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MECHANISM OF ASSOCIATION OF HUMAN PLASMA APOLIPOPROTEINS WITH DIMYRISTOYLPHOSPHATIDYLCHOLINE

EFFECT OF LIPID CLUSTERS ON REACTION RATES

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The human serum apolipoproteins are surface-active polypeptides that solubilize the lipids circulating in plasma. Under certain conditions, the isolated apolipoproteins spontaneously reassemble with phospholipids by a mechanism that appears to involve penetration into transient defects in the lipid matrix (1, 2). We have studied the kinetics of this process with a series of apolipoproteins and a synthetic apolipopeptide. The behavior of all of these peptides is similar. When mixed with the synthetic lecithin, dimyristoylphosphatidylcholine (DMPC), several physical changes occur as a consequence of lipid-protein association. The peptide transforms from a random coil to an α-helix, the tryptophan residues transfer from a polar to a nonpolar environment, and the initially turbid liposomes become optically clear. We have followed the changes in the relative turbidity as a function of time and used the derived rate constants to estimate the rate of lipid-protein association. The sequences and isolation of the native apolipoproteins may be found in several reviews (3, 4). The synthesis and properties of LAP-20 have been described previously (5–7). Some typical rate data for the association of DMPC with apoA-I (mol wt 28,400), apoA-II (mol wt 17,400), apoC-III$_1$ (mol wt 9,300), reduced and carboxymethylated apoA-II (RCM-A-II; mol wt 8,700), and LAP-20 (mol wt 2,280), are shown in Fig. 1. The data shown were collected below, at, and above the solid → fluid phase transition temperature of DMPC, T_c = 23.9°C (8). These data are shown in Fig. 1 *A–C*. The most notable feature of these data is that there are large differences in the rates of association in these three temperature regions.

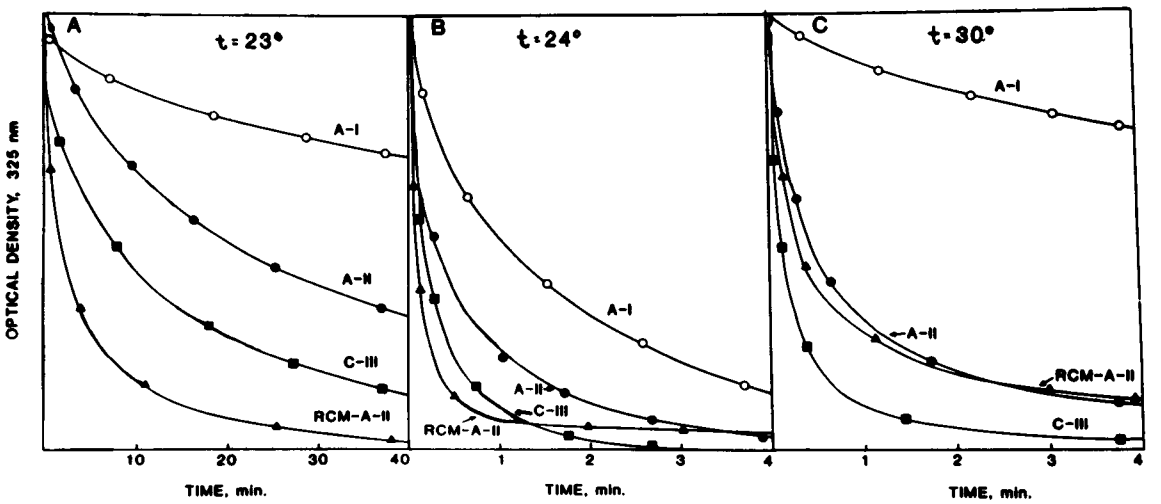

FIGURE 1 Representative traces of clarification of DMPC turbidity by apoproteins. DMPC (0.5 mg/ml) and the apoprotein (0.25 mg/ml) were preincubated for 10 min at a given temperature and equal volumes mixed at the same temperature in a 1-cm path length spectrophotometer cell. The decrease in absorbance was recorded as a continuous function of time. *A*, 23°C; *B*, 24°C; *C*, 30°C.

