review

The amphipathic helix in the exchangeable apolipoproteins: a review of secondary structure and function

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Abstract Site-directed mutagenesis and other molecular biology-based techniques are now available for probing the amphipathic α helix structural motif in the exchangeable apolipoproteins. Here we survey the published literature on lipid-binding and functional domains in apolipoproteins A-I, A-II, A-IV, C-I, C-II, C-III, and E and compare these results with recently developed computer methods for analysis of the location and properties of amphipathic helixes. This comparison suggests that there are at least three distinct classes of amphipathic helixes (classes A, Y, and G*) in the exchangeable apolipoproteins whose distribution varies within and between the seven apolipoproteins. This comparison further suggests that lipid affinity resides largely in class A amphipathic helixes (Segrest, J. P., et al. 1990. *Proteins.* **8**: 103) and that variations in structure and/or numbers of class A domains in individual apolipoproteins allow a range of lipid affinities from high to low. The positions of the four α helixes recently shown to form a 4-helix bundle globular structure in apoE (Wilson, C., et al. 1991. *Science.* **252**: 1817) correspond closely to the four amino-terminal class G* amphipathic helixes of apoE identified by our computer analysis. It is of particular interest, therefore, that all of the exchangeable apolipoproteins except apoA-II and C-I, contain amphipathic helixes of class G*. Additional implications of amphipathic helix heterogeneity for the structure and function of the exchangeable apolipoproteins will be discussed.—**Segrest, J. P., M. K. Jones, H. De Loof, C. G. Brouillette, Y. V. Venkatachalapathi, and G. M. Anantharamaiah.** The amphipathic helix in the exchangeable apolipoproteins: a review of secondary structure and function. *J. Lipid Res.* 1992. **33**: 141–166.

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The amphipathic α helix is an often-encountered secondary structural motif in biologically active peptides and proteins. An amphipathic helix is defined as an α helix with opposing polar and nonpolar faces oriented along the long axis of the helix. Amphipathic helixes were first described as a unique structure/function motif involved in lipid interaction by Segrest et al. in 1974 (1); this initial observation was confined to the apolipoproteins.

Amphipathic α helixes have been described in plasma apolipoproteins, and in other putative lipid-associating proteins, including certain polypeptide hormones (2–4), polypeptide venoms (5, 6), polypeptide antibiotics (7, 8), certain complex transmembrane proteins (9) and the human immunodeficiency virus glycoprotein (10, 11). In addition, amphipathic helixes involved in both intra- and intermolecular protein–protein interactions have been described in a number of proteins, including globular proteins (12), calmodulin-regulated protein kinases (13), and coiled–coil-containing proteins (14, 15).

Suggestions for amphipathic structural motifs in proteins have not been limited to α helixes; both amphipathic β sheets (16) and amphipathic π helixes (17) also have been proposed for the lipid-associating domains of certain apolipoproteins. This review will deal only with amphipathic α helixes and only with those found in the exchangeable apolipoproteins. Exchangeable apolipoproteins are those capable of moving from one lipoprotein particle to another

Abbreviations: HDL, high density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; Mab, monoclonal antibody; LCAT, lecithin: cholesterol acyltransferase.

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(apolipoproteins A-I, A-II, A-IV, C-I, C-II, C-III, and E) as opposed to the non-exchangeable apolipoproteins that remain with one lipoprotein particle from biosynthesis to catabolism (apolipoproteins B-100 and B-48).

While it has been known for some time that the amphipathic α helix plays a pivotal role in the structure/function of the exchangeable apolipoproteins, until recently direct experimental access was limited. However, site-directed mutagenesis and other molecular biology-based techniques are now available for probing this structural motif in apolipoproteins. Here we survey the published literature on location and properties of amphipathic helixes in the exchangeable apolipoproteins and compare these results with recently developed computer methods for location and characterization.

THE α HELIX

We begin with a brief consideration of α helixes in globular proteins, membrane proteins, and apolipoproteins. In this section we show why it is generally felt that prediction of α helical domains from primary sequence data is intuitively more reliable for lipid-associating α helical domains than for nonlipid-associating α helical domains of globular proteins. This is a fortunate situation since experimental identification of lipid-associating amphipathic α helical domains in the exchangeable apolipoproteins is thus far indirect.

In globular proteins

The α helix is formed by repetitive H-bonds between backbone CO and NH located 4 residues apart. This helix tends to be right-handed (a result of the L amino acid residues) with a pitch of 3.6 residues per turn (varies between 3.5 and 3.7 on average) and a rise of 1.5 Å and a radial rotation of 100° between residues (**Fig. 1**).

Predictions of α helical domains of globular proteins thus far have never exceeded approximately 70% accuracy (18). This is at least partly the result of a failure to adequately consider long range interactions (tertiary structure) in the prediction schemes. Since the majority of α helixes in globular proteins are half-buried on the surface, the addition of amphipathic α helix analysis to the existing predictive schemes is viewed by many as having great potential (18). Because of the complex nature of globular protein surfaces and the presence of turns and loops, amphipathic α helix analysis generally is least accurate at the ends of helixes.

In transmembrane proteins

Richardson and Richardson (19) have suggested that membrane protein structure is under much stronger and simpler constraints than globular proteins; the membrane has "a profound effect on the permissible conformations, orientations, and topologies, as well as on the hydrophobicity of the outer surface that interacts with membrane lipids." They go on to say that "Orientations documented so far are either along the membrane, as for amphi[pathic] helixes, which lie along the surface, or else closely perpendicular to the membrane and extending all the way through it." Based upon the dominant influence that membrane lipids play on allowable membrane protein structures, Richardson and Richardson (19) suggest that membrane protein structure will be more reliably predicted than globular protein structure in the near future.

It should be noted that globular protein-based secondary structural predictions for membrane proteins do not work, since all transmembrane domains are predicted to be β strand (20). An important aid to secondary structural prediction in membrane proteins is therefore amphipathic helix analysis (21).

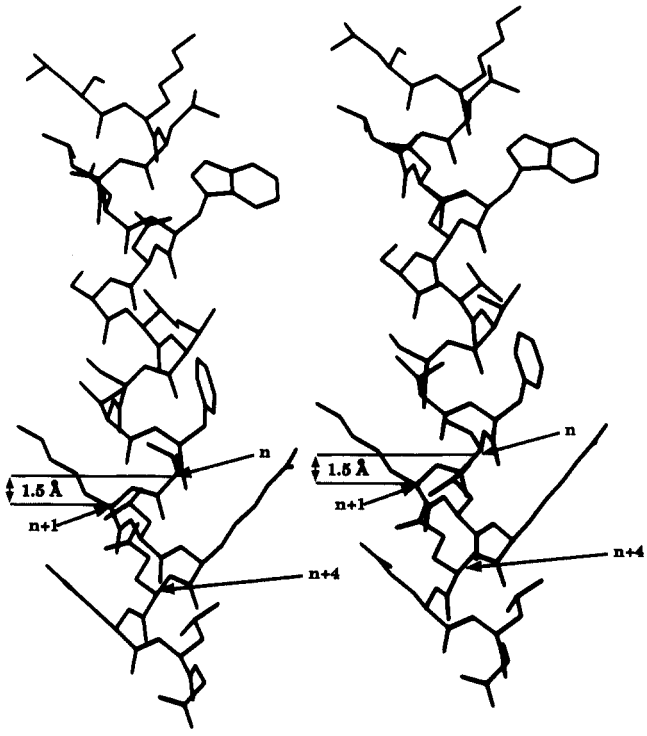


Fig. 1. Relaxed stereo projection of a model of the first 22-mer tandem repeat of apoA-I (helix 1, residues 44–65) constructed as an idealized α helix. The amino-terminal end is up. The side chains are in the fully extended conformation. The pitch (3.6 residues per turn) and the rise (1.5 Å per residue) and radial rotation (100° per residue) are indicated for residues n through n + 4 and n and n + 1, respectively. Use relaxed stereo glasses or relaxed eyes to view.

In plasma apolipoproteins

By extrapolation from the membrane protein argument, and because a lipid–water interface can leave its

signature in the form of an amphipathic helix, we suggest that algorithms for locating amphipathic helical domains in apolipoproteins can more reliably define the ends of lipid-associating amphipathic helical domains than it can define the ends of protein-associating domains.

EVIDENCE FOR AMPHIPATHIC HELIXES IN APOLIPOPROTEINS

Relationship of genomic structure to amphipathic helixes

The initial description of the amphipathic helix in apolipoproteins (1) described an α helix with the following properties: *a*) a relatively large nonpolar face which associates with the fatty acyl chains of phospholipids, half-buried at the surface of the phospholipid structure, and *b*) a polar face in which Asp, Glu, Lys and Arg are distributed so that negatively charged residues are at the center and positively charged residues are at the periphery of this face (**Fig. 2**).

The periodic pattern of an α helix with well demarcated polar and nonpolar faces is encoded into the genomic structure of the exchangeable apolipoproteins. All the human exchangeable apolipoprotein genes have been cloned and sequenced. All except apoA-IV show a remarkable similarity in having four exons and three introns (22). In addition, several of these genes are located close to each other on the

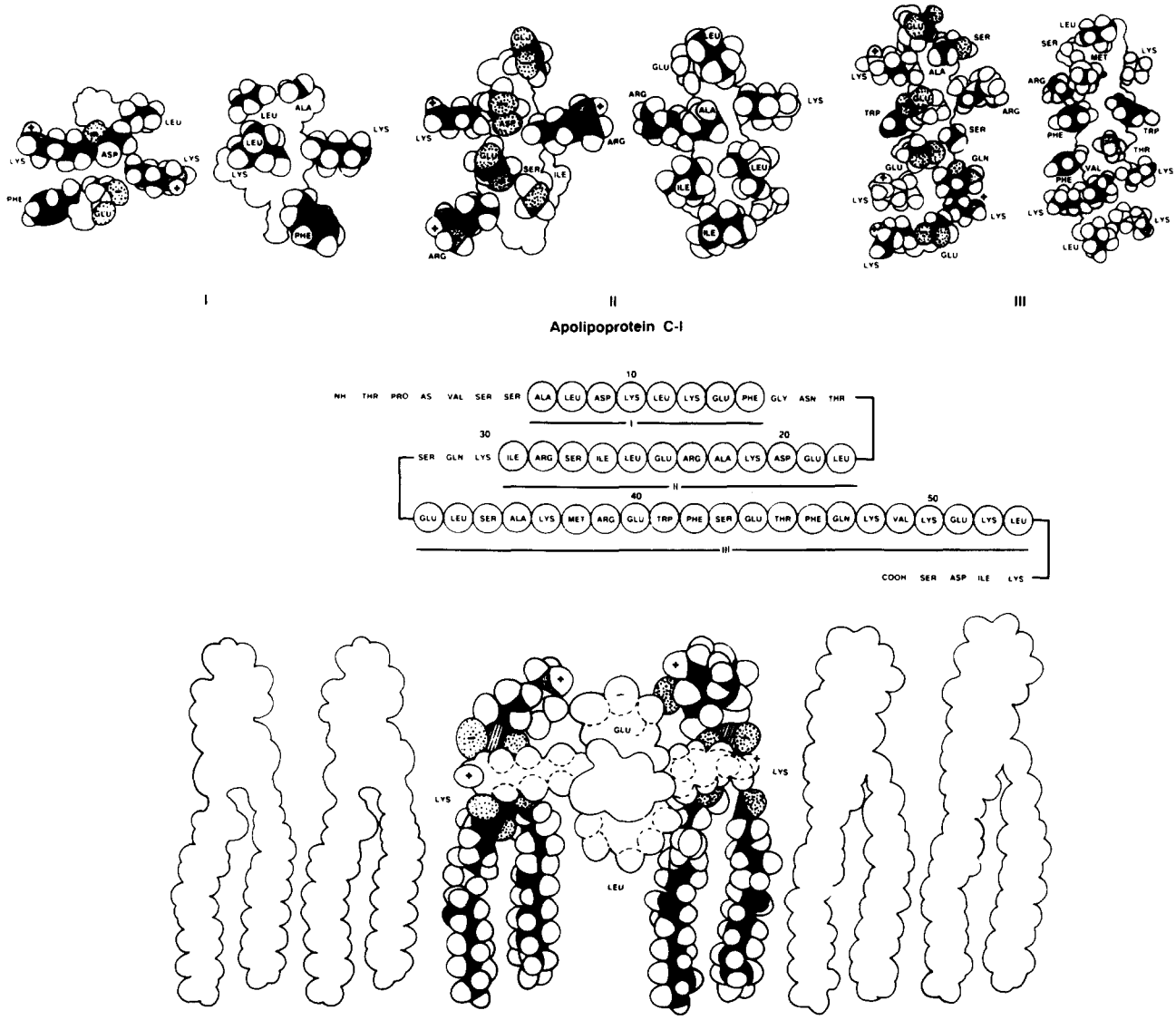


Fig. 2. Amphipathic helical domains of apoC-I as suggested in the original hypothesis (1). Top: Space-filling models of the three apoC-I domains postulated to be amphipathic α helixes adapted from Segrest et al. (1). Bottom: Suggested mechanism of interaction of the class A amphipathic helixes with phospholipid adapted from Segrest et al. (1). Note the depth of burial of the center of the α helix backbone relative to the phospholipid head groups and compare with Fig. 4.

genome (23). The most striking feature of these exchangeable apolipoproteins is the presence of internal 11-residue-long amino acid repeats (24). In apoA-I, A-IV, and E the 11-mer repeats have evolved into multiple 22-mer tandem repeats. Most of these 22-mer repeat units have the periodicity of an amphipathic α helix (11). Many of the 22-mer repeats have prolines at the first, and only the first, position. These 22-mer repeats appear predominantly in exon 4 and their number ranges from 13 in apoA-IV to 1 in apoC-III (**Fig. 3**). Based on their degree of homology and pattern of internal repeats, an evolutionary tree has been proposed (22) for the exchangeable apolipoproteins in which, through gene duplications, a single gene has evolved to the current multigene family.

