Interfacial Exclusion Pressure Determines the Ability of Apolipoprotein A-IV Truncation Mutants to Activate Cholesterol Ester Transfer Protein*

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We used a panel of recombinant human apolipoprotein (apo) A-IV truncation mutants, in which pairs of 22-mer α-helices were sequentially deleted along the primary sequence, to examine the impact of protein structure and interfacial activity on the ability of apoA-IV to activate cholesterol ester transfer protein. Circular dichroism and fluorescence spectroscopy revealed that the second structure, conformation, and molecular stability of recombinant human apoA-IV were identical to the native protein. However, deletion of any of the α-helical domains in apoA-IV disrupted its tertiary structure and impaired its molecular stability. Surprisingly, determination of the water/phospholipid interfacial exclusion pressure of the apoA-IV truncation mutants revealed that, for most, deletion of amphipathic α-helical domains increased their affinity for phospholipid monolayers. All of the truncation mutants activated the transfer of fluorescent-labeled cholesterol esters between high and low density lipoproteins at a rate higher than native apoA-IV. There was a strong positive correlation (r = 0.790, p = 0.002) between the rate constant for cholesterol ester transfer and interfacial exclusion pressure. We conclude that molecular interfacial exclusion pressure, rather than specific helical domains, determines the degree to which apoA-IV, and likely other apolipoproteins, facilitate cholesterol ester transfer protein-mediated lipid exchange.

Apolipoprotein A-IV is a 46-kDa plasma glycoprotein (1) that is synthesized by the intestinal enterocytes of mammalian species (2) during lipid absorption (3). ApoA-IV enters circulation primarily as a lipid-free protein (4, 5), but rapidly dissociates from their surface (5) and thereafter circulates primarily as a lipid-free protein (6). A broad spectrum of physiologic functions has been proposed for apoA-IV in human lipid metabolism (7, 8), including specific roles in intestinal lipid absorption (8), intravascular lipoprotein metabolism (9–12), cellular cholesterol efflux (13, 14) by interaction with the ABCA1 transporter (15, 16), and regulation of the activity of two key proteins involved in the process of reverse cholesterol transport: lecithin-cholesterol acyltransferase (17, 18) and cholesterol ester transfer protein (19, 20).

Cholesterol ester transfer protein (CETP) is a 74,000-dalton plasma protein that facilitates the exchange of non-polar lipids among circulating lipoproteins (21). CETP-mediated transfer of cholesterol esters from HDL to very low density lipoprotein is central to the process of reverse cholesterol transport (22). It is well established that lipoprotein lipid composition modulates CETP activity (23), but the role of apolipoproteins is less clear. In studies that used lipid emulsions as model lipoproteins, apolipoproteins A-I, A-II, A-IV, and E stimulated CETP-catalyzed lipid exchange equally well (20, 24, 25). However, studies that assayed CETP activity using native lipoproteins found that apoA-IV may have distinct concentration-dependent effects on CETP-catalyzed lipid exchange between HDL and LDL (26) and among HDL subfractions (19, 27).

Although like all apolipoproteins, apoA-IV has a high content of amphipathic α-helical structure (28, 29), the amphipathic helices in apoA-IV are very hydrophilic (29), are predominantly of the Y-class (30) and are incapable of deeply penetrating lipid monolayers (31, 32). With increasing surface pressure, these helices are sequentially excluded from the interface (33, 34). Consequently, the interaction of apoA-IV with lipoproteins is very labile and is sensitive to interfacial pressure (35). We have proposed that these properties enable apoA-IV to act as a barostat which maintains lipoprotein surface pressure within a critical range required for optimal activity of lipolytic enzymes and transfer proteins (35, 36, 37). In this regard, apoA-IV possesses dynamic interfacial properties that are optimal for stabilizing surface tension and lipid packing at expanding lipid/aqueous interfaces (34).

