A MOLECULAR THEORY OF LIPID–PROTEIN INTERACTIONS IN THE PLASMA LIPOPROTEINS

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1. Introduction

Lipid–protein interactions are of fundamental importance in the structure of biological membranes and of plasma lipoproteins. From previous studies [1–3] it seems reasonable to assume that the interactions between phospholipids and lipoprotein–protein (apoprotein) constituents are fundamental to the binding of neutral lipid by the plasma lipoproteins. For example, there is an insignificant binding of cholesteryl ester by the apoproteins of human HDL * in the absence of phospholipids. We have recently presented studies describing the probable location of phospholipid-binding site(s) in the MN-glycoprotein of the human red cell membrane [4–6] and of plasma lipoproteins [7–12]. Phospholipid-binding regions do not appear to be uniformly distributed along the length of the polypeptide chain [7–11,13]; certain fragments of apoLP-Ala, apoLP-Gln-I and apoLP-Gln-II preferentially bind phospholipid as compared to other fragments [7–11]. We have found that the binding of phospholipid is accompanied by circular dichroism changes that are consistent with an increase in the α-helical content of the protein or peptide [7–11]. Under identical conditions, peptide fragments that do not bind phospholipids show no changes in circular dichroism. We have therefore reexamined the known sequences (fig. 1) of the plasma apoproteins [14–16] to determine whether there are any structural features which might account for both the helical transitions and the binding of phospholipids.

Based on our findings, we present a new theory, suggesting that the apoproteins contain specific amino acid sequences with amphipathic regions that can assume the α-helical conformation and which possess the following properties: (a) a relatively large apolar face which associates with the fatty acid chains of phospholipids, and (b) a polar face in which side-chains of aspartate, glutamate, lysine and arginine are disposed so that negatively charged groups are at the center and positively charged groups at the periphery of this face. This configuration permits close orientation of the zwitterionic polar head group with the ion pairs on the protein. It also allows interaction of the carboxyl-terminal portion of the fatty acid chains with the hydrophobic face of the helix. Models are

* *Abbreviations*: VLDL, very low density lipoproteins; HDL, high density lipoproteins; apoprotein, a lipid-free protein component from a lipoprotein; apoLP-Ala and apoLP-Ser, two apoproteins from human VLDL, with carboxyl-terminal alanine and serine, respectively; apoLP-Gln-I (A-I), and apoLP-Gln-II (A-II), the two major apoproteins of human HDL, each with carboxyl-terminal glutamine.