The 11-mer/22-mer evolutionary pathway for apolipoproteins can be explained as a result of the 3.6 amino acid residues per turn periodicity of an α helix: 11 residues equal three complete turns of an α helix. Consequently, tandem duplication of an 11 residue amphipathic α helix produces a 22 residue amphipathic α helix in which there is little twist (20° or less) between the polar and nonpolar faces of the two identical 11-mer halves (11, 25). This 11-mer/22-mer motif also means that continuous amphipathic helixes significantly longer than 22 residues can exist, e.g., there will be a twist of 40° or less between the polar and nonpolar faces of two tandem identical 22-mer amphipathic helixes.

Studies with de novo-designed synthetic peptide analogs

Based on the key structural features predicted for the amphipathic helix by the original model (1), three laboratories independently studied the properties of de novo-designed peptide analogs of the amphipathic helix (26–28). The strategy was based, not on the primary sequence of naturally occurring apolipoproteins, but on incorporating the periodicity of the

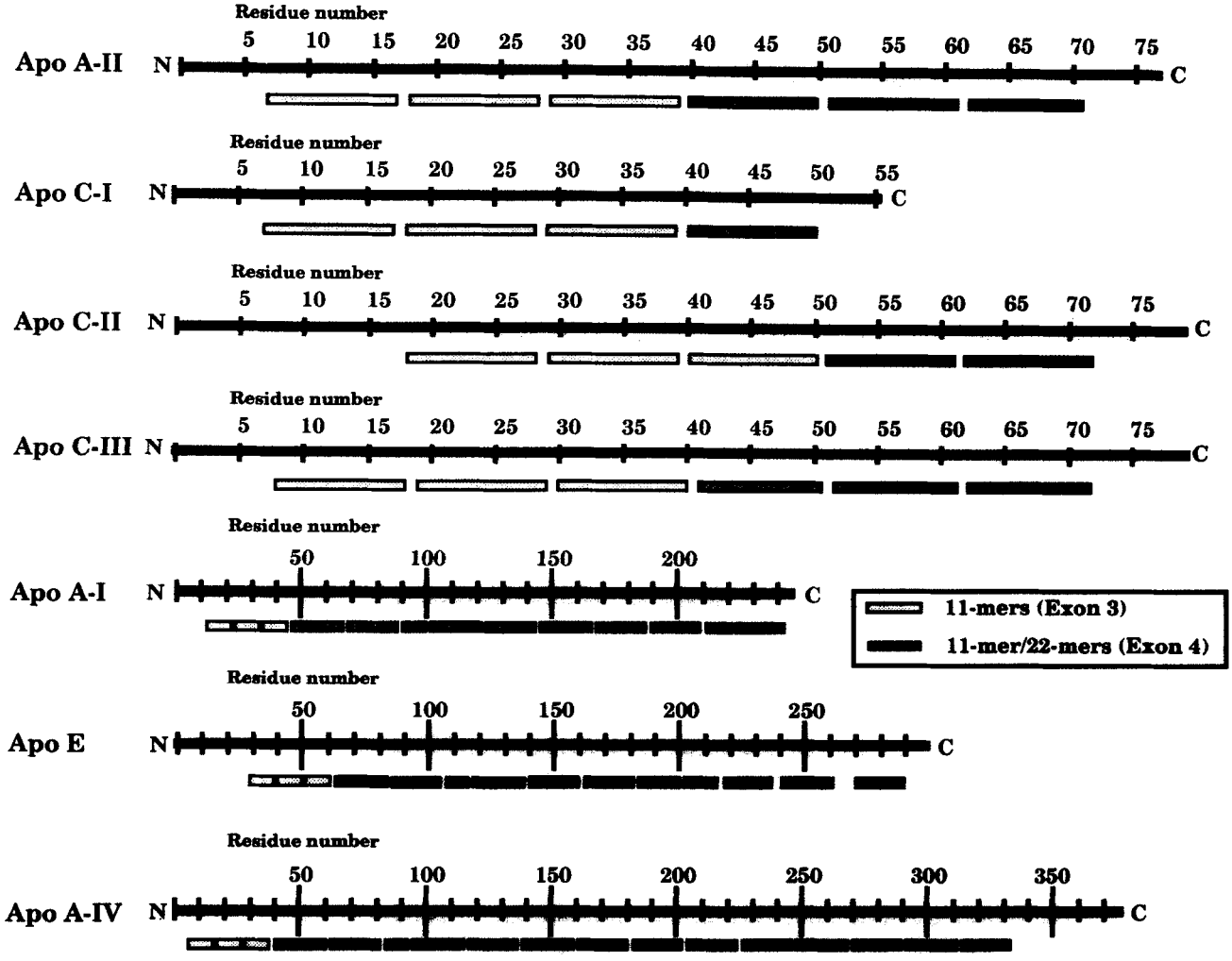


Fig. 3. Location of the tandem 11-mer/22-mer repeats in the exchangeable apolipoproteins.

secondary structural features of the amphipathic helix motif into the sequences of the peptide analogs.

One of these studies (26) focused on the question of whether or not the position of charged residues on the polar face of de novo-designed amphipathic helixes plays a role in lipid affinity and will be discussed below. The second study involved peptides synthesized by Fukushima and colleagues (27). The 22-residue peptide was synthesized entirely from Glu, Lys, and Leu arranged periodically in the typical apolipoprotein distribution shown in Fig. 2 to form an amphipathic α helix with equal polar and nonpolar faces. As studied by quantitative ultrafiltration, gel permeation chromatography, and circular dichroism, the peptide was shown to associate effectively with phospholipid and mimicked some of the physical and chemical properties of apoA-I (2, 3, 27, 29). A dimer of this 22-residue peptide was later found to more closely mimic apoA-I than the monomer; based on these results, the authors suggested that 44-mers represented the minimal functional domain in apoA-I (29).

The third study involved model amphipathic peptides synthesized by the Baylor group (28). Peptides called LAP-16, LAP-20, and LAP-24 (16, 20, and 24 amino acid residues long, respectively) were shown to associate with phospholipid. Others have shown that peptide analogs of the amphipathic helix as short as 10 to 12 residues in length have the ability to interact with phospholipid (30).

A variation of LAP-16 was also synthesized in which the amino-terminus of LAP-16 was blocked with fatty acyl chains of varying lengths (31). These peptides were synthesized to study the role of hydrophobicity. They were shown to interact with lipid to form stable lipoprotein complexes and associated with high density lipoproteins (HDL) both in vitro and in vivo (32). In vivo injection of reassembled HDL containing a series of radiolabeled acylated peptides showed that the plasma half-life increased with the acyl chain length. From these results the authors concluded that the rates of clearance of the exchangeable apolipoproteins are a predictable function of their lipid affinity (32).

Studies of de novo-designed amphipathic peptides, excluding for the moment the question of the role of charged residues, have demonstrated that: *1*) the degree of amphipathicity correlates with the ability of peptides to interact with phospholipid, e.g., an increase in the hydrophobicity of the nonpolar face increases the lipid affinity (26–28, 33); *2*) lipid association increases the α helicity of the peptides (26–28, 32); *3*) inclusion of a Pro within the sequence of the putative helix decreases the lipid affinity of the peptide (34); and *4*) amphipathic helixes synthesized entirely from all D-amino acids are equally as efficient in associating with lipid as those synthesized from all L-amino acids (35).

Model amphipathic peptides synthesized by Sparrow et al. (28) were designed with polar faces organized in a manner unlike the amphipathic helical domains associated with exchangeable apolipoproteins. Our laboratory has focused on the question of whether or not the position of charged residues on the polar face plays a role in lipid affinity. Peptide analogs were designed to mimic the amphipathic helical domains of apolipoproteins with respect to the distribution of charged residues. The peptide mimics were designed to have positively charged residues at the polar–nonpolar face interface and negatively charged residues at the center of the polar face.

In the second step of this strategy, these mimics were compared with peptide analogs with reversed charge distribution, i.e., the negatively charged amino acids were at the polar–nonpolar interface and the positively charged residues were at the center of the polar face. Consistently it has been found that peptides with the reversed charge distribution have decreased lipid affinity relative to the mimics (26, 36–39). Our explanation for these results is as follows (11). The bulk of the van der Waals' surface areas of the positively charged residues are hydrophobic and thus are amphipathic. We suggest that these amphipathic basic residues, when associated with phospholipid, extend ("snorkel") toward the polar face of the helix to insert their charged moieties into the aqueous milieu (**Fig. 4**). Thus, essentially the entirety of the uncharged van der Waals' surface of the amphipathic helixes of the apolipoproteins can be buried

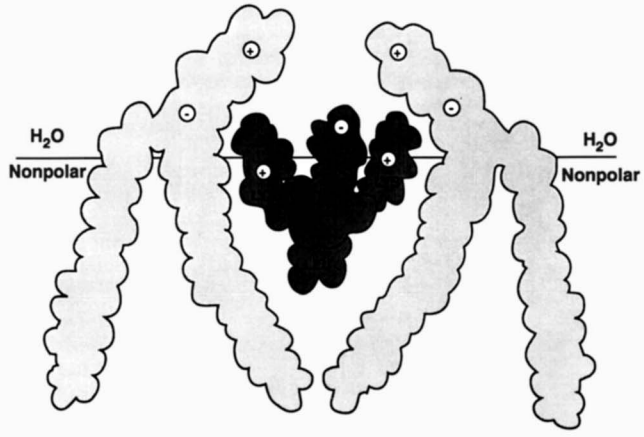


Fig. 4. Schematic diagram of the snorkel model of an amphipathic helix of the class A motif showing postulated insertion into a phospholipid monolayer. Long axis of the amphipathic helix is perpendicular to the plane of the page. Dimensions are approximately to scale. Note snorkeling to the aqueous surface by the interfacial amphipathic Lys residues. In this model the shorter negatively charged residues are localized to the center of the polar face because of the close proximity of this portion of the helix edge to the aqueous surface. Note the depth of burial of the center of the α helix backbone relative to the phospholipid head groups and compare it with Fig. 1.

within the hydrophobic interior of a phospholipid monolayer.

More recently, we have incorporated charged unnatural amino acids with varying alkyl chain lengths into de novo-designed peptide analogs to further test the snorkel hypothesis. Independently of the charge on the amino acid residue, increased alkyl chain lengths of residues located at the polar–nonpolar interface resulted in increased lipid affinity; increased alkyl chain lengths of residues located in the middle of the polar face had no effect on lipid affinity (39–41).

Studies of native apolipoprotein sequences by fragmentation and synthetic peptides

A number of laboratories have examined the lipid affinity of hydrolytic fragments and synthetic peptide analogs of apolipoproteins. **Fig. 5** contains a diagrammatic summary of the locations in the exchangeable apolipoproteins of lipid-associating domains (lines with double arrowheads) and nonlipid-associating domains (lines without arrowheads) suggested by these experiments.

ApoA-II. Human apoA-II is a homodimer of two 77-residue-long monomers. Both the dimer and the carboxymethylated monomer associate with lipid to form lipoprotein complexes (42). Synthetic peptides apoA-II[47–77] and apoA-II[40–77] associate with phospholipid, while peptides apoA-II[65–77] and apoA-II[56–77] have essentially no lipid affinity (43). In other studies, peptide apoA-II[17–31] failed to associate with lipid but the addition of five more residues, apoA-II[12–31], resulted in lipid association (44, 45). This suggests that there are at least two lipid-associating domains in apoA-II located at opposite ends of the molecule (Fig. 5).