Using a panel of recombinant human apoA-IV truncation mutants, Emmanuel et al. (38) found that a specific α-helical domain located between residues 117 and 160 determines the catalytic efficiency of apoA-IV in the lecithin:cholesterol acyltransferase reaction. Given the impact of distinctive α-helical domains in determining the interfacial properties of apoA-IV, and given the importance of interfacial phenomena in the reg-
ulation of the CETP reaction (36), we have used this same
panel of truncation mutants to examine the impact of α-helical
structure, protein conformation, and interfacial activity on the
ability of apoA-IV to modulate CETP-catalyzed cholesterol es-
ter transfer between HDL and LDL.

EXPERIMENTAL PROCEDURES
Lipids—Egg phosphatidylcholine and cholesterol oleate (Sigma)
dissolved in high performance liquid chromatography grade chlo-
roform (Aldrich, Milwaukee, WI) and stored under nitrogen at
20 °C. Phospholipid concentration was determined by phosphorous analysis (39). The self-quenching fluorescent cholesterol ester analog NBDBE
was obtained from Molecular Probes (Eugene, OR). Lipids were >99%
pure by TLC on silica gel.

Lipoproteins, Apolipoproteins, and Cholesterol Ester Trans-
fer Protein—LDL and HDL were isolated from human plasma by sequential
flocculation at 1.019–1.063 and 1.25–1.25 g/ml, respectively, and dialyzed
against 10 mM Tris, pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.025% sodium
azide. Human apoA-I was isolated from HDL (40). Human apoA-IV was
isolated from donors homozygous for the A-IV-1 and A-IV-2 alleles (41).
Recombinant human apoA-IV truncation mutants were created by site-
directed mutagenesis of a DNA construct containing the human apoA-IV
coding sequence, followed by expression in Escherichia coli (38). Recombinant human CETP was produced in a Chinese hamster
ovary cell line expressing a cloned human CETP cDNA; the specific
activity of recombinant CETP, as assayed by the transfer of 14C-choles-
teryl ester from HDL to LDL, was identical to plasma CETP (42).

Circular Dichroism Spectroscopy—Circular dichroism studies were
performed on a Jasco J-720 Spectropolarimeter. Spectra of apolipoproteins at 3 μM in 50 mM Tris, pH 7.4, 1 mM EDTA, 0.02% sodium
azide were recorded at 25 °C from 190 to 260 nm using a 1-mm thermostated
cell, 1-mm spectral bandwidth, and 2-s time constant. Buffer blanks
were digitally subtracted. Percent α-helix was calculated as previously
described (28). Thermal denaturation studies were performed by
monitoring ellipticity at 222 nm as a function of temperature from 20 to
65 °C. The enthalpy of denaturation, ΔH,D, and the thermal denaturation
midpoint, Tm, were determined from the slope of plots of ΔG versus
1/T (45).

Fluorescence Spectroscopy—Fluorescence studies were performed on
an SLM 9000C spectrofluorometer. Spectra of apolipoproteins at 3 μM in
50 mM Tris, pH 7.4, 1 mM EDTA, 0.02% sodium azide were recorded at
25 °C from 290 to 370 nm using a 1-cm cell with excitation at 280 nm,
1-s integration, and 4-nm slits on excitation and emission monochro-
mators. Spectra were excitation corrected by reference to a rhodamine
quantum counter, and corrected for scatter and Raman emission by
digital subtraction of buffer blanks. Chemical denaturation studies were
performed by addition of buffered 6 M guanidinium hydrochloride.
Quenching studies were performed by addition of buffered 6 μM tryptophan.
Stern-Volmer quenching constants, Ksv, were obtained from plots of I/Io
versus [I]; fractional tryptophan exposure was calculated as Ksv(apolipo-
protein/Ksv(N-acetyltryptophanamide) (46).

