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Proposed folding pattern for apolipoprotein A-II based on a structural analogy with uteroglobin

(sequence analogy/computer modeling/tertiary structure/lipid binding)

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ABSTRACT **The tertiary structure observed in the crystalline state for uteroglobin, a small steroid binding protein, is used as a template to build an approximated model for apolipoprotein A-II. The presence of four proline residues and four hydrophobic clusters located at similar positions in apolipoprotein A-II and uteroglobin is taken as the major source of stability in such tertiary structures. A brief description of plausible specific binding sites appearing on the model of apolipoprotein A-II is given. It is suggested that the internal cavity and the four surface pockets observed for uteroglobin and postulated for apolipoprotein A-II might be used to insure specific binding of triglycerides, phospholipids, or cholesterol.**

The folding of apolipoproteins and their specific interactions with triglycerides, phospholipids, free cholesterol, and cholesteryl esters play a crucial part in the metabolism of lipoproteins (1, 2). Although it has been suggested (3) and observed (4) that the presence of amphipathic α-helices in apolipoproteins is a prerequisite for binding to lipids, there does not exist presently a detailed description, at the molecular level, of specific lipid–protein binding sites. The lack of information in this field is mainly due to the failure, until now, to obtain crystalline material to solve the problem by x-ray diffraction analysis. Thus, another way to obtain valuable structural information is clearly needed.

In this paper, we point out the possibility of obtaining a detailed molecular description of putative lipid binding sites on apolipoproteins A-I, A-II, C-I, C-II, C-III, etc., by using the x-ray structural information available about rabbit uteroglobin, which is a small steroid binding protein. The tertiary structure of this little protein, 70 amino acid residues long, was recently refined at 1.34-Å resolution (5). In the crystalline state, uteroglobin is a dimer (Fig. 1), and its tertiary structure consists of 8 α-helices arranged in an antiparallel framework. The two monomers are held together by two disulfide bridges. Human apolipoproteins C-I, C-II, and C-III are single polypeptide chains made of 57, 78, and 79 residues; human apolipoprotein A-II is a dimer made of two identical chains of 77 residues connected by one intermolecular disulfide bridge; and human apolipoprotein A-I is a single polypeptide chain containing 243 amino-acid residues. The similarities between the length of the uteroglobin monomer and the lengths of apolipoproteins C-I, C-II, and C-III and the monomer of apolipoprotein A-II, as well as the high α-helical content of these molecules, led us to look for deeper possible structural analogies between these different entities, which are known to bind molecules showing some similarities (triglycerides, phospholipids, steroids, etc.). Indeed, looking at sequence similarities, we discovered at key positions the presence of identical amino acid residues that play a primary role in assuring the correct folding of uteroglobin. In this paper, we show how this information could be used to build an approximated model for apolipoprotein A-II and to give a preliminary description of the putative lipid binding sites that appear on this model.

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Molecular Modeling of Human Apolipoprotein A-II by Using the Tertiary Structure of Rabbit Uteroglobin as Template

Primary and Secondary Structures. The search for structural analogy between uteroglobin and the various apolipoproteins by hydrophobic cluster analysis (6) showed that it is apolipoprotein A-II that displays the strongest similarity with uteroglobin. Therefore, we choose to build an approximated model for this latter molecule, using the tertiary structure of uteroglobin as template.

In the sequence of uteroglobin (Fig. 2), five proline residues located at positions 4, 18, 30, 49, and 67 play a crucial part in delineating the four α-helical segments (Figs. 3 *Upper* and 4 *Upper*). In apolipoprotein A-II, four proline residues occupy positions 5, 32, 51, and 74 (Fig. 2). It appears, therefore, that proline residues are located in the sequences of uteroglobin and apolipoprotein A-II at similar positions with the exception of Pro-18, which is not present in apolipoprotein A-II.

The distribution of hydrophobic residues along the sequence of the two proteins is also quite similar (Fig. 3). The presence of hydrophobic clusters, giving rise to amphipathic helices delineated by proline residues is clearly apparent in both cases. When the sequence of apolipoprotein A-II is aligned on the tertiary structure of uteroglobin, one observes the presence of four hydrophobic clusters spatially located in apolipoprotein A-II at similar positions as those occurring in uteroglobin (Fig. 4).

Stability of the Tertiary Structure. In the tertiary structure of the uteroglobin monomer, the four amphipathic α-helices are arranged in such a way that almost all hydrophobic residues are located on the same side of the molecule, giving rise to a well-defined hydrophobic surface (Fig. 4 *Upper*). The stability of uteroglobin in water and in the crystal is brought about by the formation of a dimer (Fig. 1) in which the hydrophobic surfaces of the monomers are buried inside the molecule, while hydrophilic residues are located on the surface in contact with water. Furthermore, the amino- and carboxyl-terminal ends of the two monomers are linked together by two disulfide bridges (Cys-3 to Cys-69′ and Cys-3′ to Cys-69).

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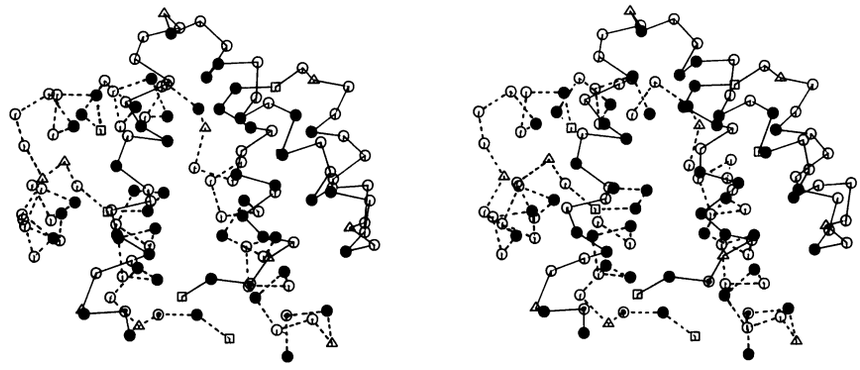

FIG. 1. Stereoview of the tertiary structure adopted by the dimer of uteroglobin in the crystalline state. ●, Hydrophobic amino acid residues; △, proline; □, glycine; ⊛, cysteine; ○, others.