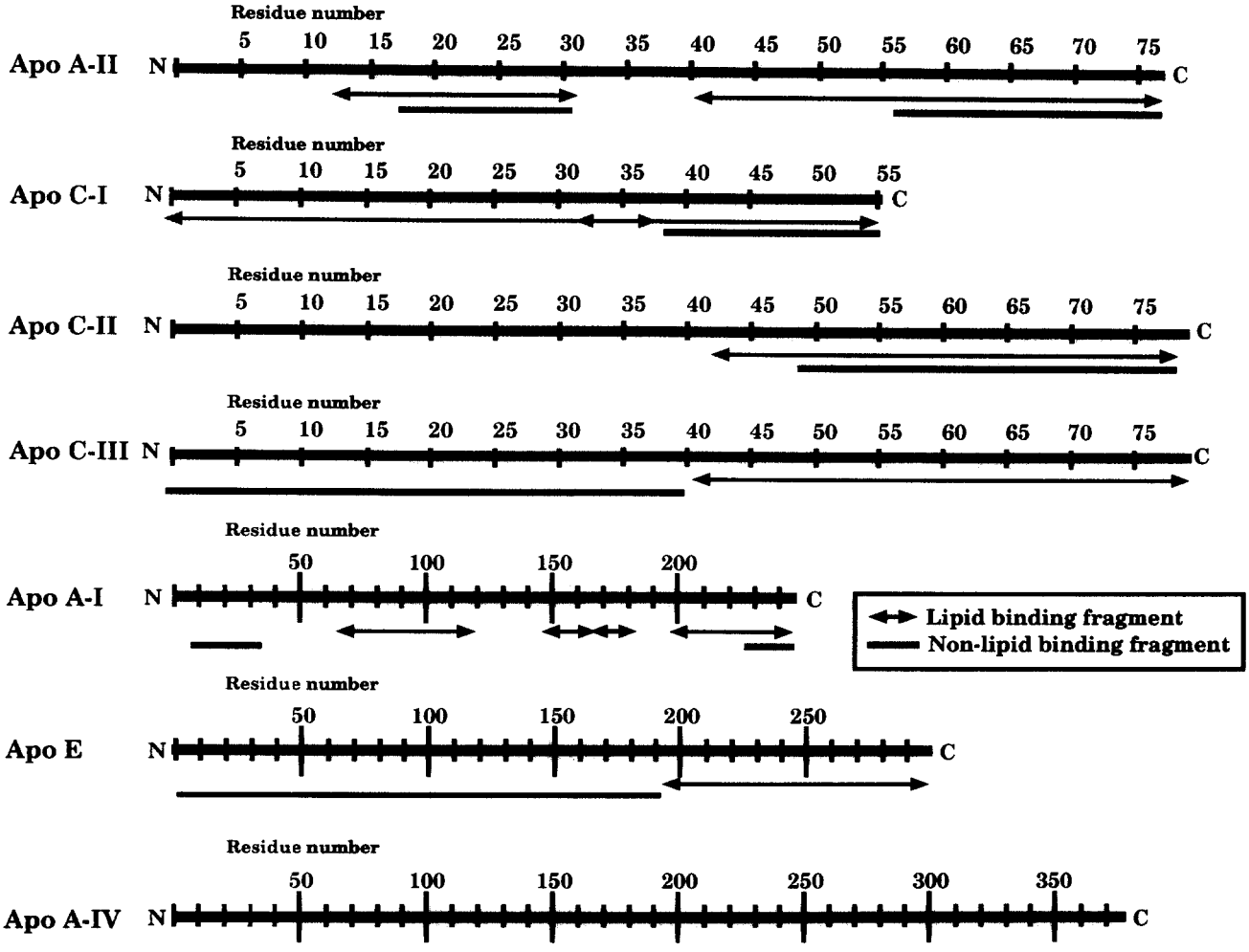


Fig. 5. Schematic representation of the experimentally determined lipid-binding and non-lipid-binding fragments of the exchangeable apolipoproteins. The lines with arrows indicate the limits of the lipid-binding fragments and the lines without arrows the limits of the non-lipid-binding fragments determined by fragmentation and/or synthetic peptide experiments.

ApoC-I. CNBr treatment of apoC-I produced two fragments, apoC-I[1–38] and apoC-I[39–57]; the amino-terminal fragment apoC-I[1–38] had the stronger lipid affinity (46). In another study the synthetic peptide apoC-I[32–57] was found to associate with phospholipid (47). Therefore, there appear to be at least two lipid-associating domains in apoC-I located between residues 1–31 and 32–57 (Fig. 5).

ApoC-II. Several synthetic peptide fragments of apoC-II have been examined for lipid-association. ApoC-II[50–78] and shorter peptides did not associate with phospholipid but apoC-II[43–78] was able to form phospholipid–peptide complexes (48), suggesting that a lipid-associating domain was at least partially located between residues 43 and 50 (Fig. 5). No studies of the lipid-associating properties of the amino-terminal half of the apoC-II have been reported.

ApoC-III. Based on the proposal that the amphipathic helical region of apoC-III was located between residues 40 to 67 (1), Sparrow, Gotto, and Marrisett (49) synthesized apoC-III[1–79], apoC-III[55–79], apoC-III[48–79], and apoC-III[41–79]. Using fluorescence and circular dichroism changes in the presence of lipid, these authors concluded that the lipid-associating domain of apoC-III is located between residues 41 and 79. In other supporting experiments, apoC-III was cleaved at the Arg40–Gly41 peptide bond into two fragments using thrombin (50); apoC-III[41–79], but not apoC-III[1–40], was found to interact with phospholipid (51) (Fig. 5).

ApoA-I. This is the major protein component of HDL. Our laboratory synthesized two amino-terminal peptide fragments, apoA-I[1–33] and apoA-I[8–33]. Although a previous computer analysis suggested an amphipathic helix in this region (52), the synthetic fragments associated weakly, if at all, with lipid (53). In the same study, apoA-I[66–120] was found to associate well with phospholipid (53). Kroon et al. (54) synthesized apoA-I[147–168] and apoA-I[158–168] and found that only the longer peptide associated with phospholipid. Finally, it has been reported that synthetic peptides apoA-I[165–185], apoA-I[218–243], apoA-I[202–243], and apoA-I[195–243] all associate with phospholipid but apoA-I[225–243] does not (55). The lipid-associating domains of apoA-I, therefore, appear to be localized to multiple sites in the carboxyl-terminal three quarters of the molecule (Fig. 5).

ApoE. Proteolysis of apoE by thrombin treatment of hypertriglyceridemic VLDL produces two apoE fragments, designated E-12 and E-22, with molecular masses of 12 and 22 kDa, respectively (56). Fragment E-22 corresponds to apoE[1–191] and E-12 corresponds to apoE[192–299]. While E-12 remained associated with VLDL, E-22 dissociated from thrombin-treated VLDL. As noted in the next section X-ray crystallography studies of the E-22 fragment indicate the presence of a 4-helix bundle globular structure (57). These results indicate that the lipid-associating domain(s) of apoE appear to be located on the carboxy-terminal half of the molecule (Fig. 5).

ApoA-IV. To the best of our knowledge, no experimental data have been published suggesting localization of the lipid-associating regions of apoA-IV.

Studies in intact apolipoproteins

Protein–lipid interactions. When exchangeable apolipoproteins are mixed with certain phospholipids, the two components spontaneously associate to form small protein-lipid complexes (a form of mixed micelle); circular dichroism indicated a concomitant increase in α helicity of the apolipoprotein (58). Many of these studies have been performed with apoA-I. This apolipoprotein interacts spontaneously with hydrated dimyristoyl phosphatidylcholine to form small complexes (< 200 Å in diameter) (59) shown, on the basis of negative stain electron microscopy and X-ray scattering data (60), to be discoidal in shape.

Based on thermodynamic considerations and differential scanning calorimetry, it has been suggested that recombinants of apoA-I with dimyristoyl phosphatidylcholine represent unilamellar bilayer discs whose otherwise thermodynamically unstable edges are lined by amphipathic helical domains of apoA-I (61, 62). Low-angle neutron and X-ray scattering data support this model (63, 64). Differential scanning calorimetry (36) and ^{13}C-NMR (65, 66) suggest that amphipathic helixes, under certain circumstances, also can associate with the planar phospholipid at the center of these discoidal structures. The precise organization of the amphipathic helixes at the disc edge has been the subject of much speculation and discussion but will not be discussed in this review.

Apolipoproteins A-II, C-I, C-II, C-III and E (67), and probably apoA-IV (68), also interact with dimyristoyl phosphatidylcholine to form discoidal particles. The exchangeable apolipoproteins thus act as protein detergents (63). Studies of intact and fragmented apolipoproteins and model peptides, described earlier, support the idea that amphipathic helixes serve in general as the lipid-associating protein detergent domains for the exchangeable apolipoproteins.

Cooperativity. An additional determinant of lipid affinity for individual apolipoproteins is the presence of multiple amphipathic helixes per protein monomer and the possibility of cooperativity between them (29, 36, 52, 69). Thus, a given apolipoprotein can have its lipid affinity increased not only by increasing the affinity of individual amphipathic helixes but also by increasing the number; one apolipoprotein may have a greater lipid affinity than another by having more am-

phipathic helical domains, even though those of the former may be of lower lipid affinity.

Hinged-domains. Regions termed hinged-domains have been postulated for apoA-I. As shown in **Fig. 6A**, these are suggested to be one or more amphipathic α helical segments that undergo conformational changes to produce alternate lipoprotein bound or unbound conformations (65, 70). These hinged-domains have been hypothesized to regulate both the size and apolipoprotein composition of lipoprotein particles and the process of conversion of nascent discs to mature spheres (70, 71). Several lines of evidence now support this hypothesis.

The original observation from which the hinged-domain hypothesis was developed came from the characterization of discoidal particles formed between apoA-I and dimyristoyl phosphatidylcholine (65). Several discretely sized particles were found in complexes containing a constant number of apoA-I molecules per particle. The "step-size" between the particles was constant and the change in circumference of the discoidal particles was found equal to the diameter of two α helixes (65). Thus it was proposed that the particle size is controlled by conformational changes in apoA-I that result in the all-or-none binding of complete α helical domains. This size quantization of particle classes has since been shown to be a general characteristic of apoA-I:lipid complexes reconstituted with a variety of phospholipids in the presence and absence of cholesterol (72–74).

Physical–chemical studies. The hinged-domain hypothesis has been tested using apoA-I/palmitoyl, oleoyl phosphaditidylcholine/cholesterol particles purified by gel filtration to a single-sized species (73). Par-

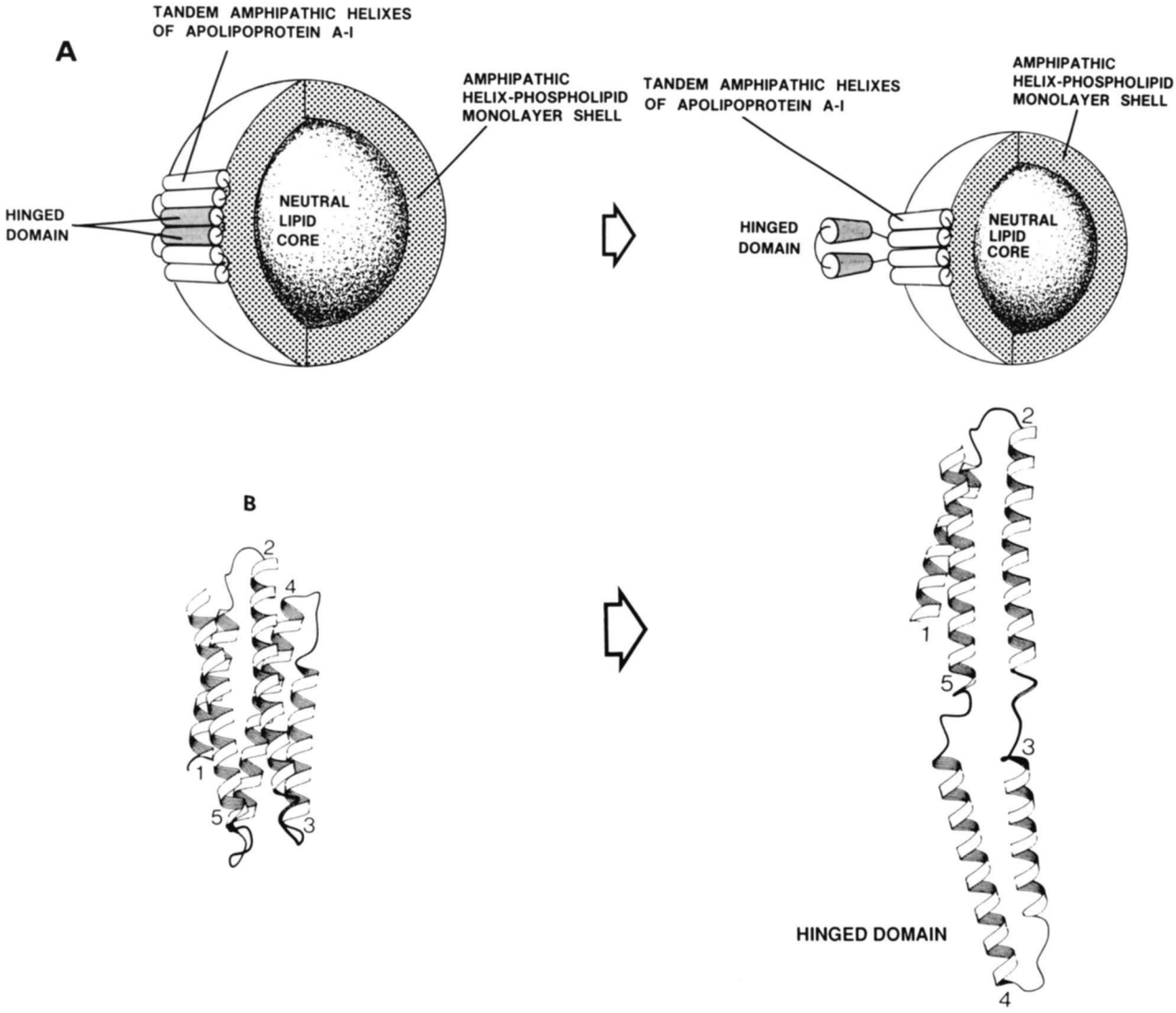


Fig. 6. Models for hinged-domain: (A) in apolipoprotein A-I, adapted from Cheung et al. (70), and (B) in insect apoLp-III, adapted from Breiter et al. (83) with permission. The arrows indicate a transition from the hinge closed to the hinge open conformation.

ticles differing in size, but containing the same number of A-I molecules, were shown to contain apoA-I in different conformations. Because the four Trp residues of apoA-I are located in the amino-terminal portion of the protein (residues 8, 50, 72, and 108), changes in fluorescence polarization with particle size suggested that this region of the protein contained reversible binding domains. Subsequently, the smaller apoA-I/ palmitoyl oleoyl phosphaditidylcholine particles, those thought to contain regions of apoA-I not directly in contact with lipid, were shown to be less stable to guanidine hydrochloride denaturation and to be more reactive to apoA-I monoclonal antibodies than the larger complexes, further supporting a difference in the A-I conformation for particles of different size (75).