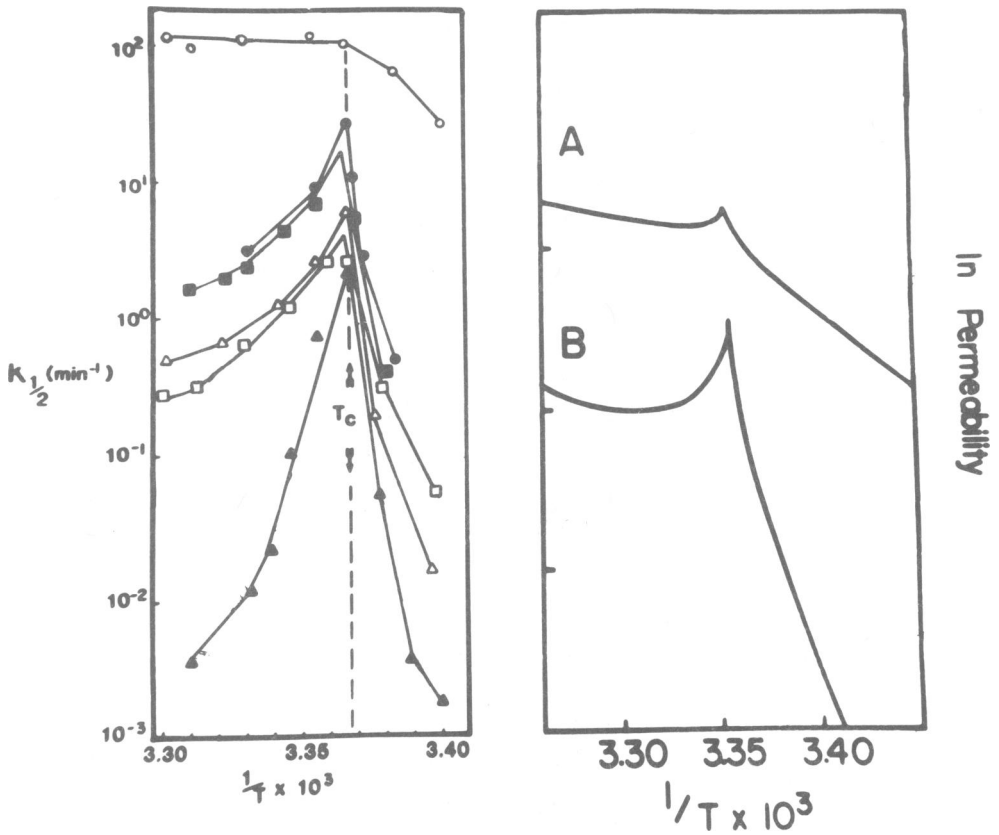

FIGURE 2 Arrhenius plots of the rate of clarification of liposomal turbidity by apoproteins and LAP-20. Data were taken from Fig. 3 and a larger body of data collected in the same way at other temperatures. (○) LAP-20; (●) RCM-A-II; (■) apoC-III; (□) apoA-II; (▲) apoA-I.