Determination of Interfacial Exclusion Pressure—The interfacial ex-
clusion pressure (Πex) of recombinant apolipoproteins at the phospha-

dotable 1

| Apolipoprotein | Modification | Classab | Deleted helices | Remaining molecule |
|----------------|--------------|---------|----------------|-------------------|
| r-AIV          | MRS6(H)1     |         | 0.27 0.22      | −0.22 0.36        |
| r-AIVAN12      | Δ1–12        |         | −0.07 0.83     | −0.25 0.36        |
| r-AIVh1–2      | Δ13–61       | G/Y     | 0.29 1.02      | −0.21 0.34        |
| r-AIVh3–4      | Δ62–116      | Y/A     | −0.35 1.48     | −0.21 0.34        |
| r-AIVh5–6      | Δ117–160     | α/γ     | −0.24 0.98     | −0.22 0.36        |
| r-AIVh7–8      | Δ161–204     | Y/γ     | −0.26 0.75     | −0.22 0.36        |
| r-AIVh9–10     | Δ195–238     | Y/Y     | −0.26 1.27     | −0.22 0.35        |
| r-AIVh11–12    | Δ249–288     | Y/Y     | −0.15 0.84     | −0.23 0.36        |
| r-AIVh13–14    | Δ289–332     | Y/Y     | −0.15 0.84     | −0.23 0.36        |

a Amphipathic α-helix class (30).
b Mean residue hydrophobicity (44).
ApoA-IV truncation mutants were much more sensitive to thermal denaturation, as evidenced by \( \Delta H_D \) values that were 19.6–51.0 kcal/mol lower than either native or the parent recombinant apoA-IV. Interestingly the \( \alpha \)-helicity of all the truncation mutants was higher, perhaps reflecting recruitment of some local secondary structure with disruption of ordered folding. The tryptophan fluorescence emission in all the deletion mutants was red-shifted compared with native and r-AIV, although iodide accessibility was lower. Moreover, none of the truncation mutants displayed the increase in quantum yield with denaturation that is a signature of native human apoA-IV (28, 46).

Comparison of the interfacial exclusion pressure of the recombinant construct to facilitate purification (38). Taken together, these findings demonstrate that deletion of any of the \( \alpha \)-helical domains in apoA-IV significantly disrupts its tertiary structure and impairs its molecular stability.

**TABLE II**

| Apolipoprotein | \( \alpha \)-Helix | \( \Delta H_D \) | \( T_m \) | \( \lambda_{\text{max}} \) | \( I_{\text{max}}/I_0 \) | \( k_{r/k_{\text{m}}\text{IR}} \) |
|---------------|------------------|-------------|--------|----------------|----------------|----------------|
| h-AIV-1       | 56\(^a\)         | 63.6        | 48.1   | 332            | 1.26           | 0.24\(^b\)   |
| r-AIV         |                 | 70.8        | 50.3   | 328            | 1.19           | 0.18          |
| r-AIV\(\Delta\)N12 | 57               | 51.2        | 48.3   | ND             | 1.00           | ND            |
| r-AIV\(\Delta\)h1-2 | 59               | 27.9        | 48.4   | 331            | 1.00           | 0.09          |
| r-AIV\(\Delta\)h3-4 | 63               | 22.8        | 53.2   | 334            | 1.00           | 0.10          |
| r-AIV\(\Delta\)h5-6 | 69               | 27.7        | 45.6   | 337            | 1.00           | 0.14          |
| r-AIV\(\Delta\)h7-8 | 63               | 25.5        | 47.0   | 336            | 1.01           | 0.09          |
| r-AIV\(\Delta\)h9-10 | 60               | 19.8        | 52.3   | 334            | 1.00           | 0.09          |
| r-AIV\(\Delta\)h11-12 | 70              | 30.7        | 47.8   | 334            | 1.00           | 0.10          |
| r-AIV\(\Delta\)h13-14 | 69              | 30.8        | 48.8   | 332            | 1.03           | 0.13          |

\(^a\) Weinberg and Spector (28).
\(^b\) Weinberg and Spector (46).
\(^c\) ND, not determined.

**FIG. 1.** Difference in the water/phosphatidylcholine interfacial exclusion pressure of recombinant apoA-IV truncation mutants compared with the parent recombinant apoA-IV, for which the exclusion pressure was 34.3 mN/m.

