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KINETICS OF ANTIBODY-DEPENDENT ACTIVATION OF THE FIRST COMPONENT OF COMPLEMENT ON LIPID BILAYER MEMBRANES

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SUMMARY: We have determined the rate constants for the activation of the first component of complement by fluid and solid liposomes containing nitroxide spin-labeled lipids in the presence of rabbit anti-nitroxide antibodies. The rate constant for the overall activation process is approximately four times greater for fluid liposomes (dimyristoylphosphatidylcholine at 32°) than for solid liposomes (dipalmitoylphosphatidylcholine at 32°). The data suggest that the difference in activation of the first component of complement by fluid and solid membranes may be due to a difference in lateral diffusion of antibody bound to the spin-labeled lipids in these membranes.

INTRODUCTION: Several years ago it was shown that the antibody-dependent depletion of complement by liposomal membranes containing lipid haptens is dependent on the physical state of the membrane (1-4). Complement depletion was found to be more efficient with fluid than with solid membranes. On the other hand, in a subsequent study it was found that the equilibrium binding of C1q (the sub-component of C1 responsible for the binding of C1 to antibodies) is the same for fluid and solid haptenated liposomes in the presence of equal concentrations of specific antibodies (5). This result suggested that the difference in efficiency

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Abbreviations: DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine. Complement components are identified according to WHO Recommendations (1968) A bar over the number of a complement component designates an activated state not expressed by the native protein molecule.

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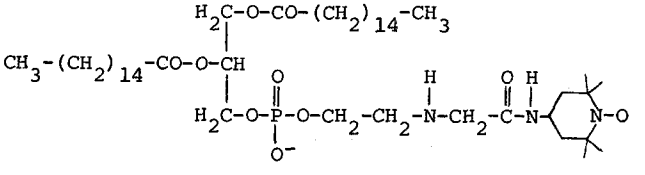
of complement depletion by fluid and solid liposomes was not due to differential binding of C1 to the liposomal membranes. Recently it was discovered that the dependence of complement depletion on the physical state of the membrane was due to a difference in the activation of C1 (6). This result coupled with the fact that C1q binds equally well to fluid and solid membranes led us to the conclusion that the difference in C1 activation was probably kinetic and did not reflect a difference in the total amount of C1 which could be activated by the two systems. The present study was undertaken to test the validity of this conclusion in order to understand further the mechanism of C1 activation by these liposomal systems. For recent reviews on the structure and function of C1 and the complement system, see references 7-9.

MATERIALS AND METHODS: Anti-nitroxide antibodies were prepared as an IgG fraction from rabbit antisera as previously described (10). The total IgG concentration was determined by absorbance at 280 nm to be 1.3×10^{-4} M, and the specific anti-nitroxide IgG concentration was determined by the binding of soluble nitroxide spin labels to be 3.4×10^{-6} M. Preparation of ^{125}I-C1 and the measurement of C1 activation by monitoring cleavage of ^{125}I-C1s have been previously described (11). The spin-labeled lipid hapten (I) was prepared by the method of Brûlet and McConnell (1). Liposomes containing 0.1% spin-label hapten were prepared essentially by the method of Schwartz and McConnell (12) as previously described (10). This procedure results in a broad size distribution of liposomes with ∿5% of the total lipid exposed to the bulk aqueous solution. For the measurement of the kinetics of C1 activation, liposomes, antibodies, and C1 reagent were mixed thoroughly at 0° in a glass test tube. The test tube was then transferred to a 32° water bath and 70 µℓ aliquots were removed and quenched with an equal volume of a solution of 8M urea, 2% sodium dodecyl sulphate, and 2% 2-mercaptoethanol at times corresponding to the data points in Figs. 1 and 2.