More subtle effects are better illustrated in the Arrhenius plots of Fig. 2 *A*, which show that there are large differences in the rates as a function of the molecular weight of the polypeptide. If we assume that the polypeptides associate with DMPC by penetration into preformed transient holes in the lipid matrix, then the temperature dependence of the rates should simulate that of the permeability. These effects can be understood in terms of the cluster theory of lipid phase transitions, which has been used to predict the temperature dependence of the permeability of phospholipids in the vicinity of T_c (9). In Fig. 2 *B* the temperature dependence of permeability is shown for the two extremes in the size of permeants. Our data are in good qualitative agreement with all aspects of the cluster model, and all of the salient features of the theoretical permeability curves in Fig. 2 *B* can be recognized in the measured rates of lipid-peptide association in Fig. 2 *A*. (*a*) The rate of association is always fastest at the transition temperature. (*b*) Rates are asymmetric about T_c, such that there is a stronger temperature dependence at $T<T_c$ than at $T>T_c$. (*c*) The temperature range of rapid association decreases in the order of increasing molecular weight. (*d*) The rate of association increases in order of decreasing molecular weight. These results support our previous assignment (1) of the association of apolipoproteins with preformed "holes" in the lipid matrix, and extends the utility of the cluster model to understanding lipid-protein association.

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APOLIPOPROTEIN A-II AND SERUM HIGH-DENSITY LIPOPROTEINS

A Model System for the Study of Protein-Lipid Interactions at a Natural Hydrophilic-Hydrophobic Interface

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The precise role played by apolipoproteins in the overall structural organization of plasma lipoproteins is still unknown (1). The A apolipoproteins are the principal constituents of the high density lipoprotein (HDL) class. In man and nonhuman primates HDL contains both apo A-I and apo A-II. Human apo A-II consists of two identical chains linked by a single disulfide bridge, whereas in the monkey, apo A-II is a single chain molecule due to the absence of cisteine. The only disulfide of human apo A-II is readily amenable to reduction and alkylation, thus permitting the in vitro production of human single-chain apo A-II. In lower animal species, HDL contains no apo A-II; yet this lipoprotein has structural features which closely resemble those of man.

RESULTS

Based on the above information and on the fact that the functional role of apo A-II is yet undetermined, we elected to use canine HDL as a model to study the structural and functional effects caused on this lipoprotein by the addition of graded amounts of two- or one-chain apo A-II. These studies were favored by the knowledge of the properties of apo A-I and apo A-II in solution showing a different mode of self-association for apo A-I and apo A-II and between two-chain or one-chain apo A-II, whether naturally occurring (rhesus monkey) or obtained from the reduction and alkylation of human apo A-II (2). We also have evidence for a greater affinity of apo A-II relative to apo A-I for the hydrophilic:hydrophobic interface. At the glass bead:water interface, it has been estimated[1] that the ΔG_{aff} for apo A-I is -9.1 kcal/mol and that of apo A-II is -10.5 kcal/mol.

The addition at room temperature of lipid-free human apo A-II (two-chain) to canine HDL results in the uptake of this apoprotein by HDL and a concomitant displacement of apo A-I into the aqueous solution (3). The reaction, which is attended by no loss of lipid, is stoichiometric in that by varying the initial free apo A-II-apo A-I (HDL) ratio, one can show that per mole of apo A-I displaced, there are 2 mol apo A-II occupying the HDL surface. Thus, one obtains hybrid particles having the lipids of canine HDL and varying proportions of human apo A-II and canine apo A-I at the surface. In the extreme case, all of the protein mass in the hybrid particle is apo A-II with a calculated maximal occupancy of 6 mol/particle (~ 220,000 mol wt). In such a system apo A-II is never found free in solution even in the presence of phospholipid vesicles, which act as scavengers of the released apo A-I.

To a lesser degree, single-chain apo A-II either from natural sources (rhesus apo A-II) or reduced and alkylated human apo A-II are also effective (4) in displacing apo A-I from the surface of canine HDL, and the hybrid products formed exhibit differences which are both quantitative and qualitative. As shown in Fig. 1, the end products of the interaction between single-chain apo A-II and canine HDL are the HDL-apo A-II hybrid and free apo A-I. In turn, the incubation of two-chain apo A-II with canine HDL results in an additional component likely representing an apo A-I:apo A-II complex containing ~ 7% phospholipids. This component is particularly evident when two-chain apo A-II is incubated with HDL in apo A-II:apo A-I molar ratios exceeding maximal apoprotein

[1]Shen, B. Unpublished results.