This work was supported by grant Q-343 from the Welch Foundation and grants HL-15648 and HL-17269 from the United States Public Health Service.

Received for publication 18 May 1981.

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THERMODYNAMICS OF LIPID-PROTEIN ASSOCIATION IN HUMAN PLASMA LIPOPROTEINS

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The plasma lipoproteins are water-soluble macromolecules which are the primary transport vehicles for lipids in blood (1). We have been working on a thermodynamic model of lipid-protein association that would correctly predict the free energy of this process. That the major polar components are water soluble and freely exchange between lipoproteins can be taken as tacit evidence for their equilibrium distribution between lipoproteins (1–3).

In aqueous solutions, the unitary free energy of association, ΔG_a, of an amphiphile with a lipoprotein may be

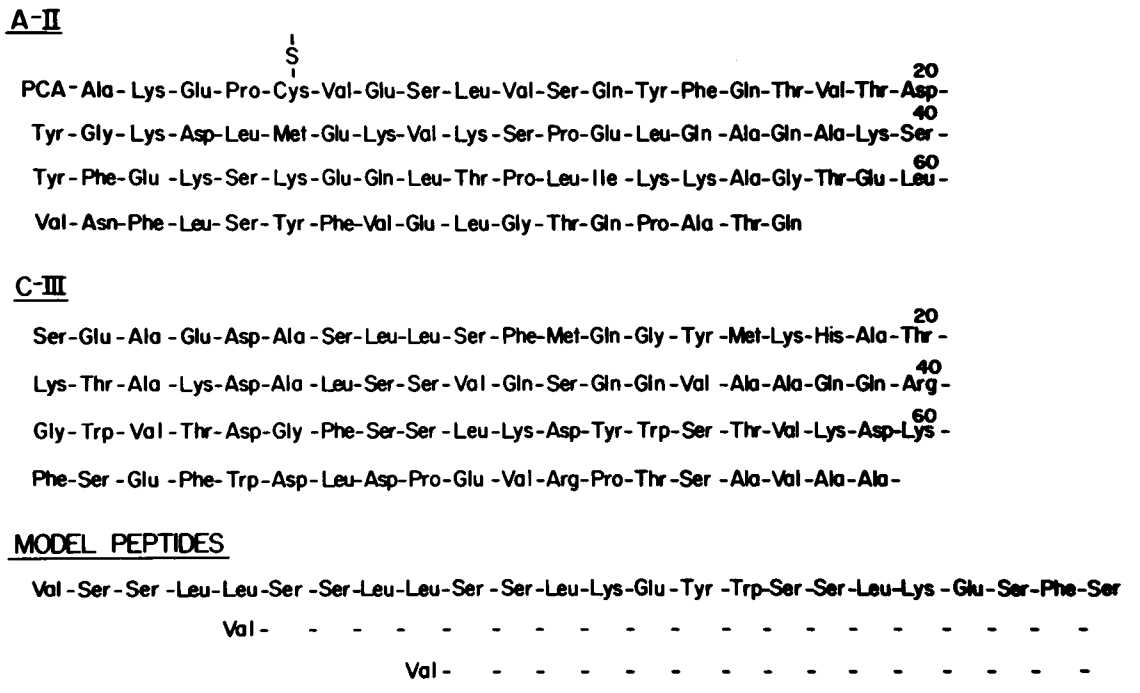

FIGURE 1 Primary structure of the polypeptides of this investigation. Reduced apoA-II monomer is carboxymethylated at Cys-6. The model peptides are, from top to bottom, designated in the text as LAP-24, LAP-20, and LAP-16.

TABLE I
FREE ENERGY OF ASSOCIATION, ΔG_a, OF LIPID-ASSOCIATING PEPTIDES WITH DMPC

Peptide	Measured	ΔG_a	
		B&B calculated*	L calculated‡§
		kcal	
ApoC-III	−10	−61	+12.1 (−53)
+0.3M Gdm·Cl	−9.3		
RCM-A-II‖	−8.0, −7.2	−66.6	−1.6 (−53.8)
+0.3M Gdm·Cl	−6.2		
LAP-16§	−6.5	−16.7	−1.4 (−14.2)
LAP-20	−8.9	−22.4	−4.4 (−17.8)
LAP-24	−9.5	−28.1	−7.4 (−21.4)

*Based upon Bull-Breese parameters (5) and Eq. 4.
‡Based upon Levitt parameters (6) and Eq. 4.
§Values in parentheses neglecting contribution of hydrophilic side chains.
‖First value is for association with single bilayer vesicles and the second was obtained with multilayers.
¶Measured in 1.0 M NaCl.

calculated from

$$\Delta G = -RT \ln K \tag{1}$$

$$K = X_L/X_W \tag{2}$$

where X_L and X_W are the respective mole fractions of the amphiphile in the lipoprotein and aqueous phases.