Monoclonal antibody studies. Differential interactions between monoclonal antibodies (Mabs) and HDL support the hypothesis that the conformation of apoA-I is not the same in all HDL subspecies. Mabs to apoA-I produced against intact HDL have a differential ability to precipitate HDL, a result that suggests immunogenic and conformational heterogeneity of HDL-associated apoA-I (76–78), a heterogeneity apparently related to the size of the HDL subpopulations (76).

Curtiss and Smith (77) have localized one Mab epitope (AI-18) to residues 95–105 of apoA-I. This Mab binds more strongly to reconstituted apoA-I/cholesterol/phospholipid complexes than to either free apoA-I or HDL. The lipid-bound state of apoA-I also affected recognition of Mabs developed by Piot et al. (79). Silberman et al. (80) described a Mab localized to residues 87–124 of apoA-I (Mab-30) that recognizes only lipid-bound apoA-I and binds to HDL3 more effectively than to HDL2. Finally, Marcel et al. (81) have studied a number of Mab epitopes of apoA-I and have concluded that most epitopes are discontinuous and define regions of tertiary structure. Further, they observed that several Mabs are reactive to an epitope localized to residues 99–120. One possible explanation for the particularly high antigenicity of this domain and another adjacent domain, according to the authors, is a conformational flexibility of this domain, "consistent with the existence of mobile or hinged pairs of [helixes]." The sum of the data suggests that this hinged region is centered on residue 100 of apoA-I.

Chemical modification. Recent preliminary data from our laboratory (unpublished results) provides additional support for the hinged-domain model. All three Met residues in apoA-I are on the nonpolar face of amphipathic helical domains and two of the three are found at the end of helix 2 (residue 86) and the middle of helix 3 (residue 112). Methylation of the Met residues in apoA-I, which induces a formal positive charge into the Met residues, inhibits the ability of the modified apoA-I to induce step-size quantization in recombinants with dimyristoyl phosphatidylcholine.

Evidence to date thus supports the existence of a conformationally flexible domain in the amino-terminal portion of apoA-I, at or about residue 100, that allows the protein to expand or contract to accommodate changes in particle diameter. The hinged-domain hypothesis was proposed to describe a possible structure for this region as at least one pair of flexible or hinged helical segments (65, 70). Supporting the importance of the region of apoA-I around residue 100 is a report that the domains between residues 66–120 are phylogenetically the most conserved regions of apoA-I (82). As discussed in the next section, a pair of amphipathic helixes have recently been identified in the first X-ray crystal structure of an entire apolipoprotein that are postulated to undergo a hinged-movement (Fig. 6B) in order to bind to lipid (83).

It is interesting that the region of protein that seems most likely to be associated with the putative hinged-domain function is close to, if not identical to, a putative LCAT-activating domain proposed from synthetic peptide studies (25) and described in a later section. In this regard, the methylated apoA-I described above not only lost its putative hinged-domain activity but also lost over 50% of its LCAT-activating ability.

X-ray structure determinations. Insects have only one major kind of lipoprotein, lipophorin, that contains two apoB-like apolipoproteins, apolipophorin I and II (84); several of the insects also have a third apolipoprotein of 18–20 kDa (84) referred to as apolipophorin III (apoLp-III). The suggested function of apoLp-III is to help move lipid from the fat body to flight muscles during prolonged flight. ApoLp-III in the resting insect circulates in the hemolymph in the form of a lipid-free globular protein monomer. During flight apoLp-III is postulated to bind to hydrophobic defects created by expansion of the lipophorin particles, allowing continued lipid loading (84).

Breiter and coworkers (83) recently have described a molecular structure for apoLp-III from hemolymph of the African migratory locust, *Locusta migratoria* determined at 2.5 Å resolution. The structure determined for apoLp-III is that of five long amphipathic α helixes connected by short loops to form a 5-helix bundle globular protein. A hinged-movement of two helixes, as shown in Fig. 6B, was proposed to account for the partitioning of apoLp-III from its solution phase 5-helix bundle globular structure to a lipid-bound unfolded conformation on the surface of lipophorin. This proposed conformational change is quite similar to the hinged-domain movement postulated for apoA-I (65, 70, 71).

Even more recently, the 191 amino acid residue amino-terminal segment of human apoE (E-22, see

below) has been crystallized and its structure has been determined at 2.5 Å resolution. This fragment of apoE, containing the LDL receptor-binding domain, was determined to have a 4-helix bundle structural motif (57). The up-and-down helixes of this globular domain were located at residues 24–42, 54–81, 87–122, and 130–164, with the latter containing the LDL receptor-binding domain (85). A fifth α helix at residues 44–53 forms a short link between the first two α helixes of the 4-helix bundle. The E-22 fragment of apoE is known to associate only weakly with lipid (56).

COMPUTER ANALYSIS OF AMPHIPATHIC HELIXES

A number of methods have been used to detect and characterize putative amphipathic helical domains. These methods include: the Schiffer-Edmundson helical wheel (86), the helical net of Lim (87), the helical hydrophobic moment of Eisenberg, Weiss, and Terwilliger (88), and the related Eisenberg plot (88), hydrophobic moment plots of periodicity angles between 0° and 180° (89), comparison matrix methods (90), and cross-correlation methods of Kubota et al. (91). Each of these methods was described in some detail in our recent review article (11) and will not be discussed here.

There have been no comprehensive attempts to locate the amphipathic helical domains in the exchangeable apolipoproteins other than through analysis of the tandem repeats in these proteins. To rectify this deficiency, in this section we use several recent computer implementations of the helical wheel and the helical net algorithms to locate and classify, on the basis of polar face charge distribution and other physical chemical properties, the putative amphipathic helical domains of the exchangeable apolipoproteins.

Class A amphipathic helixes

Amphipathic helical domains have been reported in a variety of other proteins. In our previous review article we grouped amphipathic helixes into seven distinct classes (A, H, L, G, K, C, and M) based upon a detailed analysis of their physical–chemical and structural properties (11). In this classification, class A represented the amphipathic helical domains of the exchangeable apolipoproteins.

The most distinctive feature of the class A amphipathic helix, as defined (11), is the unique clustering of positively charged amino acid residues at the polar–nonpolar interface and negatively charged residues at the center of the polar face. As noted above, this distribution is the origin of the snorkel concept (11, 39–41). The original model for the amphipathic helical domains of the apolipoproteins was class A in its structural motif (1). On average, this original assumption remains true for the exchangeable apolipoproteins.

However, detailed analyses of the structural motifs of each of the amphipathic helical domains of the exchangeable apolipoproteins show considerable diversity. The positive–negative charge clustering motif found in class A amphipathic helixes does not exist to the same extent in all apolipoproteins nor does it exist equally in all amphipathic helical domains of individual apolipoproteins. It is known that lipid affinity varies perceptibly between the different exchangeable apolipoproteins (92–94) and between different regions within a given apolipoprotein (55). We have proposed the hypothesis that this lipid affinity is correlated with the extent to which a given amphipathic helical domain in an apolipoprotein sequence fits the class A snorkel motif.

Identification and classification of amphipathic helical domains

To test the class A-based working hypothesis, a computer-based strategy was used to quantify amphipathic helix diversity.

1. Amino acid sequences for the apolipoproteins analyzed in this study were obtained from the National Biomedical Research Foundation (NBRF) database.

2. Computer programs were developed to compare the degree to which amphipathic helical domains in the exchangeable apolipoproteins fit the class A snorkel motif. The programs WHEEL (helical wheel) and HELNET (helical net) analyze individual amphipathic helixes, and the programs COMBO (addition of helical wheels), COMNET (addition of helical nets), and CONSENSUS (average sequence and physical properties of helical wheels) analyze groups of amphipathic helixes. The basic features of these five computer programs are outlined in the legends to Fig. 8 (WHEEL and HELNET), Fig. 9 (COMBO), and Fig. 10 (COMNET and CONSENSUS) and are described in detail in an accompanying article (95).

3. Putative amphipathic helical domains in apolipoproteins A-I, A-II, A-IV, C-I, C-II, C-III, and E were identified from the NBRF database using the WHEEL and HELNET programs via a defined search and select algorithm.

4. Finally, all five computer programs were used to analyze and classify each domain.

In the remainder of this section we briefly describe the algorithms used to localize the amphipathic helical domains in the exchangeable apolipoproteins A-I, A-II, A-IV, C-I, C-II, C-III, and E, discuss the results of computer analyses of these domains, and compare these results with the relevant literature on lipid-binding and

functional domains. At the end of this review conclusions will be drawn from these results and the possible implications of these conclusions for the structure and function of these important apolipoproteins will be discussed.

Algorithm for locating domains for analysis. The following algorithm was used to define the location and limits of each amphipathic helical domain. *1*) Each of the 11-mer tandem amino acid repeaters described by Luo and colleagues (22) was individually analyzed by WHEEL and HELNET. *2*) Contiguous 22-mer and 33-mer segments of the basic 11-mer motif were individually analyzed by WHEEL and HELNET. *3*) Amino acid sequences outside the 11-mer repeats, including overlap regions with adjacent 11-mer repeats, were analyzed by WHEEL and HELNET. *4*) Amphipathic helical domains were terminated based on the rules shown in **Table 1**. *5*) A normalized hydrophobic moment > 0.20 per residue was required for inclusion as an amphipathic helix.

Locations and properties of class A amphipathic helixes

Fig. 7 includes diagrammatic representations of the results of the computer-based analysis of the amphipathic helical domains in the apolipoproteins. To facilitate comparison, Fig. 7 also includes information from Fig. 3 (location of tandem repeats) and Fig 5 (location of lipid-associating and nonlipid-associating domains).

Based upon the properties of their class A amphipathic helixes, the exchangeable apolipoproteins fall into three separate groups: apoA-II, C-I, C-II, and C-III with well-defined class A amphipathic helical domains defined as class A_2 domains; apoA-I and E with typical but less well-defined class A amphipathic helical domains defined as class A_1 domains; and apoA-IV with atypical class A amphipathic helical domains defined as class A_4 domains.

TABLE 1. Rules for termination of an amphipathic α helix

Occurrence of	Termination at
Pro	Pro -1
Phe, Met, Ile, Leu, Val, Trp, or Cys within 40° of center of polar face	Residue prior to Phe, etc.
Lys, Arg, Glu, or Asp within 40° of center of nonpolar face	Residue prior to Lys, etc.
Gln or Asn within 20° of center of nonpolar face	Residue prior to Gln, etc.
Cassette of four or more of the following consecutive neutral or polar residues (Thr, Gly, Ser, His, Gln, Asn)	Residue prior to first residue of cassette

ApoA-II, C-I, C-II, and C-III (class A_2). Eight separate class A amphipathic helical domains were identified in these four apolipoproteins: three in apoA-II, one in apoC-III, and two each in apoC-I and C-II. **Figs. 8A and B** are WHEEL and HELNET analyses of residues 7–32 from apoC-I that are given as an example of an individual class A amphipathic helical domain from this group.

