DYNAMICS OF APOLIPOPROTEIN-PHOSPHOLIPID INTERACTIONS

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Apolipoprotein A-I (apo A-I), the major polypeptide of high density lipoprotein (HDL), interacts spontaneously with hydrated dimyristoylphosphatidylcholine (DMPC) to form putative discoidal complexes. We recently completed high field (400 MHz) ^{1}H-NMR studies of the dynamics of interaction of apo A-I with small unilamellar vesicles (SUV). Using spin echo NMR to increase peak resolution, choline proton peaks from the inner and outer portions of small unilamellar phosphatidylcholine vesicles can be completely resolved, even in the absence of shift reagent (Fig. 1 *A*). Following the addition of apo A-I to egg PC, the inner and outer asymmetry is maintained, indicating

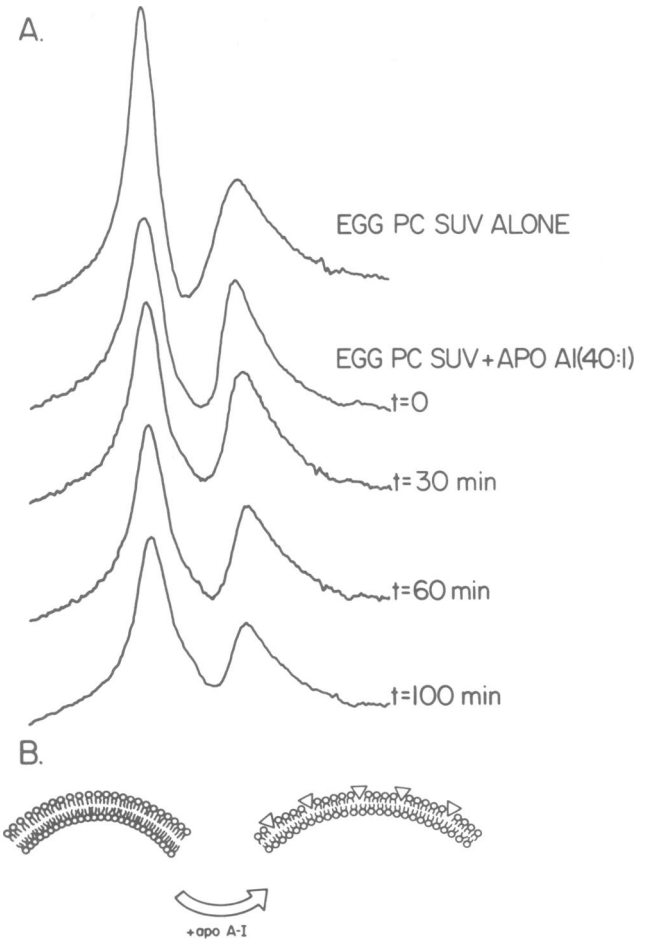

FIGURE 1 High field ^{1}H-NMR study of the interaction of apolipoprotein A-I with egg PC small unilamellar vesicles. *A*, Kinetics of changes in choline methyl resonances; the outer cholines are shifted downfield by ~0.185 ppm from the inner cholines. *B*, postulated model to explain the NMR changes seen in *A*.

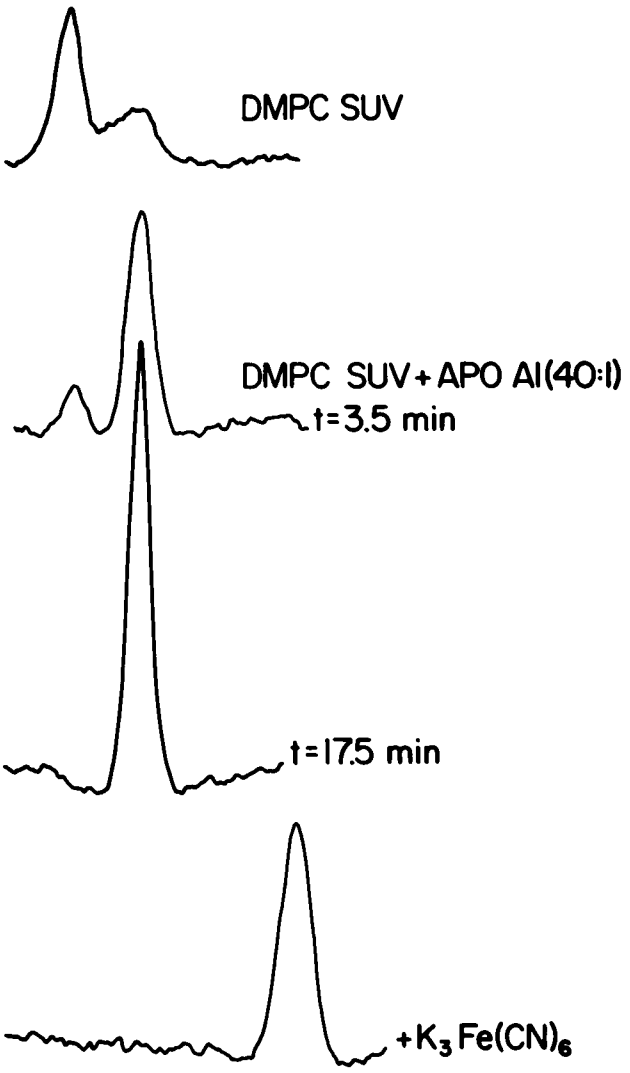

FIGURE 2 High field ^{1}H-NMR study of the kinetics of apolipoprotein A-I interaction with dimyristoyl phosphatidylcholine (DMPC)SUV.

