles and labeled with [^{125}I]TID. Subsequent SDS–PAGE analysis of thrombin-cleaved C9 showed that only C9b was associated with the radioactive probe whereas C9a was not (Figure 6, right panel). This observation was corroborated with a different membrane-restricted probe ([^{3}H]PTPC; Brunner et al., 1983). In the case of the trypsin fragments, both polypeptide chains became labeled (Figure 6, left panel). The same results were obtained by inducing lipid insertion of proteolyzed C9 by means of vesicle-bound C5b-8 (data not shown).

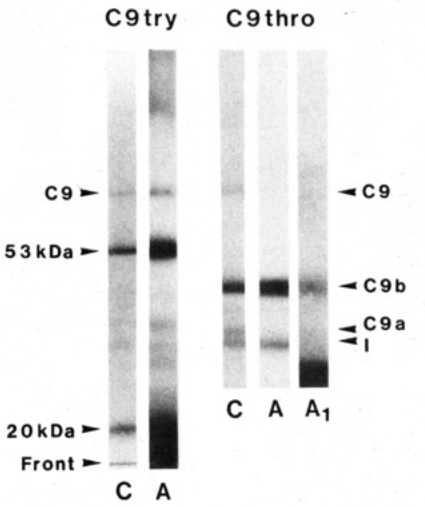

FIGURE 6: Analysis of proteolytic fragments of C9 interacting with membrane-restricted probes. C9 fragments were generated by trypsin ($C9_{try}$: 53- and 20-kDa fragments) and by thrombin ($C9_{thro}$: C9b and C9a) and reacted with vesicles containing a membrane-restricted probe. (C) Coomassie blue stained SDS–polyacrylamide gels run under nonreducing conditions; (A) autoradiogram. Vesicles contained [^{125}I]TID (A) or the photoactivatable phospholipid [^{3}H]PTPC [see Brunner et al. (1983)] as membrane-restricted probe (A1). (I) See Figure 4.

Discussion

Considering the pronounced differences in the photochemical properties of the various reagents used to label MAC constituents, there is surprisingly good agreement in the overall labeling of MAC proteins (Table II). In all studies, labeling of C6 and C7 is marginal (5% of the label incorporated in protein), and the role of C9 in the formation of transmembrane channels is clearly reflected in the strong labeling of this protein, accounting for as much as 65–87% of the label bound to protein. However, due to the multiplicity of C9 bound per C5b-8, results of C9 labeling must be treated with caution. Assuming that between three and six C9 per C5b-8 complex migrate as monomeric and dimeric C9 in SDS–PAGE, the extent of labeling of an individual C9 is not much greater than that of C8. The different labeling efficiencies of C9 dimer (disulfide linked) and C9 monomer (although both derived from nontubular C9) in our study suggest a different accessibility to the reagent for these two forms. One difference between the study of Steckel et al. (1983) and our results exists: in contrast to our experiments, Steckel et al. found no labeling of C8β.

It is evident that the source of membranes used for the insertion of the MAC may play a crucial role in the study of lipid–protein interaction by lipophilic probes. One difference between MAC labeled in artificial vesicle membranes and MAC labeled in red blood cell membranes is the degree to which C5b is labeled. With vesicles, C5b (the β subunit) is clearly labeled by the radioactive probe, whereas labeling does not occur at all with erythrocyte membranes. Either of the following explanations could account for the results obtained. First, [^{125}I]TID diffuses from the membrane more easily in the artificial membrane system; consequently, slightly hydrophobic domains of proteins that are not in contact with the lipid bilayer could be labeled. It is also possible that the smooth surface of vesicles allows lipid interaction of hydrophobic domains of C5b. C5b is firmly attached to the vesicles, since it remains bound after floatation in the C5b-6 complex. In contrast, C5b-6 does not interact with red blood cells (data not shown).