(I)

RESULTS AND DISCUSSION. Figure 1 gives the percent of human ^{125}I-C1 activated as a function of time in the presence of 5.7 mg/ml heat-aggregated IgG and in the absence of any activator at 32°. These

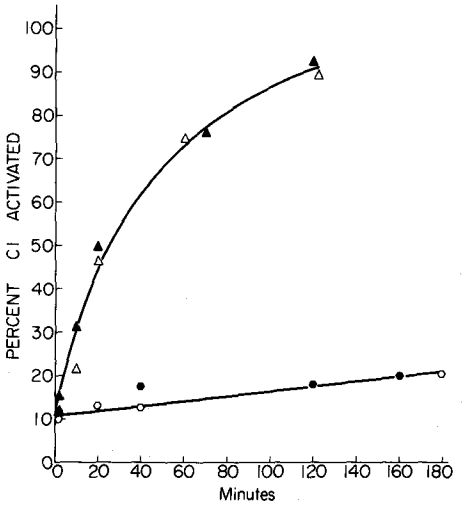


Figure 1. Activation of C1 as a function of time. Activation of $\sim 10^{-8}$ M C1 is measured in the presence of 0.57 weight percent heat-aggregated IgG (Δ) or in the absence of any activator (○). The C1 reagent and activators were mixed at 0° and transferred immediately to a 32° water bath (open symbols) or incubated for 3 hrs at 0° prior to being transferred to 32° (solid symbols).

conditions result in the fastest and slowest activation rates that we have observed. Decreasing the concentration of heat-aggregated IgG by a factor of eight resulted in no decrease in the rate of C1 activation under comparable conditions. The data points in Fig. 2 represent the activation of C1 in the presence of "fluid" liposomes (DMPC at 32°), and "solid" liposomes (DPPC at 32°). The fluid and solid liposomes contained 0.1 mol% spin-label hapten and C1 activation was measured in the presence of specific rabbit anti-nitroxide antibodies. See the legend to Figs. 1 and 2 for experimental details. The data in Fig. 2 establish that the previously reported difference in the degree of specific antibody-dependent C1 activation after a given period of time for fluid and solid liposomes (6) is due to a difference in the kinetics of C1 activation. It is, therefore, likely that the greater degree of antibody-dependent complement depletion for fluid relative to solid liposomes (1-4) is likewise caused by a greater rate of C1 activation.

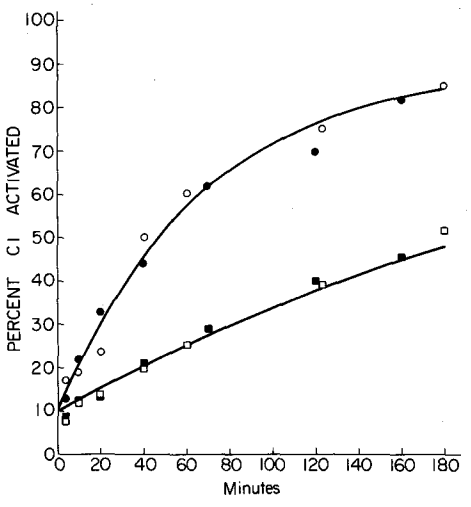


Figure 2. Liposomes containing 0.1% nitroxide spin label lipid hapten were mixed with rabbit anti-nitroxide IgG and C1 reagent at 0° to give 3.2×10^{-4} M lipid, 7.5×10^{-7} M specific anti-nitroxide IgG, and $\sim 10^{-8}$ M C1. Samples were transferred immediately or after a three-hour preincubation at 0° to a 32° water bath. Data points represent percent of C1 activated at the times indicated after transfer to 32°. Samples are DMPC with no preincubation (O), DMPC with three-hour preincubation (●), DPPC with no preincubation (□), and DPPC with three-hour preincubation (■). The solid lines are appropriately scaled first-order curves according to Eq. [1] using the rate constants 0.90 sec^{-1} for DMPC and 0.22 sec^{-1} for DPPC.

We have analyzed the kinetics of C1 activation in terms of a pseudo first-order rate process,

$$-d(C1)/dt = k(C1) \qquad [1]$$

where k is the pseudo-first-order rate constant. The loss of C1 results in the formation of activated C1, $\overline{C1}$. (Specifically, in our case ($\overline{C1}$) refers to the concentration of the (C1s) cleavage product (11).) The fit of the experimental data points to Eq. [1] is shown by the solid curves in Fig. 2. These curves were generated assuming that 10% of the C1 reagent was activated prior to mixing with liposomes and that the maximum amount of C1 which could be activated was 90%. The two first-order rate constants used are $k_{(DMPC)} = 0.90\ sec^{-1}$ and $k_{(DPPC)} = 0.22\ sec^{-1}$. Several factors evidently play a role in determining these rate constants. One