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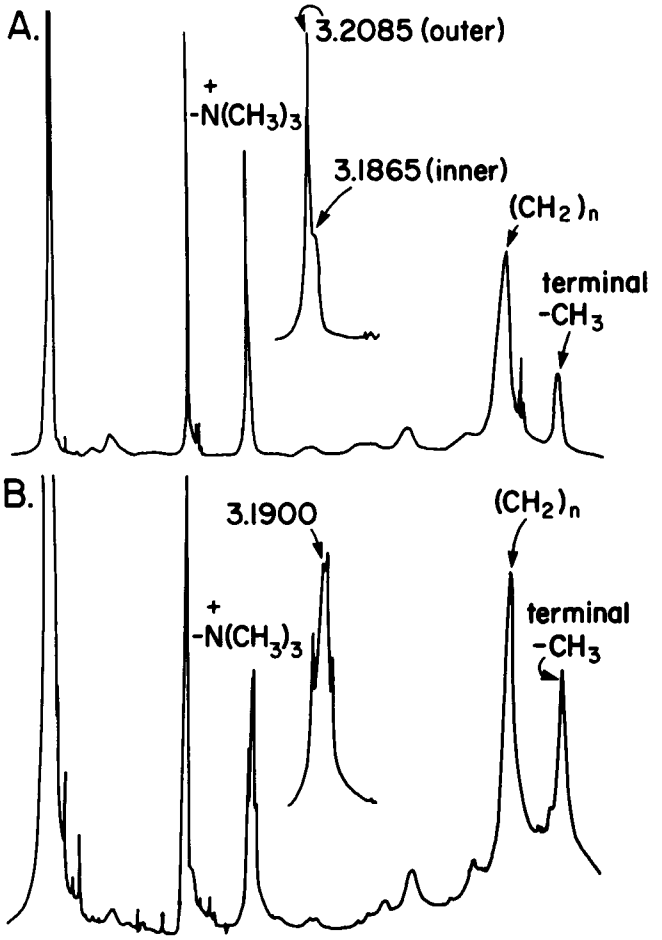

FIGURE 3 High field ^{1}H-NMR spectra after 253 scans. *A*, small unilamellar egg PC vesicles. *B*, concentrated apolipoprotein E-rich nascent HDL obtained from human alcoholic hepatitis plasma by single vertical spin ultracentrifugation (× 2).

retention of vesicular structure. However, the vesicles are now leaky (over a period of several hours) to shift reagent, and the outer peak is broadened and the inner narrowed relative to the respective peaks in liposomes alone (Fig. 1 *A*).

Our interpretation of these results is that apo A-I interacts with the outer monolayer of the egg PC vesicles via its amphipathic helical domains, as shown in Fig. 1 *B*. Insertion of amphipathic helical domains into the outer monolayer of the egg PC vesicles results in line broadening of the outer choline signal. This process also increases the total surface area of the outer monolayer. Since hydrocarbon regions of bilayers are essentially incompressible, the inner monolayer must also increase its surface area. As a

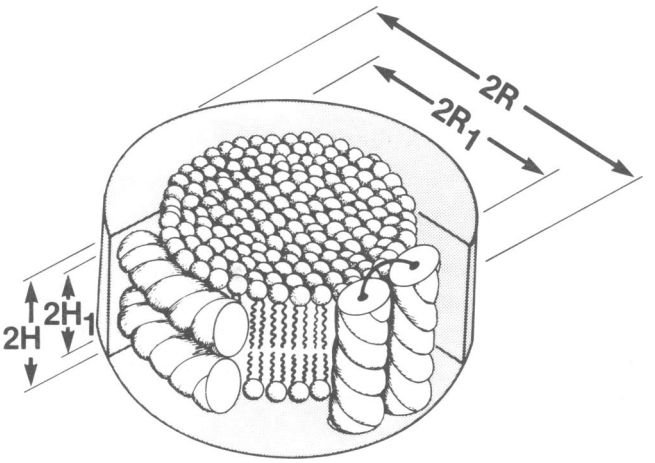

FIGURE 4 Annular protein–bilayer disk model for apo A-I:DMPC recombinants and nascent HDL.

consequence of this head group spreading, there is increased motion of the inner choline head groups, accounting for the narrowing of the inner choline signal (Fig. 1 *B*).

In contradistinction to egg PC, addition of apo A-I to DMPC-SUV results in the rapid disappearance of the outer choline peak. A single peak appears at the position of the inner, which is approximately equal to the sum of the outer and inner peaks (Fig. 2). Addition of shift reagent produces a virtually instantaneous shift of 100% of the signal, suggesting that all the DMPC cholines are directly exposed to the solvent. We consider these results strong support for a planar bilayer micelle structure for DMPC:A-I recombinants.

Fig. 3 shows the high field ^{1}H-NMR spectrum of apo E-enriched nascent HDL obtained from the plasma of a patient with alcoholic hepatitis compared to that of sonicated small unilamellar egg PC vesicles. The chemical shift of the choline methyls in nascent HDL (3.1900 ppm, Fig. 3 *B*) appears to be quite close to that of the inner choline methyls of the egg PC SUV (3.1865 ppm, Fig. 3 *A*). This finding suggests that nascent HDL has a structure similar to apo A-I:DMPC complexes, i.e., a presumptive annular protein-bilayer disk structure (Fig. 4).

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