It is now generally accepted that the MAC or poly(C9) is not a homogeneous multiprotein complex but rather one containing different amounts of C9 protomers [1 (2)–18] (Tschopp, 1984a). In the case of the MAC, the ratio of C9 to C5b-8 determines the extent of C9 polymerization. Polymerization is self-limiting due to ring closure. Tubular poly(C9) exhibits two features not shared with nontubular C9: (a) the inside of the tubule is completely separated from the surrounding lipid bilayer, whereas nontubular poly(C9) exposes

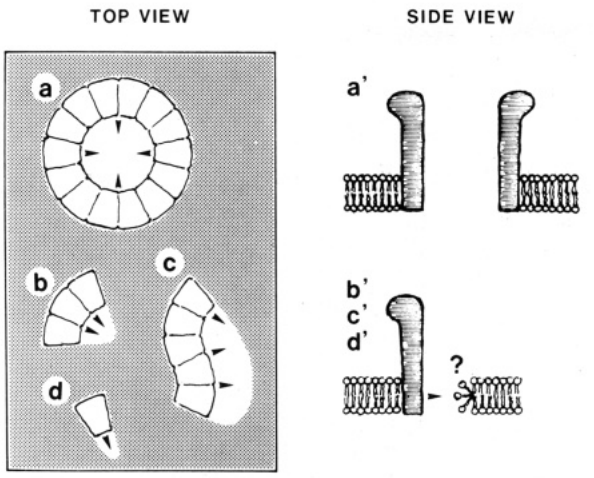

FIGURE 7: Hypothetical model of transmembrane channels formed by poly(C9). (a) The inside of tubular poly(C9) is devoid of any lipid due to the hydrophilic domains of C9 facing the inside of the protein channel, thus allowing passage of macromolecules of up to 10 nm. (b) Nontubular poly(C9) is attached by the same lipid binding domains, as shown in (a). The hydrophilic sites aligned on one side are now facing the hydrocarbon core of the lipid bilayer. Lipids are repelled (arrows), and a mixed protein–lipid channel is formed. The structure of the lipid bilayer facing the hydrophilic areas of C9 remains to be determined. It is feasible that these mixed protein–lipid channels could form transmembrane channels even with one C9 (d) or with noncircular aggregates (b and c).

the hydrophilic interior of the semicircular polymer to the hydrophobic lipid bilayer; (b) because of the tubule closure, tubular poly(C9) exhibits a much higher stability than nontubular poly(C9). On the basis of this differential stability, poly(C9) can be separated into the two forms by SDS–PAGE. Our study shows that both forms of poly(C9) are labeled by the membrane-restricted probes with no difference in intensity, indicating that (a) tubular and nontubular poly(C9) are inserted into the membrane and (b) only one side of nontubular poly(C9) exhibits lipid contact, because nontubular poly(C9) being embedded on both sides in the hydrocarbon core of the lipid bilayer would exhibit approximately twice as much [^{125}I]TID labeling as tubular C9. This suggests that nontubular poly(C9) may form transmembrane channels that are delimited by a combination of protein and lipids (Figure 7), thus confirming ultrastructural data (Tschopp, 1984).

It is astonishing that membrane leakage may be created by polymers with hydrophobic domains aligned on one side interacting with the hydrocarbon core of the membrane and hydrophilic surfaces aligned on the other side repelling the lipid molecules. This mechanism requires that the lipid–protein interaction is extremely strong in order to avoid the ejection of the proteins due to the unfavorable exposure of hydrophilic domains to the lipids. Different channel sizes could be generated depending on the degree of polymerization; indeed, functional data show that the functional pore size of the MAC is linked to the C8/C9 ratio (Mayer, 1982). Other proteins known to form transmembrane channels may use the same mechanism; for instance, streptolysin O, a bacterial cytolysin, forms nonclosed circular polymers with a diameter of 30 nm (Bhakdi et al., 1984). T effector cells release perforins/cytolysins known to induce leakage in the target membrane via pore formation (Podack, 1985).