factor which must be considered is diffusion of C1 to the liposomes. Assuming that the average radius of a liposome is r_0 = 0.25 μm (as judged by optical microscopy) and that the liposome binds every C1 with which it collides, an upper limit for this reaction rate can be estimated by assuming that each liposome acts as a perfect sink for C1, that is, every C1 striking the liposome is activated. The first-order decay constant for this process can be derived from the equations for the diffusion of heat from a surrounding medium to a sphere at 0° (13).

$$k' = 4\pi r_0 DL, \qquad [2]$$

where L is the number of liposomes per cm^3. Using $r_0 = 0.25 \times 10^{-4}$ cm, $D \simeq 5 \times 10^{-8}$ cm^2/sec, and $L = 8.6 \times 10^9$ liposomes/cm^3, we obtain $k' \simeq 0.14$ sec^{-1}. The values for both D and L are only estimates. The similarity of this diffusion-limited rate constant to observed pseudo first-order rate constants indicates that this three-dimensional diffusion may in fact limit the observed rates. This view is further supported by the earlier study of C1 activation after a one-hour incubation that shows increasing levels of activation with increasing liposome concentration (cf. Eq. [2]). See Fig. 3 of reference 6.

The above calculation and experimental results lead to the conclusion that the efficiency of C1 activation per C1-liposome collision is high. Nonetheless, the specific antibody-dependent activation by haptenated DMPC liposomes is significantly higher than by haptenated DPPC liposomes. Cytofluorographic examination of DMPC and DPPC liposomes prepared using similar techniques to those employed here showed very similar low-angle light scattering intensity profiles (unpublished data). Thus in our opinion the difference in reaction rate of C1 with DMPC and DPPC liposomes cannot be attributed to size differences. We believe

that the difference in the rate of C1 activation can be understood as sketched below.

We assume the following reactions,

$$Cl + L \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ClL$$

$$ClL \xrightarrow[k_3]{} \overline{Cl}L.$$

Assuming that the concentration of bound ClL is sufficiently low that $d(ClL)/dt = 0$, then

$$\frac{d(\overline{Cl})}{dt} = k_3 k_1 (Cl)(L)/(k_2+k_3). \qquad [3]$$

If k_1 is of the order of the diffusion-limited reaction rate (Eq. [2]), but k_3 is comparable to or less than k_2, and larger for DMPC than for DPPC, then we account qualitatively for the difference in C1 activation. This difference which resides in k_3 may involve a difference in antibody diffusion rates on the liposomal surface so as to provide two or more antibodies that are presumably required for C1 activation (14), or possibly two binding sites for C1 sub-components (15). Note especially that the rate constant k_3 could be antibody diffusion limited but because of the two-dimensional nature of the diffusion it need not be linearly dependent on the bound antibody diffusion constant (16). Alternatively, other molecular motions necessary for C1 activation may be favored by DMPC relative to DPPC.

In a previous communication (6) it was concluded that lateral diffusion of bound antibodies to lipid membranes could not be rate-limiting in C1 activation since (i) comparable degrees of specific antibody-dependent C1 activation were observed for haptenated liposomes containing 15 and 25 mol% cholesterol in DPPC, and (ii) lateral diffusion coefficients of both hapten and bound antibody measured by periodic pattern

photobleaching for these cholesterol concentrations differ by at least one order of magnitude (17,18). This argument against the possible role of lateral antibody diffusion in C1 activation in these cholesterol-containing membranes is now known to be invalid, since it has been discovered that at the temperature (32°) used for the C1 activation experiments, both 15 and 25 mol% cholesterol mixtures contain a high proportion of fluid lipid, having high diffusion rates over distances relevant to antibody diffusion for C1 activation (19-21). The fluid lipid domains in the 15 mol% cholesterol samples are not easily detected in the pattern photobleaching experiments (17) since the widths of the fluid and solid domains are small compared to the period of the photobleaching pattern. These widths are, however, large on the size scale of antibody and C1 molecules (19).

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