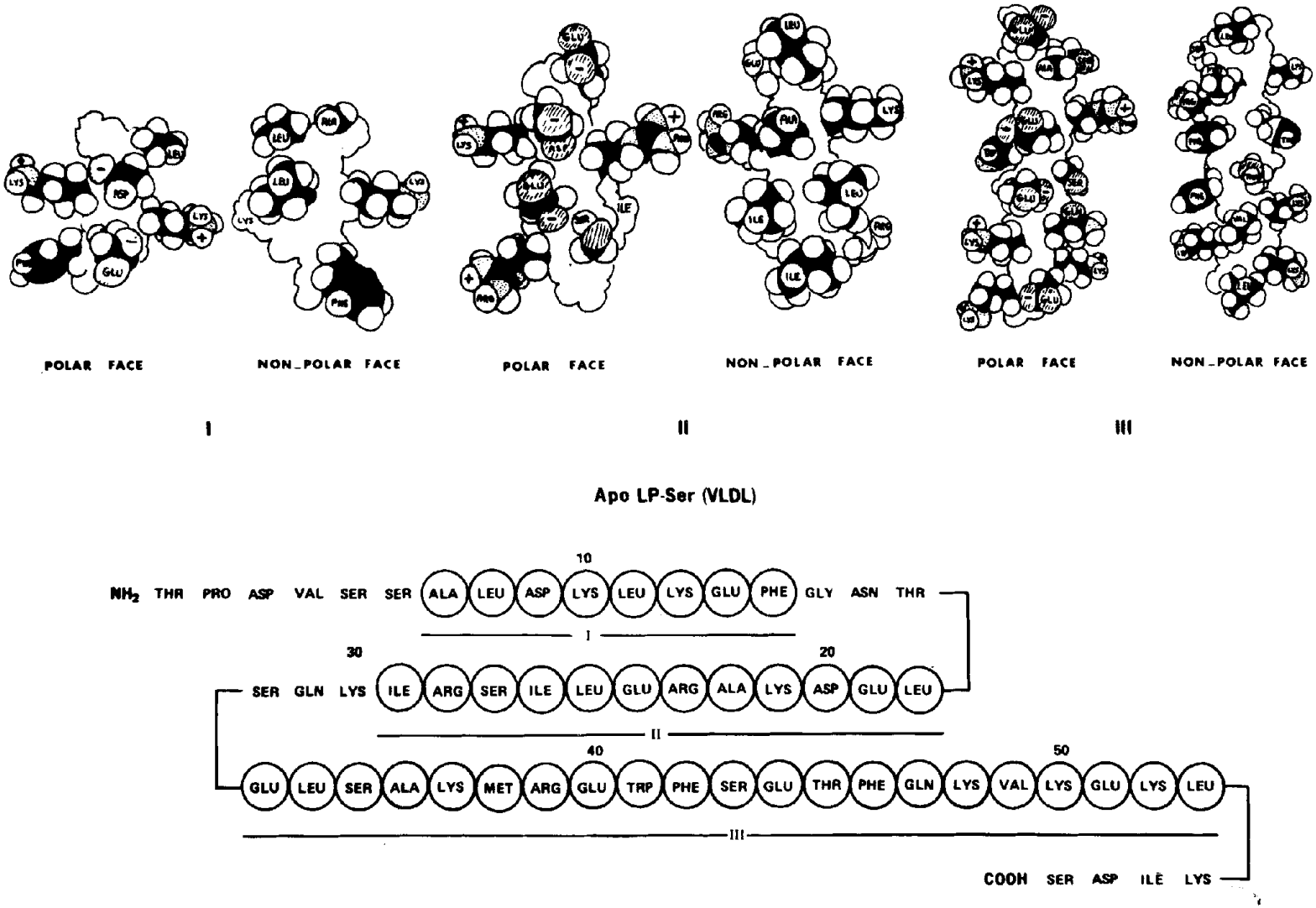
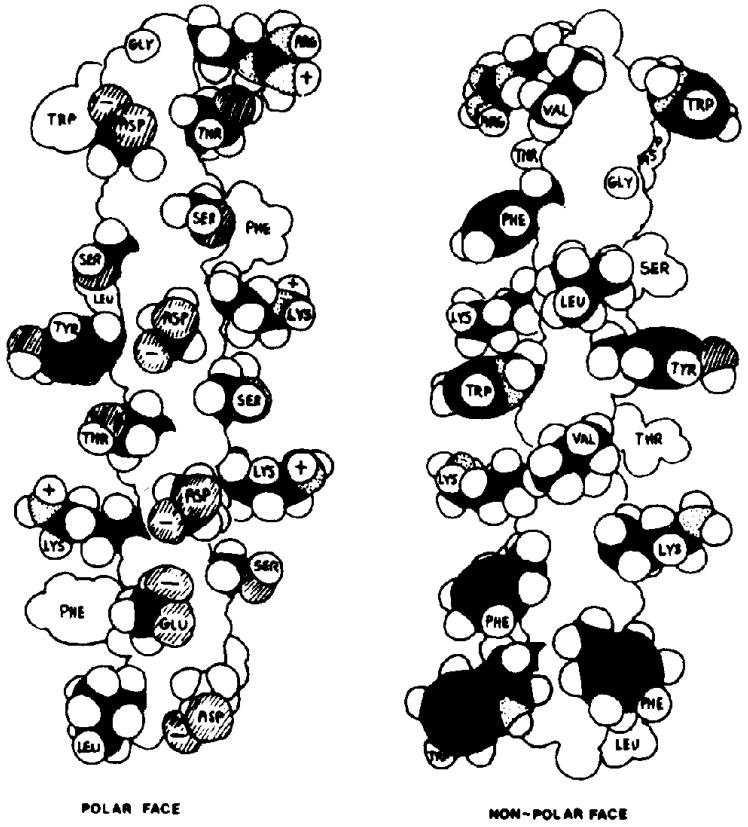