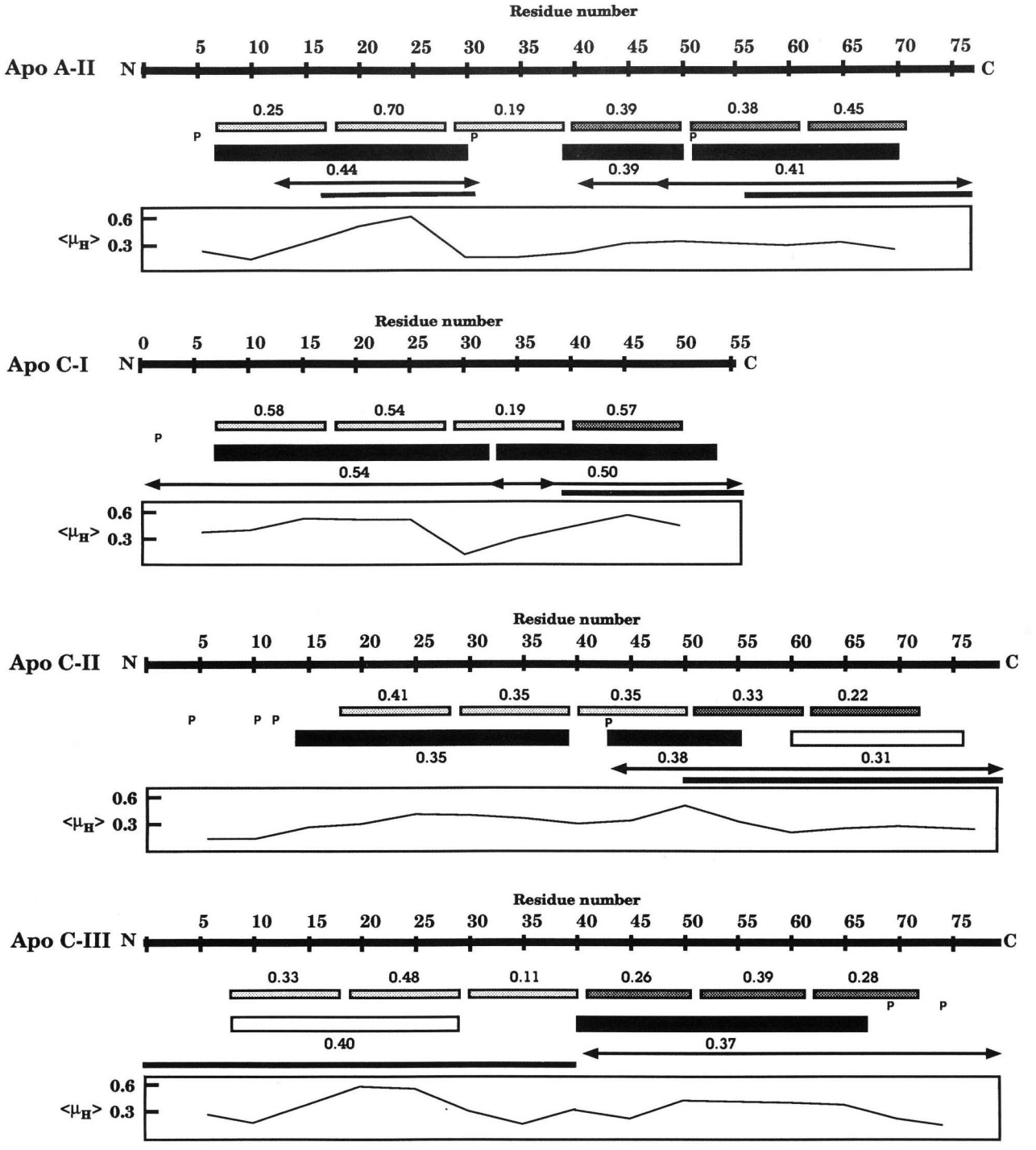
Figs. 9A and B are COMBO/SNORKEL and COMBO analyses, respectively, of the eight sequences making up class A_2. The COMBO program contains an algorithm that analyzes clustering in the positive and negative combo wheel diagrams; filled circle and error bars indicates the average angle and its standard deviation, subtended by each putative positive and negative residue cluster (see ref. 95).

It can be seen in these eight amphipathic helixes that the positive/negative charge-clustering motif is exact, the midpoints of the positive charge clusters are symmetrically distributed at + and –100° (SD of ± 17.5° of arc), and the separation between the charge clusters is virtually complete. Unlike apoA-I and E (see below), there is relatively little difference in the results whether helical wheel orientation is by the hydrophobic moment algorithm or the snorkel algorithm; the average of the standard deviation of the two cluster analyses of the positive clusters is only ± 3.5° of arc greater for the former than for the latter. COMNET analysis (**Fig. 10A**) shows that there is no significant charge clustering along the length of the helical axis but indicates clearly the cross axial charge clustering.

The degree of charge separation for amphipathic helixes in apoA-II, C-I, C-II and C-III is well demonstrated by CONSENSUS/SNORKEL (Fig. 10B). This algorithm also defines several meaningful elements of a consensus sequence for class A_2: four Lys residues cluster at and below (on the polar side of) the polar–nonpolar interface and three Glu residues cluster in the center of the polar face.

Table 2 is a compilation of physical-chemical properties derived from COMBO analyses of the different sets of potential amphipathic helical classes from all exchangeable apolipoproteins. Two of these physical chemical properties distinguish the class A_2 amphipathic helixes from the rest: *a*) both the mean hydrophobic moment ($<\mu_H>$) and the hydrophobicity of the nonpolar face are maximal and *b*) the Lys/Arg ratio is several times greater than 1.0.

ApoA-I and E (class A_1). ApoA-I and apoE were identified as having six and two potential class A amphipathic helical domains, respectively. COMBO/SNORKEL and COMNET analyses of these eight class A amphipathic helixes are shown in Figs. 9C and 10C. The class A motif is typical in these two apolipo-



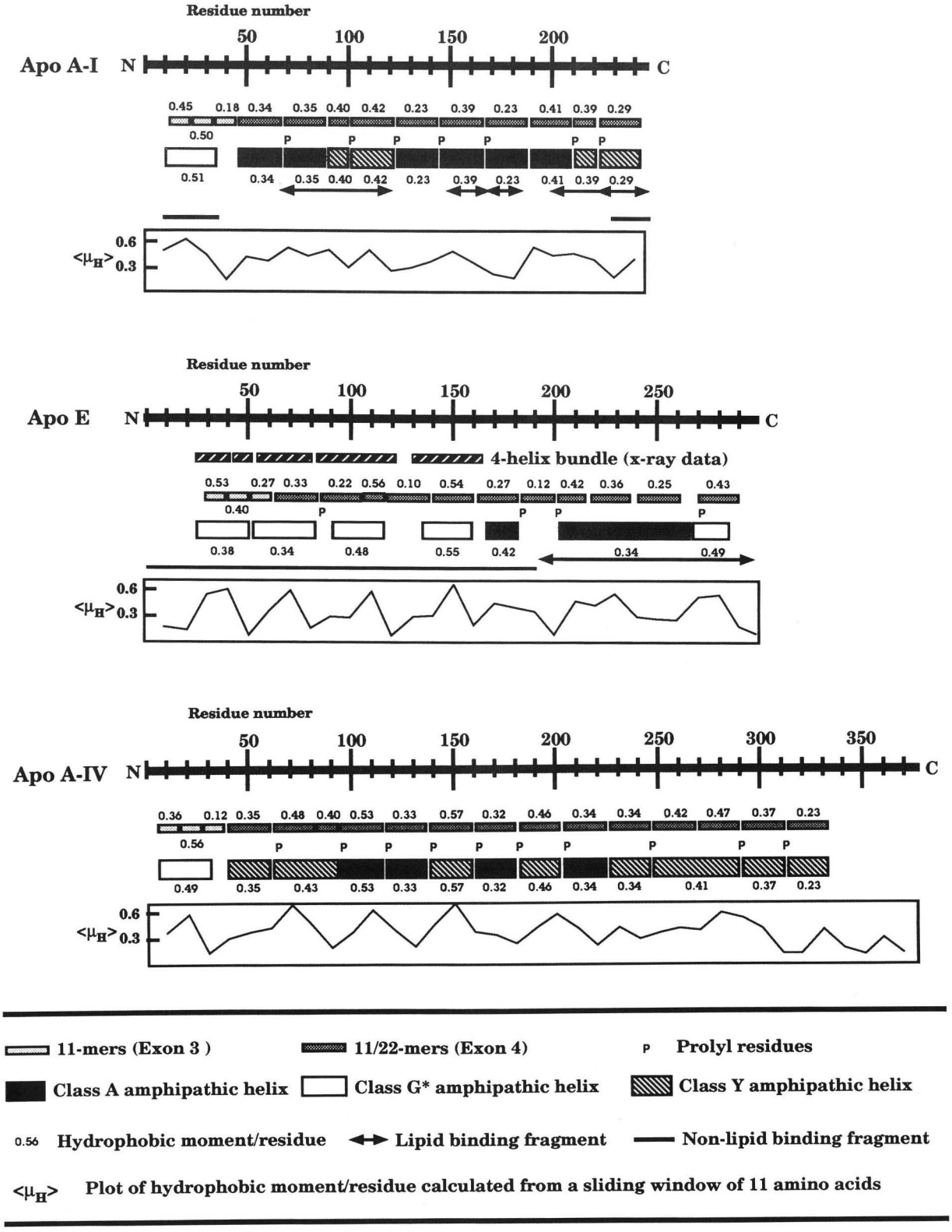


Fig. 7. Diagrammatic representation of the distribution of experimentally and analytically derived properties along the amino acid sequence of the exchangeable apolipoproteins (apoA-I, A-II, A-IV, C-I, C-II, C-III, and E). The following features are represented: *1*) locations of lipid-associating and non-lipid-associating domains suggested by experiment; *2*) computer-derived locations of amphipathic helixes of class A, class G*, and class Y; *3*) tandem repeats in Exon 3 (11-mers) and Exon 4 (11-mers and 22-mers); *4*) positions of all Pro residues; *5*) hydrophobic moment/residue calculated using a normalized GES hydrophobicity scale (118) for the tandem repeats and predicted amphipathic helical domains; *6*) plot of every 5th point (apoA-II, C-I, C-II, and C-III) or every 10th point (apoA-I, A-IV, and E) from a hydrophobic moment/residue calculation using a normalized GES hydrophobicity scale (118) and a sliding window of 11 amino acid residues.

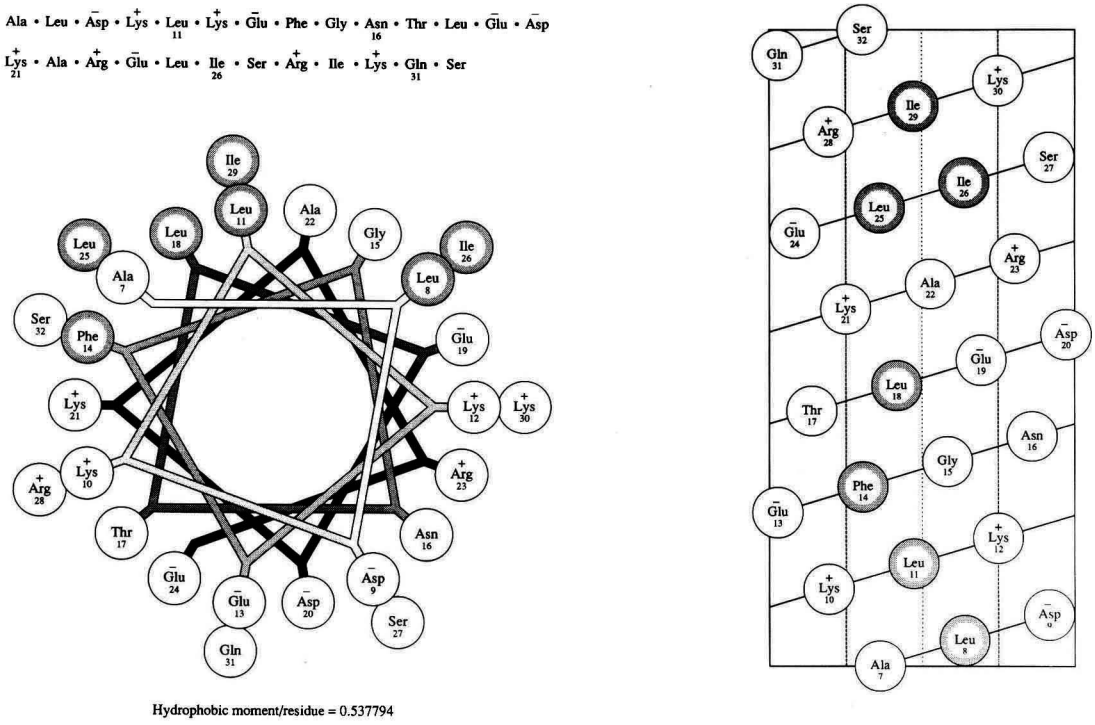


Fig. 8. WHEEL/SNORKEL and HELNET/SNORKEL analyses of the class A amphipathic helix apoC-I [7–32]. The helical wheel program (WHEEL) creates a "Schiffer-Edmundson" helical wheel diagram (86) of a given sequence of amino acids arranged as an ideal α helix (100° rotation per residue) seen down the long axis. The residues are projected onto a circular figure that is rotated so as to orient the nonpolar face toward the top of the page; i.e., the hydrophobic moment (88) points toward and perpendicular to the top of the page. By specifying a program option, WHEEL/SNORKEL, the wheel orientation to the page is realigned so that the normal to the top of the page bisects the nearest positive residues to either side of the hydrophobic moment. In the results reported here we have used a normalized version of the GES scale (118), but any hydrophobicity scale can be used. The helical net program (HELNET) creates a diagram by the method of Lim (87) of the α helix seen as a cylinder cut along the center of the polar face and flattened. The center of the nonpolar face, determined by the hydrophobic moment, lies in the center of the figure (dotted line) and is oriented to rise out of the page. Left: WHEEL analysis. Right: HELNET analysis.

proteins; the mean angles of the two positive clusters are at precisely + and −90°. However, the positive–negative charge-clustering motif is noticeably less well defined than for the class A_2 apolipoproteins; the standard deviations for the average of the two cluster analysis of the positive clusters and for the one cluster analysis for the negative cluster are ± 7° and ± 9° of arc greater, respectively, in class A_1 than in class A_2. When the helical wheel orientation is by the hydrophobic moment algorithm, the positive charge-clustering is still visually apparent (COMBO, not shown) but considerably less well defined than when the orientation is by COMBO/SNORKEL; the average of the standard deviation of the two cluster analyses of the positive clusters is ± 8° of arc greater for the former than for the latter.

