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Communications

Micellization of Dipalmitoylphosphatidylcholine Vesicle by Apolipoprotein A-I Without the C-Terminal Segment

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The micellization of phospholipid vesicles by a certain group of proteins is an intriguing aspect of bilayer-protein interactions and there has been an increased interest on this subject in recent years[1-3]. In a previous communication, we compared the mode of interactions between human apolipoprotein A-I (apo A-I) and dipalmitoylphosphatidylcholine (DPPC) vesicles under the conditions of vesicular complex formation and micellar complex formation[4]. It was found that only the C-terminal stretch of apo A-I protein penetrates the bilayer when the lipid/protein ratio is 5000. This was the condition under which the vesicle morphology did not change upon binding with the protein. Time-dependent labeling of apo A-I protein with 3-(trifluoromethyl)-3-(m-[^{125}I] iodophenyl) diazirine ([^{125}I]TID) during the micellar complex formation followed by fragmentation into four segments indicated that the whole length of the polypeptide chain of apo A-I is involved in the interaction with the hydrophobic interior of the vesicle from the beginning. However, even when the differences in length of these fragments were taken into account, the C-terminal segment was more extensively labeled with [^{125}I]TID than any other segment (Figure 6 of reference 4). These results suggest the importance of the C-terminal region for the micelle formation. If this is the case, there is a possibility that apo A-I protein from which the C-terminal segment is removed may show appreciably reduced capacity to form micellar complex. The present investi-

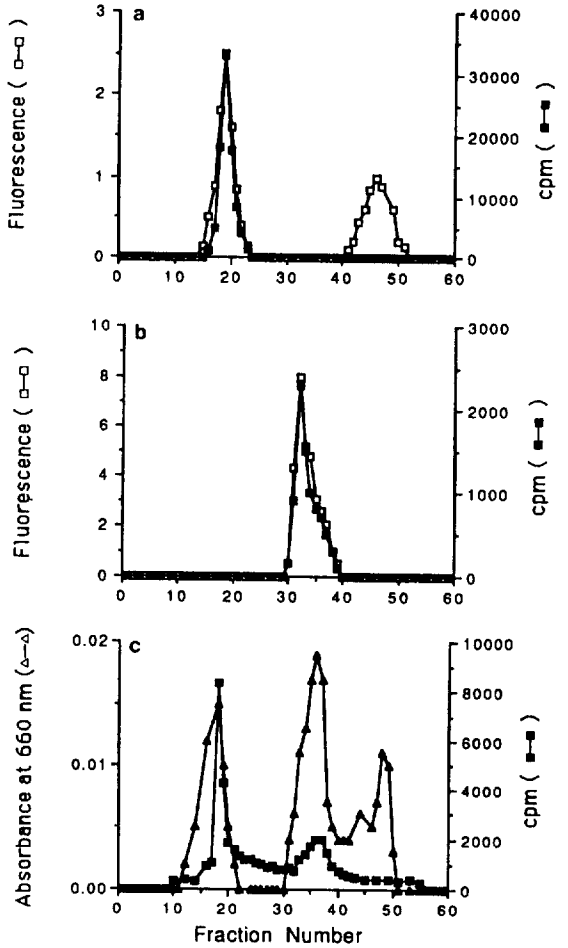

Figure 1. Elution profiles on a Sepharose CL-4B (1.8×40 cm) of DPPC/apo A-I reaction mixtures. a) DPPC/apo A-I-Dns, 5000 : 1 (mol/mol), b) DPPC/apo A-I-Dns, 100:1 (mol/mol), c) DPPC/C$^-$ apo A-I, 100 : 1 (mol/mol). Incubations were performed at 42℃ for 24 h. The fraction volumes are 1.5 m*l*. Lipid fractions were assayed by obtaining ^{3}H counts per min in 0.5 m*l* aliquots. Apo A-I-Dns fractions were monitored by means of the Dns probe fluorescence intensity. Excitation wavelength was 340 nm and emission was observed at 500 nm. C$^-$ apo A-I was analyzed by Lowry method. Apo A-I-Dns : dansylated apo A-I protein.

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gation was undertaken to address this question.

Since the segment from Leu-189 to Arg-215 labeled with [^{125}I]TID in vesicular complex[4], our interest was to cleave off the C-terminal segment including this stretch from apo A-I. For this purpose, apo A-I preparation was treated with 2 M hydroxylamine (1-5 mg/ml) in 6 M guanidine hydrochloride and the pH was adjusted to 9.6. This solution was incubated for 4 h at 45℃. Under this condition it is expected that the peptide bond between Asn-184 and Gly-185 is cleaved[5]. The reaction was terminated by introducing formic acid to bring the pH to 2-3. The C-terminal-depleted apo A-I (C^- apo A-I) was isolated using the gel elution method and its purity was confirmed by Laemmli SDS-PAGE[6]. Unilamellar ^{3}H-DPPC vesicle was prepared by the reverse phase evaporation method[7]. The size range of the vesicle was reduced to 500-700 Å in diameter with a Heat System Sonifier cell disrupter.