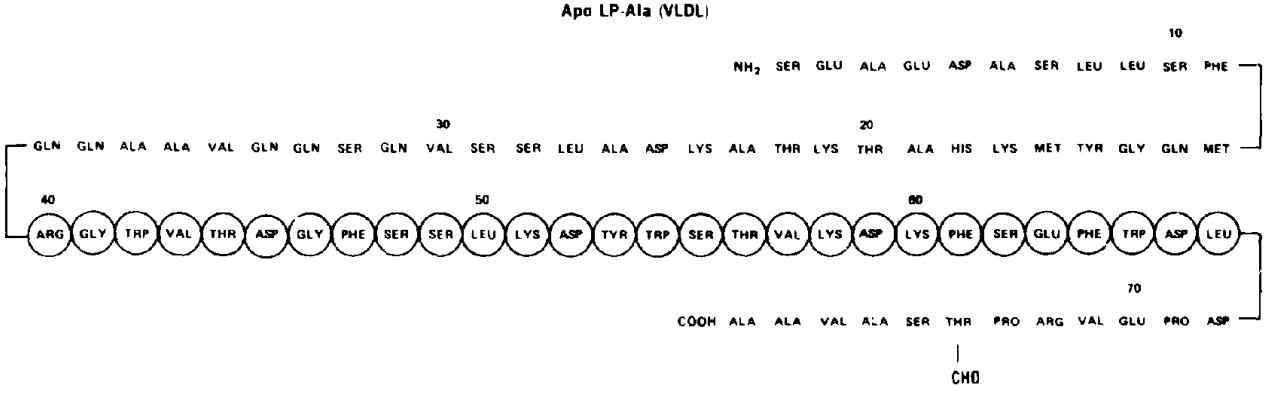
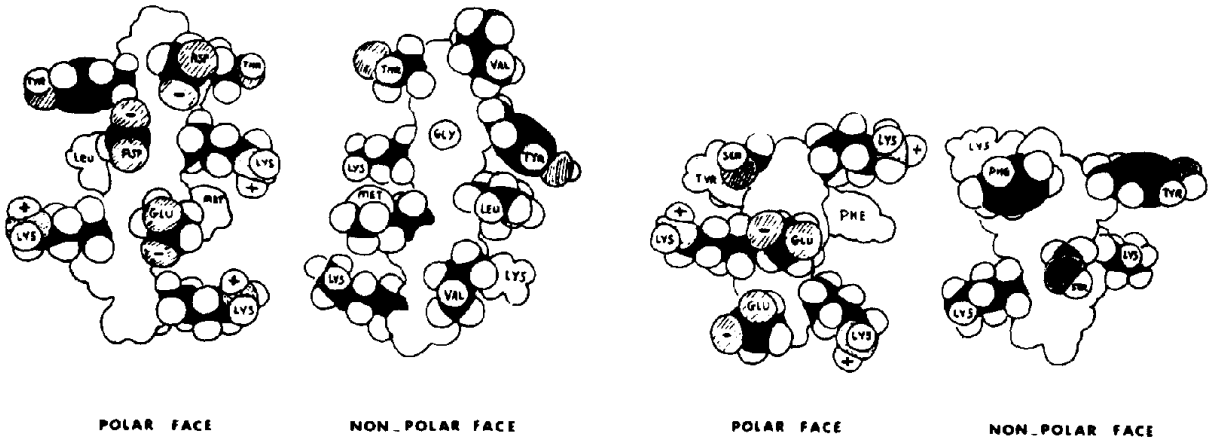