The major features defined by CONSENSUS/SNORKEL analysis of class A_1 (Fig. 10D) are two Arg residues at the polar–nonpolar interface and four Leu residues in the center of the nonpolar face. From Table 2 class A_1 has a nonpolar face hydrophobicity comparable to that of class A_2 but the mean $\langle\mu_H\rangle$ is considerably lower and the Lys/Arg ratio is significantly less than 1.0.

ApoA-IV (class A_4). Only four potential class A amphipathic helical domains were identified in apoA-IV and these are rather atypical compared to class A domains in the other exchangeable apolipoproteins. COMBO/SNORKEL analysis (Fig. 9D) suggests a A-shaped class A snorkel motif in which the centers of the two positive clusters are at + and − 120°. The class A_4 domains are distinguishable among the other class A domains in having an intermediate mean $\langle\mu_H\rangle$ and a low nonpolar face hydrophobicity (see Table 2).

Locations and properties of amphipathic helixes of other classes

As seen in Fig. 7, each of the exchangeable apolipoproteins except apoA-II and apoC-I were identified as having putative amphipathic helical domains that cannot be classified as class A in their radial arrangement of positive and negative residues. Detailed examination of the individual domains suggested that they fall into two basic types. The first type of am-

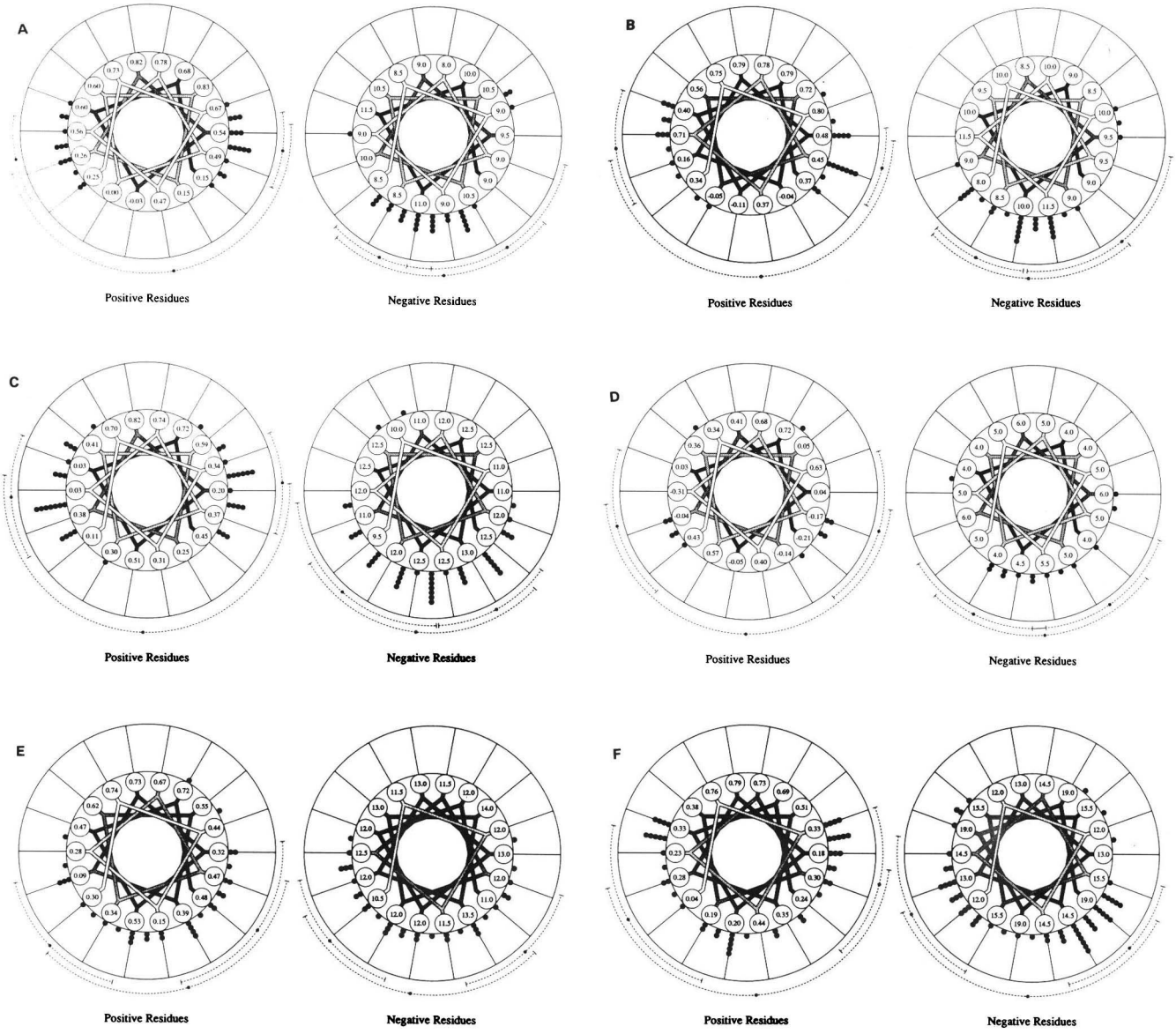


Fig. 9. Analysis of the potential helixes in the exchangeable apolipoproteins by the COMBO and COMBO/SNORKEL programs. The following sequences were analyzed: class A_2, apoA-II[7–30, 39–50, 51–71], apoC-I[7–32, 33–53], apoC-II[14–39, 44–55], apoC-III[40–67]; class A_1, apoA-I[44–65, 66–87, 121–142, 143–164, 165–186, 187–208], apoE[161–182, 203–266]; class A_4, apoA-IV[95–116, 117–138, 161–182, 205–226]; class G*, apoA-I[8–33], apoA-IV[7–31], apoE[25–51, 52–83, 91–116, 135–160, 268–285], apoC-II[60–76], apoC-III[8–29]; class Y, apoA-I[88–98, 99–120, 209–219, 220–241], apoA-IV[40–61, 62–94, 139–160, 183–204, 227–248, 249–288, 289–310, 311–332]. The program for addition of helical wheels (COMBO) superimposes and averages the wheels for specified sets of amino acid sequences. Before the helixes are superimposed, each helix is rotated so that the nonpolar face points towards the top of the page. The residues are projected onto two circular figures. The left-hand figure shows the counts of all positively charged residues and the right-hand figure shows the counts of all negatively charged residues. COMBO/SNORKEL is the sum of multiple WHEEL/SNORKEL analyses. The algorithm for cluster quantification is described in the methods article on page 000 of this issue. A. COMBO/SNORKEL analysis for class A_2; B. COMBO analysis for class A_2; C. COMBO/SNORKEL analysis for class A_1; D. COMBO/SNORKEL analysis for class A_4; E. COMBO analysis for class G*; F. COMBO/SNORKEL analysis for class Y.

phipathic helix is present in five of the seven apolipoproteins and is distinguished by a random radial arrangement of positive and negative residues. These amphipathic helixes are similar but not identical to the class G amphipathic helixes found in globular proteins; we call them class G*. The second type of amphipathic helix is present in only two of the seven apolipoproteins and is distinguished by a radial clustering of positive and negative residues into a pattern unlike that of class A; we term this a class Y motif.

Class G*

A total of nine class G* amphipathic helical domains are located in five of the seven exchangeable apolipo-

proteins, five in apoE and one each in apoA-I, A-IV, C-II, and C-III (Fig. 7). Four of these five domains in apoE are located in the amino-terminal half of the molecule and the fifth is at its carboxyl-terminal end. All but apoC-II contain an amino-terminal class G* domain largely derived from the first two of the three tandem 11-mer repeats located in exon 3. In apoC-II the single class G* domain is located at the carboxyl-terminus and is largely derived from the second of the two tandem 11-mer repeats located in exon 4.

Figs. 9E, 10E, and 10F show COMBO, COMNET, and CONSENSUS analyses, respectively, of the nine domains classified as class G* amphipathic helixes. The standard deviations of arc for the positive and negative residues are essentially those of random radial distributions confined to the polar face. COMBO/SNORKEL analysis (data not shown) gives essentially the same random radial distribution. Consistent with the lack of charged residue clustering, the only consensus feature identified is a cluster of four Leu residues on the nonpolar face. From Table 2 the class G* amphipathic helixes have a high mean $<\mu_H>$ and moderately high nonpolar face hydrophobicity.

Class Y

Eight of the thirteen putative amphipathic helical domains in apoA-IV and four of the eleven domains in apoA-I are of the Y class. Figs. 9F, 10G, and 10H are COMBO/SNORKEL, COMNET/SNORKEL, and CONSENSUS SNORKEL analyses, respectively, of these twelve amphipathic helixes. As is readily apparent from these figures, the basic features of the class Y motif are two negative residue clusters on the polar face separating the two arms and the base of the Y motif formed by three positive residue clusters. The basic features of this class Y motif are apparent even in a COMBO analysis (data not shown). From Table 2 the class Y amphipathic helixes have both an intermediate mean $<\mu_H>$ and an intermediate nonpolar face hydrophobicity compared to the other amphipathic domain classes in the exchangeable apolipoproteins.

FUNCTIONAL PROPERTIES OF AMPHIPATHIC HELIXES IN APOLIPOPROTEINS

In this section we will review the literature for evidence linking amphipathic helixes (and/or specific apolipoprotein sequences) with certain functional properties of the exchangeable apolipoproteins. The results of this review of amphipathic helix/function linkage will be compared with the map of amphipathic helix location and classification derived in the previous section and shown in Fig. 7.

Lecithin:cholesterol acyl transferase activation

Plasma enzyme lecithin:cholesterol acyl transferase (LCAT) is a water-soluble plasma enzyme that plays an important role in the metabolism of HDL by catalyzing the formation of cholesteryl esters on HDL through the transfer of fatty acids from the *sn*-2 position of the phosphatidylcholine to cholesterol (96). ApoA-I is the major co-factor of LCAT in HDL and reconstituted lipoproteins (97). The other exchangeable apolipoproteins also activate LCAT but to a lesser extent (25). Many laboratories have used different techniques such as synthetic peptide analogs (25, 98), monoclonal antibodies (99), and recombinant HDL particles (100) to attempt to identify the major LCAT-activating region(s) of apoA-I.

It is known that LCAT binds to interfaces, such as the surface of HDL. Because amphipathic helixes are surface active, they have been suggested to play a role in activation of LCAT (97, 98). However, the enzyme does not require a co-factor for the hydrolysis of water-soluble substrates such as the *p*-nitrophenyl esters of fatty acids (101). Because of this it has been suggested that the major role of amphipathic helixes is to disrupt the water–phospholipid interface to expose the buried substrate to LCAT (101).

However, all of the exchangeable apolipoproteins contain amphipathic helixes, yet apoA-I is the superior LCAT activator (25). Further, with the exception to be discussed below, all amphipathic helical peptides studied have an intrinsic upper limit to their ability to activate LCAT, approximately 30% of apoA-I (25, 98). Therefore, simple water–phospholipid interface disruption by amphipathic helixes may be necessary for LCAT activation but is clearly not sufficient. Additional structural features must be involved.

Fig. 10. COMNET and CONSENSUS/SNORKEL analyses of class A_2, A_1, G*, and Y. The program for addition of helical nets (COMNET) superimposes and averages the helical nets for specified sets of amino acid residues. The residues selected are represented by small filled circles. The nets are superimposed so that the midpoint of each helix coincides. The consensus wheel program (CONSENSUS) superposes the helixes in the same fashion as COMBO and a single figure classifies the amino acid residues into five physical–chemical groups: positive (Arg, Lys), negative (Glu, Asp), polar (Asn, Gln), neutral (Tyr, Pro, His, Ser, Gly, Thr, Ala), and hydrophobic (Cys, Trp, Val, Leu, Ile, Met, Phe). CONSENSUS uses a graduated shaded contour to plot, at 20° intervals, the scaled radial distribution of these five classes of amino acid residues. Also a consensus amino acid is shown for each 20° position if there is an amino acid residue that occurs at that position most often and at least one-third of the time. A. COMNET/SNORKEL analysis of A_2. B. CONSENSUS/SNORKEL analysis of class A_2. C. COMNET/SNORKEL analysis of class A_1. D. CONSENSUS/SNORKEL analysis of A_2. E. COMNET analysis of class G*. F. CONSENSUS analysis of class G*. G. COMNET/SNORKEL analysis of class Y. H. CONSENSUS/SNORKEL analysis of class Y.