In solution, apoA-IV adopts a loosely folded “molten globule” conformation, in which the hydrophobic faces of its multiple amphipathic \( \alpha \)-helices face inward toward the interior of the molecule (28, 46). Our present findings demonstrate that these deleted \( \alpha \)-helices collectively and cooperatively contribute to the molecular stability of apoA-IV in solution, for deletion of even short helical segments disrupts its tertiary conformation and significantly destabilizes the molecule. This is further suggested by the observation that these same deletions disrupt the otherwise strong self-association of apoA-IV (38). In solution, apoA-IV adopts a loosely folded “molten globule” conformation, in which the hydrophobic faces of its multiple amphipathic \( \alpha \)-helices face inward toward the interior of the molecule (28, 46).
Intercellular transfer of cholesterol esters from HDL to LDL versus CETP activity in vivo. Several population screening studies have observed lower HDL levels (53, 54), higher LDL levels (55, 56), and an increased LDL/HDL ratio (54) in individuals carrying an A-IV-2 allele, as would be expected with increased CETP activity (57). However, in the only population survey to directly measure CETP activity, van Eckardt et al. (58) found lower CETP activity in apoA-IV-1/2 heterozygotes. Possibly, other apoA-IV genetic variants with altered interfacial properties (59) could influence CETP activity.

Finally, these data raise several caveats regarding the use of modified recombinant apolipoproteins in biological studies. First, they illustrate how minor modifications made to facilitate protein purification may have major structural and functional consequences. In this case, addition of a hydrophobic decapetide to the amino terminus of the r-AIV construct significantly increased its surface binding affinity and its ability to activate CETP. Second, these data illustrate the need for a full biophysical characterization of recombinant apolipoproteins used to probe the function of local domains. Again, the amino-terminal modification of r-AIV appeared to have no effect on its solution structure as assessed spectroscopically, yet it had a major impact on its interfacial activity. Likewise, spectroscopic characterization of the deletion mutants, alone, provided no indication as to how their apparent structural destabilization could increase their catalytic efficacy in the CETP reaction. Failure to fully characterize the biological properties of modified recombinant apolipoproteins could also lead to erroneously attributing a specific biologic function to local structure, when in fact, it was determined by global properties, such as interfacial activity. In this regard, however, comparison of our data and that of Emmanuel et al. (38) supports their conclusion that helixes 5 and 6 (residues 117–160) in apoA-IV play a specific catalytic role in lecithin:cholesterol acyltransferase activation.

In summary using a panel of recombinant human apoA-IV truncation mutants, we have found a strong correlation between interfacial exclusion pressure and the ability to activate CETP. We conclude that molecular interfacial exclusion pressure, rather than specific helical domains, determines the degree to which apoA-IV, and most likely other apolipoproteins, facilitate CETP-mediated lipid exchanges.

REFERENCES
1. Weinberg, R. B., and Scanna, A. M. (1983) J. Lipid Res. 24, 52–59
2. Weinberg, R. B., and Scanu, A. M. (1983) J. Lipid Res. 24, 52–59
3. Hayashi, H., Nutting, D. F., Fujimoto, K., Cardelli, J. A., Black, D., and Tso, P. (1980) J. Biol. Chem. 255, 10,012–10,017
4. Green, P. H., Glickman, R. M., Saudek, C. D., Blum, C. B., and Tall, A. R. (1979) J. Clin. Invest. 64, 233–242
5. Green, P. H., Glickman, R. M., Riley, J. W., and Quinet, E. (1980) J. Clin. Invest. 65, 911–919

FIG. 2. Rate constant for the transfer of NBD-labeled cholesterol esters from HDL to LDL versus interfacial exclusion pressure for human apoA-IV-1, human apoA-IV-2, recombinant apoA-IV, apoA-IV truncation mutants, and human apoA-I. The regression line is $y = 0.06696x - 0.02111; r = 0.793; p = 0.002$.
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