Fig. 1. Each helix is shown with its axis oriented parallel to the plane of the page and its N-terminal end towards the top of the page. Two views of each helix, rotated around the helix axis by 180° relative to one another are shown. The sequence of each apolipoprotein [14, 17, 18] is shown below the helical segments whose locations are indicated by the circles. A. ApoLP-Ser (VLDL and HDL); B. ApoLP-Ala (VLDL and HDL); C. ApoLP-Gln-II (HDL).


POLAR FACE
NON-POLAR FACE

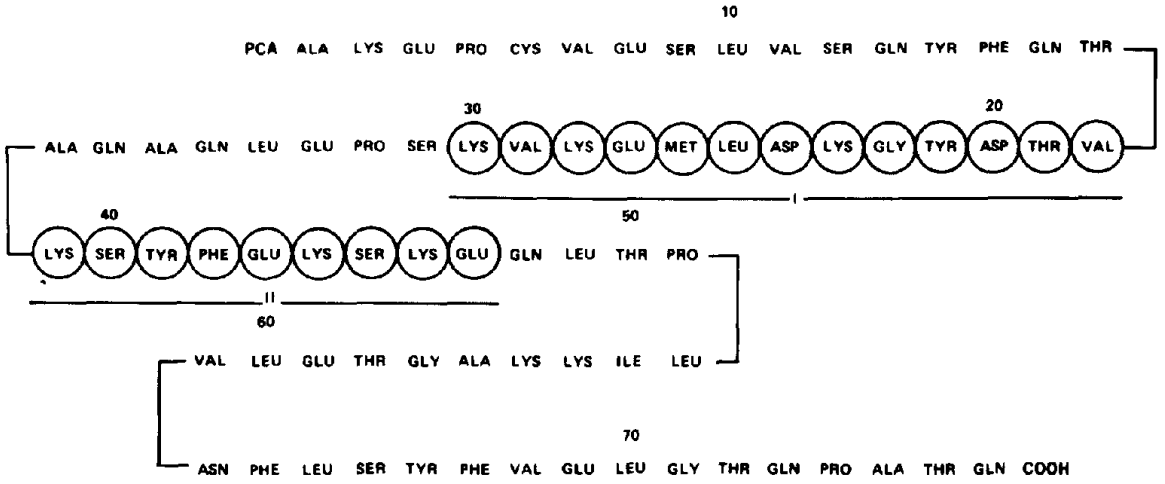

Apo LP-Ala (VLDL)
NH2 SER GLU ALA GLU ASP ALA SER LEU LEU SER PHE
GLN GLN ALA ALA VAL GLN GLN SER GLN VAL SER SER LEU ALA ASP LYS ALA THR LYS THR ALA HIS LYS MET TYR GLY GLN MET
ARG GLY TRP VAL THR ASP GLY PHE SER SER LEU LYS ASP TYR TRP SER THR VAL LYS ASP LYS PHE SER GLU PHE TRP ASP LEU
COOH ALA ALA VAL ALA SER THR PRO ARG VAL GLU PRO ASP
CHO

(Fig. 1b)



II

Apo LP-Gln II (HDL)