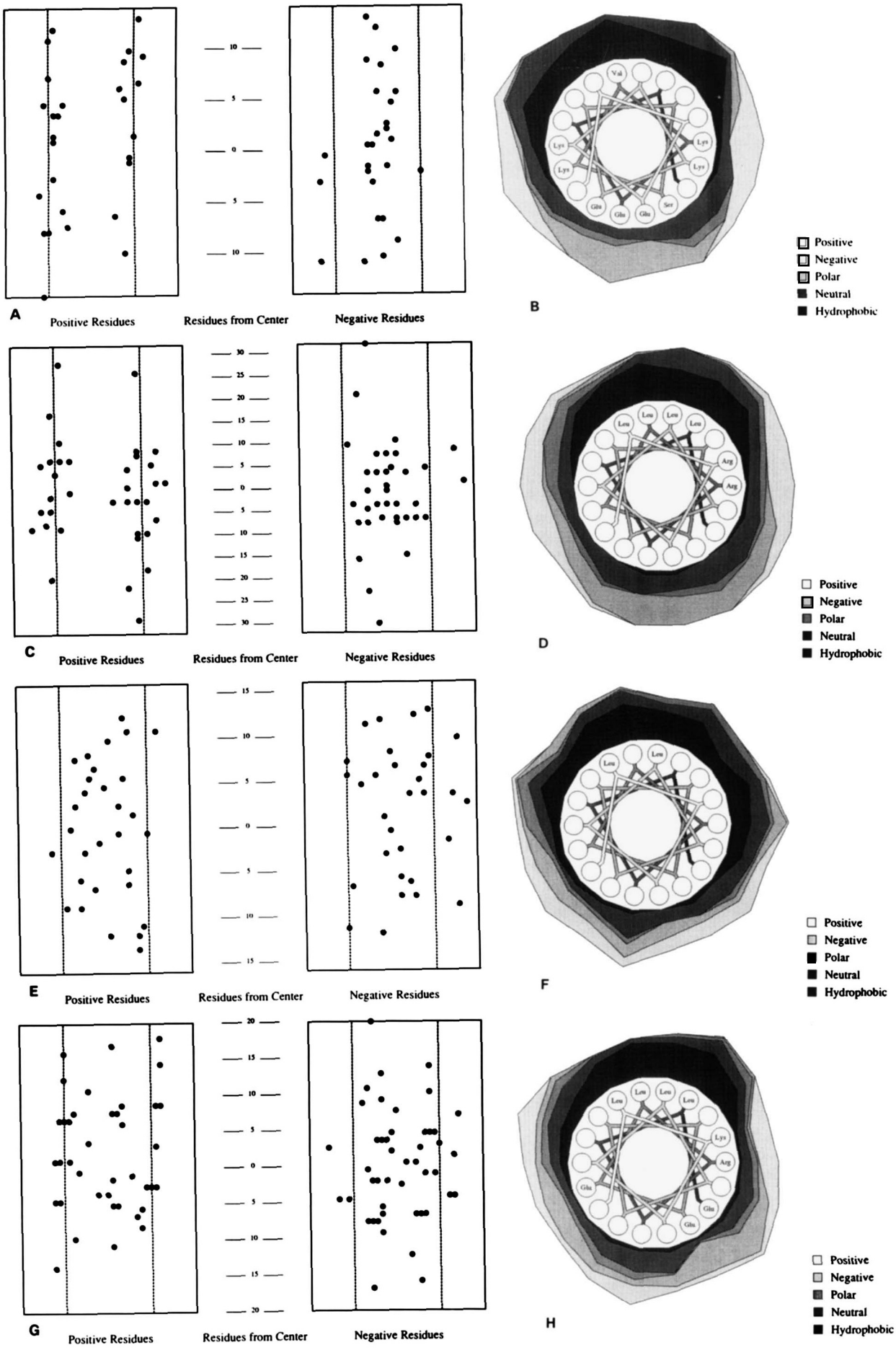


TABLE 2. Properties of amphipathic helical domains of apolipoproteins

Class	Apolipoproteins or Proteins	Mean $\langle\mu_H\rangle$ per AA[a,b]	Hydrophobicity per AA of Nonpolar Face[b,c]		No. of +AA[a]	No. of −AA[a]	Lys/Arg Ratio[a]
			CON[a]	CON/SNK[d]	Per 11 AA		
A_2	A-II, C-I, C-II, C-III	0.43	0.74	0.75	1.9	1.8	4.8
A_1	A-I, E	0.34	0.71	0.72	2.0	2.1	0.5
A_4	A-IV	0.38	0.60	0.43	1.5	1.8	1.0
G*	A-I, A-IV, E, C-II,C-III	0.44	0.69	0.61	1.6	1.6	1.0
G*	E	0.44	0.68	0.57	1.9	1.9	0.6
Y	A-IV, A-I	0.37	0.65	0.67	1.9	2.2	1.2
G	4-helix bundle	0.32	0.62	0.50	1.4	1.5	3.9
Insect	Lp-III	0.38	0.70	0.71	0.8	1.2	2.0

[a]Data derived form CONSENSUS analyses.
[b]Calculated using a normalized (unitless) GES hydrophobicity scale (118).
[c]Includes only the six residues centered on the nonpolar face.
[d]Data derived from CONSENSUS/SNORKEL analyses.

As shown in Fig. 7, structural analysis suggests the presence, starting at residue 44, of 10 tandem and structurally separate class A or class Y amphipathic helical domains in apoA-I. Several lines of evidence point to the amino-terminal region of these tandem amphipathic helixes as the predominant LCAT-activating domain in apoA-I.

1. Our laboratory has suggested that the amphipathic helical domains between residues 66 and 120 are important for LCAT activation; this region represents two 22-mer helixes and an intervening 11-mer (Fig. 3). We proposed that a unique positioning of Glu residues on the nonpolar face of helix 2 (residues 66–87) and helix 3 (residues 99–120), not present in the other apolipoproteins, are responsible for the higher LCAT-activating ability of apoA-I (25). Synthetic consensus peptides with the same positioning of Glu residues were found to be equipotent with apoA-I, on a weight basis, in activating LCAT. No other synthetic peptides are known to be as active.

2. ApoA-I specific monoclonal antibodies have been used in conjunction with synthetic peptides to suggest that part of the LCAT activation domain resides between residues 96–111 (99).

3. Earlier studies failed to identify a major LCAT-activating domain among the four fragments produced by CNBr hydrolysis of apoA-I (97). It is likely that the functional importance of the 66–120 domain was missed in these earlier studies because two of the three methionines present in apoA-I are found at the end of helix 2 (residue 86) and the middle of helix 3 (residue 112). It is likely that cleavage at these positions destroyed the conformation of the region required for LCAT activation.

4. Reconstituted apoA-I/cholesterol/palmitoyl, oleoyl phosphaditidylcholine discoidal complexes of homogeneous size activate LCAT to varying degrees depending on the complex size. Fluorescence spectroscopy of the complexes suggest that the amino terminal region of A-I (through helix 3) is not directly interacting with lipid in the complexes that are the poorest LCAT activators (75).

The mechanisms whereby the amino terminal region of apoA-I might activate LCAT are not known but could involve a combination of increased substrate assessibility and stabilization of active intermediate compounds (25).

Lipoprotein lipase activation

An amphipathic helix enhances the lipoprotein lipase activation capacity of apoC-II (48, 102). Synthetic peptide studies localized the lipoprotein lipase activation site of this protein to residues 55–78. CNBr cleavage of this protein produced peptides apoC-II[1–9], apoC-II[10–59], and apoC-II[60–78]. Lipoprotein lipase activation studies on these fragments indicate that the enzyme-activating domain is localized to apoC-II[60–78]. Comparing these experimental results with the amphipathic helix map (Fig. 7), the lipoprotein lipase-activating domain of apoC-II corresponds precisely to the predicted carboxyl-terminal class G* amphipathic helical domain (residues 60–76).

LDL receptor-binding and heparin-binding domains in apolipoprotein E

ApoE is a single polypeptide of 299 amino acids. The receptor binding domain of apoE has been localized to the region encompassing residues 130–160, and the major lipid-binding domain resides in the carboxyl-terminal one-third of the polypeptide chain (85, 103). From the amphipathic helix map (Fig. 7) the receptor domain and the lipid-binding domains of apoE correspond to class G* and class A amphipathic helical domains, respectively.

Inhibiting monoclonal antibodies have been used to localize the heparin-binding of apoE to two sites at

residues 142–147 and 243–272 (104). The first site corresponds to the LDL receptor-binding domain of apoE and is recognized both in solution and when apoE is lipid-bound. The second site is recognized only when apoE is lipid-free, suggesting that residues 243–272 are part of the major lipid-binding domain of apoE; from the amphipathic helix map (Fig. 7) the major lipid-binding domain is predicted to be the class A amphipathic helix located between residues 203–266.

Putative high density lipoprotein receptor activity

It has been proposed that HDL binds to certain cells via high-affinity saturable binding sites (105, 106) and that this binding may be mediated by a membrane protein (107). Alternatively, it has been suggested that HDL binding to cells may occur via amphipathic helix-mediated binding to membrane lipid. Human placental lactogen is a protein hormone, the release of which has been shown to be stimulated by incubation of HDL with an enriched fraction of cultured trophoblast cells; this biological activity is due to apolipoproteins A-I, A-II and C-I (108). Jorgenson et al. (109) have shown that the synthetic peptide analogs of the class A amphipathic helix mimic apolipoproteins in this biological activity. The degree of human placental lactogen release from trophoblasts by these peptide analogs is correlated with lipid affinity, thus suggesting that the role of apolipoproteins in human placental lactogen release may be mediated through an interaction of amphipathic helixes with plasma membrane phospholipids (109).

A recent study by Leblond and Marcel (110) using monoclonal antibodies supports this concept of a biological role for the direct interaction of amphipathic helixes with plasma membranes; these authors suggest that "the optimum uptake of ... HDL ... requires the ... cooperative binding of the amphipathic α helical repeats [of apoA-I] to HepG2 cell membranes." Consistent with direct interaction of amphipathic helixes with plasma membranes is the fact that apoA-I, A-II, and A-IV bind equally well even though apoA-II is made up almost entirely of class A amphipathic helical domains (Fig. 7).

Antiviral and anti-inflammatory activities of amphipathic helixes

Several novel functions of apolipoproteins have been suggested to be mediated by the amphipathic helical domains. In recent experiments, Owens, et al. (111) tested the effect of apoA-I on the HIV-mediated cell fusion, the major cytopathic effect in HIV infections. Both amphipathic peptides and free apoA-I, but not HDL, effectively inhibited the HIV-induced cell fusion (111). HSV-induced cell fusion was also inhibited by apoA-I and amphipathic peptide analogs and not HDL (53, 112), thus indicating that the amphipathic helical regions of apoA-I are involved in the fusion inhibitory effect.

Blackburn, et al. (113) have demonstrated nontoxic inhibition of neutrophil activation using physiologic concentrations of free apoA-I and not HDL. These results suggest that the lipid-associating sites of apoA-I are responsible for this biological activity. Consistent with this is the finding that the amphipathic helical model peptide analogs also inhibit neutrophil activation (113).

CONCLUSIONS AND FUTURE PERSPECTIVES

Evidence for the presence of amphipathic α helixes in all of the exchangeable apolipoproteins is sometimes indirect but convincing. First, circular dichroism data shows that association of exchangeable apolipoproteins and their peptide analogs with phospholipid produces a substantial percent increase in α helicity of the proteins or peptides (114). Second, the amino acid sequences of the putative amphipathic helical domains have the periodic patterns of α helixes containing sharply demarcated polar and nonpolar faces (52). Taken together these two observations provide strong evidence that the putative amphipathic helical domains are α helical and amphipathic when the apolipoproteins are bound to lipid.

Fig. 7 compares the location of the experimentally determined lipid-associating and non-lipid-associating regions of the exchangeable apolipoproteins with the predicted locations of the different amphipathic helical domains. This figure supports the conclusion that much, probably most, of the lipid association in all the exchangeable apolipoproteins (with the probable exception of apoA-IV) resides in the class A amphipathic helical domains.

Not only is lipid-association linked to the presence of class A amphipathic helical domains, but it seems likely that variations of the type of amphipathic helix in the exchangeable apolipoproteins, from well-defined class A to poorly defined class A to non-class A, allow a range of lipid affinities from high to low. The computer analyses described earlier suggest that the class A amphipathic helical domains of apoA-II, C-I, C-II, and C-III are more highly defined than the class A amphipathic helical domains of apoA-I and apoE, which in turn are "better" class A amphipathic helixes than the few class A amphipathic helical domains of apoA-IV: the position of the class Y amphipathic helixes in the hierarchy is uncertain but it seems probable that these have relatively weak lipid affinities. From the literature it seems clear that apoA-II (115) and apoA-IV (116, 117) are at or near the high

and low ends, respectively, of the lipid affinity spectra; the rank order lipid affinity of the remainder of the exchangeable apolipoproteins is less certain.