The hydrophobic domain of C9 interacting with the lipid membrane is located in the C9b fragment, i.e., the segment generated by thrombin that includes the C-terminus (Ishida et al., 1982). By using [^{125}I]TID, we could confirm these data, since labeling of C9a by the membrane-restricted probe was nonexistent. However, the two fragments generated by trypsin were both labeled, indicating that at least two segments are in contact with lipids. Indeed, the amino acid sequence of C9 reveals the complete absence of a long continuous hydrophobic sequence (Stanley et al., 1985; DiScipio et al., 1984). It is, therefore, likely that the domain of C9 interacting with the lipid bilayer is contained within an element of its secondary structure. Future studies must determine which amino acids of C9 contribute to this lipid binding site.

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Registry No. C5b, 80295-55-2; C6, 80295-56-3; C7, 80295-57-4; C8, 80295-58-5; C5b-9, 82986-89-8; [^{125}I]TID, 79684-41-6.

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Comparison of Physicochemical Properties of Purified Mucus Glycoproteins Isolated from Respiratory Secretions of Cystic Fibrosis and Asthmatic Patients†

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ABSTRACT: The major nonreduced mucus glycoproteins (mucins) from sputa of cystic fibrosis (CF) and asthmatic patients have been purified to electrophoretic homogeneity and subjected to physical and chemical characterization. The sputum specimens were solubilized in buffer containing 0.22 M KSCN and fractionated on Bio-Gel A-5m, followed by digestion with DNase, rechromatography on the same column, and chromatography on hydroxylapatite. Sodium dodecyl sulfate gel electrophoresis of purified mucins gave a single band. Carbohydrate analyses of the purified mucins showed no significant differences in the sugar components from the two mucins. However, the CF mucin contained substantially higher (11%) sulfate content than that observed for the asthmatic mucin (5.9%). Amino acid analyses indicated that the CF mucin had higher levels of serine plus threonine (35%) as compared to the asthmatic mucin (29%). In contrast, CF mucin contained a lower content of aspartate, glutamate, and glycine than that observed for the asthmatic mucin. Molecular weights of 3.8×10^6 and 3.5×10^6 were obtained for CF and asthmatic mucins, respectively, from light-scattering studies of mucins in the presence of 6 M guanidine hydrochloride. Reduction of the disulfide bonds of the two mucins did not alter their molecular weights. Liquid chromatographic studies on Sepharose CL2B showed that CF mucin forms aggregates sufficiently large to be excluded from the gel. As compared to the CF mucin, the asthmatic mucin formed fewer of these large aggregates under identical experimental conditions. Reduction and alkylation of the mucins resulted in their inability to form aggregates. The higher state of aggregation of CF mucin may influence the viscoelastic properties of the CF lung's mucus secretions.

Respiratory mucus secretions play an important role in the normal functioning of the lung airways. The thin mucus layer of the tracheobronchial tree of a normal individual protects the bronchial cells against airborn microorganisms and other foreign particles. This protection is accomplished through the continuous cephalad flow of mucus under the propelling action of ciliated epithelium, which helps in clearing the trapped particles and the microorganisms in the mucus lining the airways. Cystic fibrosis (CF) patients produce excessive amounts of viscous mucus secretions as compared to healthy controls (Lorin et al., 1972). The increased tenacity and viscosity of respiratory mucus in CF patients make it difficult for them to clear the secretions from the airways, thus causing pulmonary insufficiency, lung infection, and death (Gurwitz et al., 1979). The gelation and viscoelastic properties of the secretions are determined, to a large extent, by the presence of mucus glycoproteins (mucins) in the secretions (Yeager, 1971; Litt et al., 1974). Any changes in the chemical and/or physical properties of the mucins may alter the viscoelastic properties of the mucus secretions which, in turn, may influence the clearance of mucus secretions by ciliated epithelium (Chen & Dulfano, 1978; Gelman & Meyer, 1979; Giordano et al., 1978). Thus, isolation and characterization of the individual components from mucus are required to understand the relationship between mucin structure and the physical properties of the mucus.

While in recent years we have gained some knowledge from the isolation and characterization of mucins, it is not yet known

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