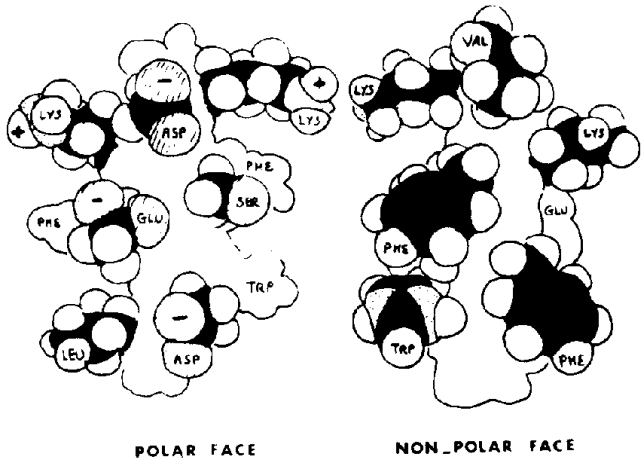


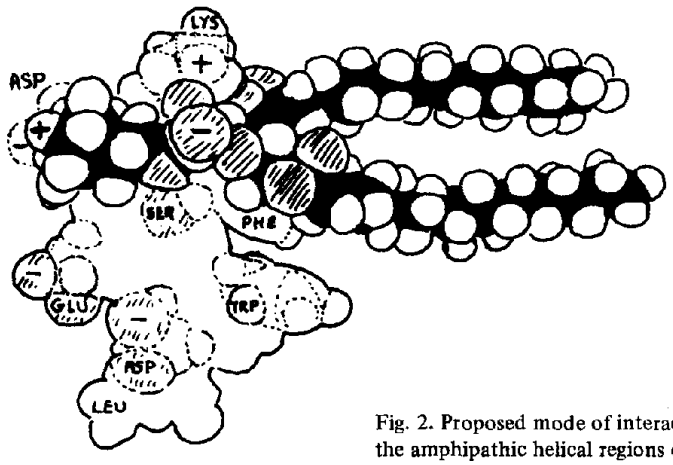
(Fig. 1c)

presented to support this amphipathic theory. The predicted location of amphipathic helical regions in three plasma apoproteins is consistent with available information about the location of phospholipid-binding sites in these molecules and their helical content as measured by circular dichroism [7–12].

2. Experimental

Helical segments of the apoproteins, apoLP-Ser, apoLP-Ala and apoLP-Gln-II were built with Ealing CPK-space-filling models. A right-handed helical backbone with 3.6 residues per turn was constructed. For each amphipathic region, amino acid residues were added onto the α-carbons in their proper order. Specific programs were designed for a computer search (J.P. Segrest and R.L. Feldman, unpublished results) of all reported amino acid sequences to determine the following: 1) the frequency of occurrence of amino acid sequences that will generate





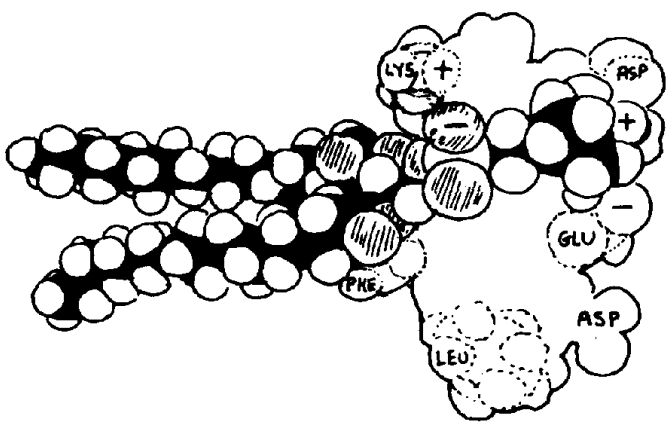


Fig. 2. Proposed mode of interaction between the amphipathic helical regions of the lipoproteins apoLP-Ser, apoLP-Gln-II and apoLP-Ala, and the zwitterionic phospholipids, phosphatidylcholine and phosphatidylethanolamine. Illustrated is an interaction between two phosphatidylcholine molecules and a portion of the amphipathic helical region (residues 57–67) from apoLP-Ala (upper figure). The phosphatidylcholine molecule in the middle figure is shown in electrostatic association with the first lysyl and first aspartyl residues from the amino-terminus of the helix (residues 58 and 59 in apoLP-Ala sequence of fig. 1B). The phosphatidylcholine molecule in the lower figure is in electrostatic association with the second lysyl and first glutamyl residues from the amino-terminus of the helix (residues 60 and 63). The upper helix has been rotated 90° to the left of its position in the upper figure (polar face) and the lower 90° to the right.

amphipathic helices, and 2) the frequency of occurrence of oppositely charged ion pairs that are in close topographic proximity in the amphipathic region. It should be noted that such pairs are not necessarily adjacent to each other in the linear sequence of the molecule.