One role of the class Y amphipathic helixes found in apoA-IV and A-I appears to be to serve as low affinity lipid-association domains. The snorkel hypothesis predicts that this class of amphipathic helix would not penetrate as deeply into phospholipid surfaces as class A and thus would have lower lipid affinity. This prediction is supported by experimental evidence (based on Trp fluorescence blue shifts, ease of Trp fluorescence quenching, and liposomal leakage) that apoA-IV sits higher in a phospholipid monolayer than the other exchangeable apolipoproteins that contain class A amphipathic helixes (117).

As an additional possibility, it may be more than a chance observation that both apoA-I and A-IV are synthesized in the intestine and are associated with the surface of nascent chylomicrons (116). Perhaps the class Y amphipathic helixes are involved in some unique fashion in chylomicron synthesis and/or metabolism, e.g., in the interaction of triglyceride-rich lipoprotein surface remnants with HDL.

With the exception of apoA-II and C-I, all apolipoproteins analyzed contain one or more amphipathic helical domains of the class G*. ApoA-I, A-IV, C-III, and E contain one or more non-class A amphipathic helical domains at their amino-terminus. As shown in the amphipathic helix map (Fig. 7), the amino-terminal class G* amphipathic helical domains of apoE, with a relatively weak lipid affinity (56), correspond closely to the position of a 4-helix bundle globular structure determined by X-ray structural analysis (85). As indicated by **Fig. 11** and Table 2, the type of amphipathic helix found in the amino-terminal domain of apoE is similar, in the first approximation, to the class G amphipathic helix found in 4-helix bundle globular proteins (11) in that both have wide zwitterionic polar faces. There are differences, however: the amino-terminal amphipathic helixes of apoE have a higher mean hydrophobic moment (0.44 versus 0.32), a higher mean nonpolar face hydrophobicity (0.68 versus 0.62), and a higher mean charge density (3.9 versus 3.0 charged residues per 11-mer) than 4-helix amphipathic helixes.

It is therefore tempting to speculate that the amino-terminal domain of apoE, including the LDL receptor-binding region, may be lipid-associated under certain conditions and globular under other conditions; the receptor-binding region has been shown to be much more active when associated with lipid than when lipid-free (85). We further suggest that the other single class G* amphipathic helical domains located in apoC-II, C-III, A-I, and A-IV interact with either lipids or proteins in a manner regulated by local environmental conditions. Consistent with this model, the class G* domain in the carboxyl-terminal region of apoC-II is involved in the activation of lipoprotein lipase by this apolipoprotein (48).

The amphipathic helix map (Fig 7) suggests a second and perhaps related possibility for the lipid association of the amino-terminal domain of apoE. The class A amphipathic helix located between residues 181–192 is disordered in the crystal structure (57) but might associate with lipid when lipid is present; this region has been shown to be less protease sensitive when the amino-terminal domain is lipid-bound (85).

The five actual amphipathic helical domains of the insect apolipoprotein Lp-III (83) analyzed by COMBO/SNORKEL and CONSENSUS/SNORKEL are shown in Figs. 11E and F, respectively, for comparison with the class G* amphipathic helical domains of apoE (Figs. 11A and B) and the class G amphipathic helical domains of 4-helix bundle proteins (Figs. 11C and D). From this figure and Table 2, it is apparent that the amphipathic helical domains of apoLp-III are similar in several important ways to the class A amphipathic helical domains of the apolipoproteins: the amphipathic helical domains of apoLp-III have a high mean $\langle\mu_H\rangle$ (0.38 per residue), a high mean nonpolar face hydrophobicity (0.71 per residue), and a well-defined negative charge cluster (the standard deviation for the one cluster analysis is $\pm 45°$ of arc versus $\pm 46°$ of arc for class A_2). There also appears to be some interfacial positive charge clustering in the amphipathic helical domains of apoLp-III but the clustering is weak. As is commonly found in the exchangeable human apolipoproteins, three of the α helixes (helixes 2, 4, and 5) are punctuated by Pro. The major difference between the amphipathic helical domains of apoLp-III and those of the exchangeable apolipoproteins is that the amphipathic helical domains of apoLp-III have a very low charge density.

The complete insect apolipoprotein Lp-III amino acid sequence was analyzed as an additional test of the algorithm for locating amphipathic helical domains. The amphipathic helical domains predicted by this algorithm versus the actual α helixes determined by X-ray crystallography (83) were quite similar but not identical (**Table 3**).

The results of the computer analyses suggest that the hydrophobic moment algorithm is not a reliable measure of the lipid affinity of lipid-associating amphipathic helixes. For example, the four class G* amphipathic helixes of the amino-terminal domain of apoE have a mean hydrophobic moment of 0.44 and a low lipid affinity, whereas the single class A amphipathic helix of the carboxyl-terminal domain of apoE has a mean hydrophobic moment of 0.34 and a high lipid affinity. Table 2 suggests that the presence

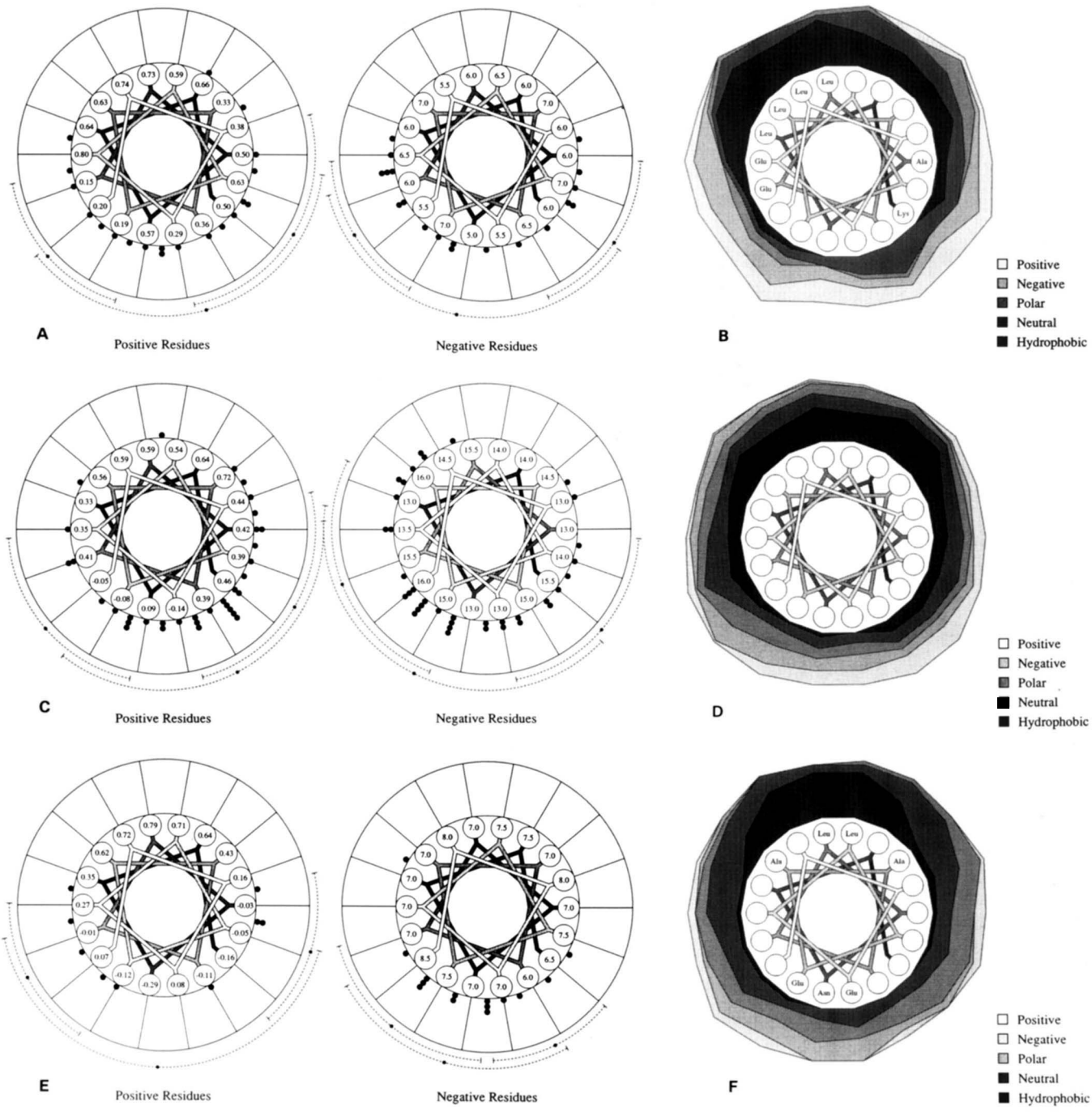


Fig. 11. Comparison using COMBO and CONSENSUS of: the four amino-terminal class G* amphipathic helixes in apoE, class G amphipathic helixes from four 4-helix bundle proteins, and the five α helixes of insect apoLp-III. The amino-terminal class G* amphipathic helical domains in apoE are [25–51, 52–83, 91–116, 135–160]. A total of twelve 4-helix bundle amphipathic α helical domains were analyzed from worm myohemerythrin [18–38, 40–62, 69–87, 93–110], bacterial cytochrome b562 [2–19, 24–45, 62–86, 88–108], worm hemerythrin. [21–37, 41–64, 69–86, 90–103], and bacterial cytochrome c3 [5–23, 42–54, 79–100, 106–117]. The amphipathic helical domains in insect apoLp-III are [7–32, 35–66, 70–86, 95–121, 129–156]. A. COMBO analysis of apoE; B. CONSENSUS analysis of apoE; C. COMBO analysis of 4-helix bundle α helixes; D. CONSENSUS analysis of 4-helix bundle α helixes. E. COMBO analysis of insect apoLp-III α helixes; F. CONSENSUS analysis of insect apoLp-III α helixes.

of a class A structural motif and a high nonpolar face hydrophobicity correlate better with lipid affinity than does the hydrophobic moment. Using these two properties from Table 2 as the ranking criteria, excluding class A_4, the predicted rank order lipid affinity is: class A_2 > class A_1 > insect > class Y > class G* > class G. This ranking does not, of course, take into account the number and cooperativity of amphipathic helixes.

TABLE 3. Sequence of amphipathic helixes in apoLp-III

From Crystal Structure	Predicted by Algorithm for Location of Amphipathic Helixes
7–32	6–32
35–66	35–66
70–86	71–90
95–121	95–113
129–156	129–159

The measurements of nonpolar face hydrophobicity are also relevant to the snorkel concept. For all of the class A amphipathic helical subclasses (except A_4), including that of the insect apoLp-III, the hydrophobicity of the nonpolar face is higher when the helixes are oriented by the snorkel algorithm than when oriented by the hydrophobic moment algorithm; this is also true for the unusual class Y amphipathic helical domains found in apoA-IV and A-I, which presumably also represent lipid-associating domains. On the other hand, for amphipathic helical domains with weak or zero lipid-affinity, the class G* found in the apolipoproteins and the class G amphipathic helixes found in 4-helix bundle proteins, the hydrophobicity of the nonpolar face is higher on average when the helix is oriented by the hydrophobic moment algorithm than when oriented by the snorkel algorithm. These results are consistent with the snorkel model in that they suggest that the snorkel orientation more accurately reflects the orientation of lipid-associating helixes than does orientation by the hydrophobic moment.

Each of the class A amphipathic helical domains in apoA-I and A-IV are exactly 22 residues in length (although two of the class Y amphipathic helical domains in apoA-IV are 44 residues in length). ApoE contains the longest and apoC-III the second longest continuous class A amphipathic helical domains among the apolipoproteins examined, with lengths of 64 and 28 residues, respectively. ApoC-I, C-II, and A-II contain continuous class A amphipathic helical domains with lengths of 26, 26, and 24 residues, respectively. It seems reasonable that the length of class A amphipathic helical domains may play a role in regulation of the relative affinity of individual helixes for different lipoprotein classes: longer helixes (e.g., apoE), depending upon local conditions, may preferentially target larger, less curved particles (triglyceride-rich lipoproteins); shorter helixes (e.g., A-I), again depending upon local conditions, may preferentially target the smaller, more highly curved particles (HDL).

In summary, we have shown that the predicted locations and properties of class A and class G* amphipathic helixes shown in Fig. 7 are in good agreement with the existing experimental data. We suggest that the limits of lipid-associating amphipathic helical domains can be more accurately defined by helical wheel-based algorithms than can the limits of amphipathic helixes involved in protein associations. In any case, the preliminary amphipathic helix map should prove useful as a guide for future experimentation. It provides, for example, a working model for the design of site-specific mutations to formally map the structure/function relationships of the different amphipathic helical domains in the exchangeable apolipoproteins.

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