Using equilibrium methods we have measured ΔG_a for the polypeptides given in Fig. 1. RCM-A-II is the monomeric species produced by reduction and carboxymethylation of human apoA-II; apoC-III is a major apolipoprotein of the high and very low density lipoproteins, and the LAP series is a group of model apolipopeptides whose design was based, in part, on the theory proposed by Segrest et al. (4). The free energies of association of these peptides with dimyristoyl phosphatidylcholine (DMPC) are given in Table I. In addition we have tabulated the calculated ΔG_a based upon the sum of the free energies of transfer (δG_i) (hydrophobicities) of the individual amino acids according to

$$\Delta G_a = \sum_i \delta G_i. \tag{3}$$

The values of δG_i were taken from the studies of Bull and Breese (5) and those of Levitt (6).

The effect of Gdm·Cl on the ΔG_a suggests that the association is hydrophobic. The trend in the ΔG_a of the homologous series of peptides composed of LAP-16, LAP-20, and LAP-24 is in the direction expected for hydrophobic bonding, since LAP-20 and LAP-24, respectively, have two and four more leucine residues than LAP-16. One of the main differences between the Levitt and the Bull-Breese free energy scales is that the former gives positive values for the polar amino acid residues and in this way reflects the unfavorable transfer of those residues from an aqueous to a hydrocarbon phase. However, according to Segrest et al. (4), the polar amino acid residues of apoproteins in a lipoprotein remain at the surface where they are in contact with water. Thus, according to this model, we should not include the δG_a of the polar amino acid residues in the calculation of ΔG_a. Using the Levitt scale and neglecting the contribution of polar residues, we calculate the ΔG_a given in the last column of Table I. Although consistently smaller by 15–20%, this gives, as expected, values similar to those based upon the Bull and Breese parameters. These values are still much more exergonic than those observed.

The polarity change at the lipid-water interface is gradual, as is best illustrated in the electron spin resonance studies of Griffith et al. (7). With this view, the thermodynamic role of both polar and nonpolar amino acid residues becomes more apparent. In a real or reassembled lipoprotein the apoprotein is in an α-helical conformation with its polar residues facing the aqueous phase and the nonpolar residues penetrating part way into the hydrocarbon region of the lipid. The charged residues have an unfavorable ($\Delta G_a > 0$) free energy of transfer from water to the hydrophobic region and remain in contact with water. This term is responsible for holding the protein at or near the surface of the lipoprotein. The polar residues have a relatively high affinity for water whereas the hydrophobic amino acids partition into any less polar phase. These amino acid residues are typically distributed throughout an apolipoprotein such that when placed in an α-helix, a polar and a hydrophobic face are formed. The collective effect of the preferential association of the polar residues with water and nonpolar residues with environments less polar than water is that a driving force for helix formation is provided if there is an interface with which the apoprotein can associate. Because the hydrophobic residues are located near the surface where the environment is not much less polar than water, the ΔG_a is not fully expressed and peptide transfer from water to the phospholipid surface is less exergonic than predicted.

Received for publication 20 April 1981 and in revised form 12 June 1981.

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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.

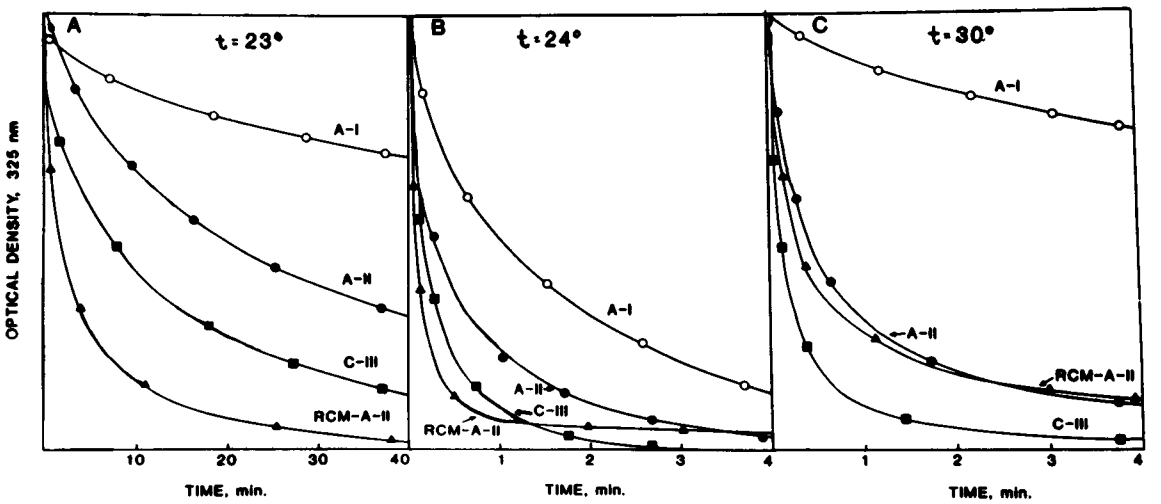

FIGURE 1 Representative traces of clarification of DMPC turbidity by apoproteins. DMPC (0.5 mg/ml) and the apoprotein (0.25 mg/ml) were preincubated for 10 min at a given temperature and equal volumes mixed at the same temperature in a 1-cm path length spectrophotometer cell. The decrease in absorbance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