3. Results and discussion

The first structural feature that we wish to point out is the relatively frequent occurrence of the apoprotein sequences of regions in which charged residues are juxtaposed with apolar residues in a sterically confined locus, e.g., residues 40–68 of apoLP-Ala. We have detected six such sequences in the apoproteins shown in fig. 1. We have constructed space-filling models of these regions, making the assumption that the amino acid residues in these segments are in an α-helix. Each α-helix is shown to be amphipathic, that is, it contains two clearly defined faces, one which is polar and the other apolar or hydrophobic. Perutz et al.[17]have previously observed from X-ray crystallography that apolar amino acid residues may be buried along the edge of an α-helical sequence. An unusual structural feature of the model presented here is that the hydrophobic face of each helix is not buried along an edge or confined to a narrow strip (fig. 2), but rather occupies fully one-half of the cylindrical surface of the helix. A computer search of all reported amino acid sequences indicates that amphipathic regions occur in 38 of approximately 200 different classes of protein (in this analysis, proteins such as cytochromes are defined as a class). Highly helical proteins such as hemoglobin and myoglobin do not necessarily contain amphipathic regions, although the probability of finding such a region is theoretically higher in helical proteins, since amphipathic segments must in general be related to an α-helix.

An important topographic feature of these helices is the distribution of charged amino acid residues on the polar face of the amphipathic segment. The frequency of occurrence of oppositely charged side chains which are sterically near each other (e.g., Glu-44 and Lys-48, of apoLP-Ser, fig. 1), in amphipathic regions is significantly higher for the three apoproteins shown in fig. 1 than for the amphipathic regions in other proteins whose sequences have been reported. Our computer search indicates a total of 76 such ion pairs occur in amphipathic regions of proteins of known amino acid sequence. Eighteen of these ion pairs occur in the three apoproteins shown in fig. 1.

In addition, the distribution of charged amino acid residues on the polar faces is quite striking. The negatively charged residues, Glu and Asp, invariably occur in a narrow strip along the center of the polar face, while the positively charged residues, Lys and Arg are located on the lateral edge of the polar faces, alternating from side to side. In general, the sums of the negative and positive residues on each segment of α-helix are equal. While members of ion pairs are not necessarily adjacent in the primary amino acid sequence, the members of such pairs become topographically close when the region is placed in an α-helix. There are at least two ion pairs which are not associated with an amphipathic helical region, viz., in apoLP-Ala, residues 24, 25 and in apoLP-Gln-II, residues 3 and 4. Thus the occurrence of adjacent pairs of zwitterions does not necessarily result in the generation of amphipathic helices. There must be juxtaposed in the amino acid sequence apolar residues in a sterically confined region. As a corollary of this model, the positive charged Lys and Arg are at the periphery so that their relatively apolar side chains are available for hydrophobic interactions with phospholipids.

The model for phospholipid–protein interactions suggested here is one in which a helical region of the apoprotein is half-buried at the surface of the phospholipid structure, such as a micelle. This permits close steric contact between a charged amino acid side-chain and the oppositely charged groups of the phospholipid (fig. 2). The long axis of the protein would be oriented perpendicular to the hydrocarbon chains of the phospholipid which is consistent with X-ray diffraction studies of HDL [18, 19]. The non-polar face of the helix would be buried in the hydrocarbon milieu of the micelle and the polar face exposed to the polar ends of the phospholipids as well as to the aqueous surroundings. The amphipathic model presented here is consistent with binding studies between phospholipids and peptide fragments of apoLP-Gln-II and apoLP-Ala [7. 11, 13]. If the degree of hydrophobicity of the nonpolar faces of all amphipathic regions selected by the computer search are calculated [6],

each of the six regions from the apoproteins shown in fig. 1 ranks in the top one-third of the list. Each is significantly more hydrophobic than the region of the MN-glycoprotein which presumably spans the red cell membrane [4–6].

It is possible that the occurrence of ion pairs along the middle and lateral portions of the 6 amphipathic regions of the apoproteins (fig. 1) are related to lipid-binding in some manner other than as postulated here. However, in lieu of further experimental data to the contrary, one has to be impressed with the topographical fit of this model to the topography of the phospholipid molecules. There are several other potential important aspects of lipid–protein interaction that are not covered by this theory, including the effects of adding neutral lipid, lipid–lipid interactions and the influence of long range forces within an apoprotein. Short helical segments, such as those illustrated in fig. 1 might require stabilization. It seems plausible to assume that there are additional cooperative and/or long-range effects necessary for the phospholipid-binding by the amphipathic sequences. In addition, quite different mechanisms of lipid–protein interactions may also be operative.

Acknowledgements

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