Figure 1 shows the elution profiles of DPPC vesicle/apo A-I protein complexes at molar ratios of 5000 and 100, respectively, and that of DPPC vesicle/C^- apo A-I protein complex at the molar ratio of 100. These profiles were obtained by incubating vesicle/protein mixture for 24 h at 42℃ and then passing through a Sepharose CL-4B column (1.4×40 cm). The lipid concentration was determined by liquid scintillation counting. The concentration of the apo A-I protein was monitored by measuring fluorescence intensity of dansylated protein[8]. The C^- apo A-I concentration was obtained by the Lowry method[9].

Figure 1c, when compared with Figure 1a and b, shows that C^- apo A-I is present in the vesicular complex as well as in the micellar complex. This may, in turn, mean that the C-terminal section of apo A-I is not indispensable for breaking down the vesicles. The reduced micellization capability of C^- apo A-I as compared to that of intact protein may be simply due to the decreased length of the polypeptide chain.

In view of an earlier obsevation that only the C-terminal segment of apo A-I protein interacts with the vesicles when the lipid/protein value is large[4], the appreciable binding of C^- apo A-I to the vesicles as shown in Figure 1c is somewhat unexpected. The only explanation we have now is that parts other than the C-terinal region, which remains attached to the vesicles when digested with trypsin, also initially bind to the vesicle. Further digestion experiments with C^- apo A-I protein is required to shed light on this problem.

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Reaction of *arachno*-$S_2B_7H_8^-$ with $(CO)_5Cr\{C(OCH_3)R\}$: Synthesis and Characterization of *arachno*-4-RCH_2-6,8-$S_2B_7H_8$ (R=CH_3, IIa; C_6H_5, IIb).

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Arachno-$S_2B_7H_8^-$ has been shown to react with a variety of polarizable organic compounds[1] such as nitriles and ketones to generate the corresponding *hypho*-$CH_3CNS_2B_7H_8^-$[2] and *hypho*-$S_2B_6H_9$,[-3] respectively.

$$S_2B_7H_8^- + CH_3CN \rightarrow \textit{hypho}\text{-}CH_3CNS_2B_7H_8^- \quad (1)$$

$$S_2B_7H_8^- + (CH_3)_2CO \rightarrow \textit{hypho}\text{-}S_2B_6H_9^- \quad (2)$$

The result of the reactions above suggests that the *arachno*-$S_2B_7H_8^-$ anion might also readily attack other polarized multiple bonds. We have found that *arachno*-$S_2B_7H_8^-$ anion readily reacts with Fisher-type carbene complexes[4] at room temperature. In contrast to the reactions with nitriles and ketones, cage addition results in the production of new alkyl substituted thiaboranes, *arachno*-4-RCH_2-6,8-$S_2B_7H_8$ (R=CH_3 **IIa**; C_6H_5 **IIb**), in good yield.

In a typical experiment, a solution of $Na^+S_2B_7H_8^-$ was prepared by the reaction *in vacuo* of excess NaH(~0.1 g, 4.2 mmol) with *arachno*-6,8-$S_2B_7H_9$[5] (0.45 g, 3 mmol) in tetrahydrofuran (~25 mL) at ~−20℃. To this solution 0.80 g (3.2 mmol) of $(CO)_5Cr\{C(OCH_3)CH_3\}$[6] in THF was added at −78℃ and allowed to warm slowly to room temperature and continued to stir overnight. The solution gradually turned dark green, suggesting the formation of a chromathiaborane complex. Protonation with HCl followed by extraction with hexane gave a reddish-yellow solid. Subsequent separation was performed by flash chromatography with hexane to give 0.18 g (1.01 mmol) of *arachno*-4-CH_3CH_2-6,8-$S_2B_7H_8$ **IIa**. This corresponds to a 34% yield based on consumed *arachno*-6,8-$S_2B_7H_9$.

In an analogous reaction, 0.45 g (3 mmol) of *arachno*-6,8-$S_2B_7H_9$, ~0.1 g (4.2 mmol) of NaH, and 1.0 g (3.2 mmol) of $(CO)_5Cr\{C(OCH_3)C_6H_5\}$[7] were reacted in ~30 mL of THF *in vacuo*. The reaction mixture was initially warmed to −20 ℃ whereupon the solution also gradually turned dark green.