The alignment of the sequence of apolipoprotein A-II on the tertiary structure of uteroglobin (Fig. 4 *Lower*) shows that the hydrophobic residues of apolipoprotein A-II are also located on the same side of the molecule. However, for apolipoprotein A-II the upper surface is not entirely hydrophobic. A few polar residues, Ser-9, Gln-13, Ser-45, and Glu-59, replace the hydrophobic residues Val-9, Leu-13, Leu-45, and Leu-59 encountered in uteroglobin. On the other hand, the hydrophobic contact between helix 1 and helix 4 seems to be reinforced by the presence of Val-11, Ile-53, Leu-60, and Val-61 instead of Glu-11, Arg-53, Thr-60, and Gln-61. The formation of a dimer for apolipoprotein A-II, following exactly the folding scheme of uteroglobin, has to accommodate the replacement of Phe-6 by Cys-6 and the closure of a disulfide bridge between Cys-6 and Cys-6′. The distance between C_β^6 and $C_\beta^{6'}$ in uteroglobin is 10.2 Å. Although this distance is more than twice the distance required to allow the formation of a S—S bond (3.9 Å), it is clear that small distortions and a possible deletion or insertion occurring, for example, in the structure of the bends between helices could enable the closure of a disulfide bridge between Cys-6 and Cys-6′.

Specific Lipid Binding Sites. A very peculiar property of the particular assembly encountered in the tertiary structure of uteroglobin is the occurrence inside the molecule of a great cavity. The size, shape, and chemical characteristics of this cavity suggest that it might be the steroid binding pocket, although it has not been possible to date to confirm experimentally this hypothesis. With the present capability of x-ray structural analysis, the electron density observed in the cavity has been interpreted as being due to the presence of 14 not-well-ordered water molecules (5).

The internal cavity that probably should result from the assembly of the dimer of apolipoprotein A-II should be a little less hydrophobic than the one observed in uteroglobin and more accessible to solvent because of the presence of only one instead of two disulfide bridges. As triglycerides and phospholipids are less hydrophobic and more flexible than steroids, it is quite possible that the hypothetical cavity briefly described here might be adequate to accommodate at best a given lipid molecule.

The calculation of the molecular surface of the uteroglobin dimer (7) indicated that, in addition to the central cavity, there are four surface pockets located between helix 1 and helix 4 or 4′ and between helix 1 and helix 2 or 2′. It was suggested that these pockets could play a role in providing intermediate attachment sites (5). For example, the association of two uteroglobin dimers, each facing two surface pockets, creates an interstitial cavity that could readily accommodate a progesterone molecule.

A similar scheme of interaction might be an efficient way to link together various apolipoprotein subunits using triglycerides, phospholipids, or cholesterol as intermediate links.

Conclusions

In this paper we suggest that the tertiary structure observed for uteroglobin in the crystalline state could be used as an initial template to build an approximate model for apolipo-

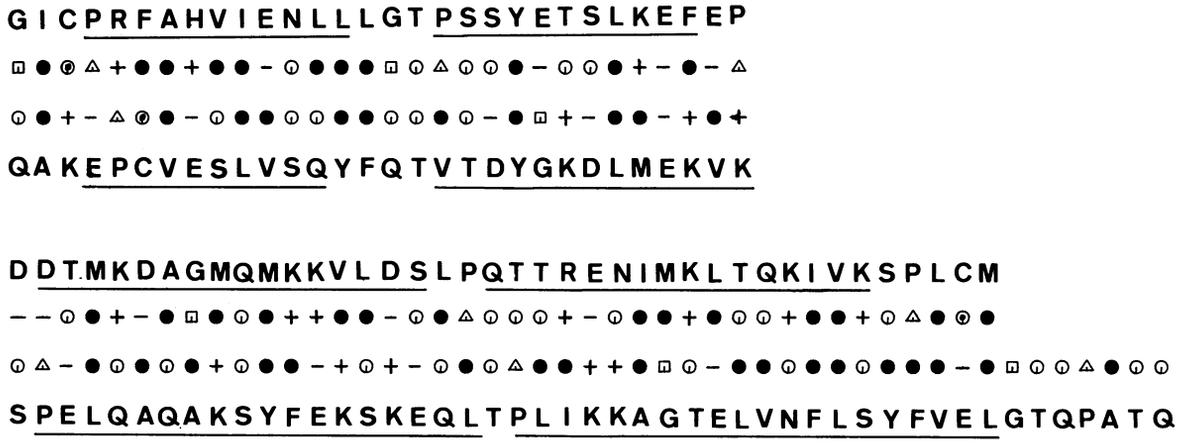

FIG. 2. Comparison between the amino acid sequences of uteroglobin (*Upper*) and apolipoprotein A-II (*Lower*). Classes of residues: ●, hydrophobic; −, acidic; +, basic; □, glycine; △, proline; ⊛, cysteine; ○, others. Helical regions are underlined. For uteroglobin, they correspond to the helices observed in the crystalline state, while for apolipoprotein A-II, they were predicted from the algorithm of Chou and Fasman.

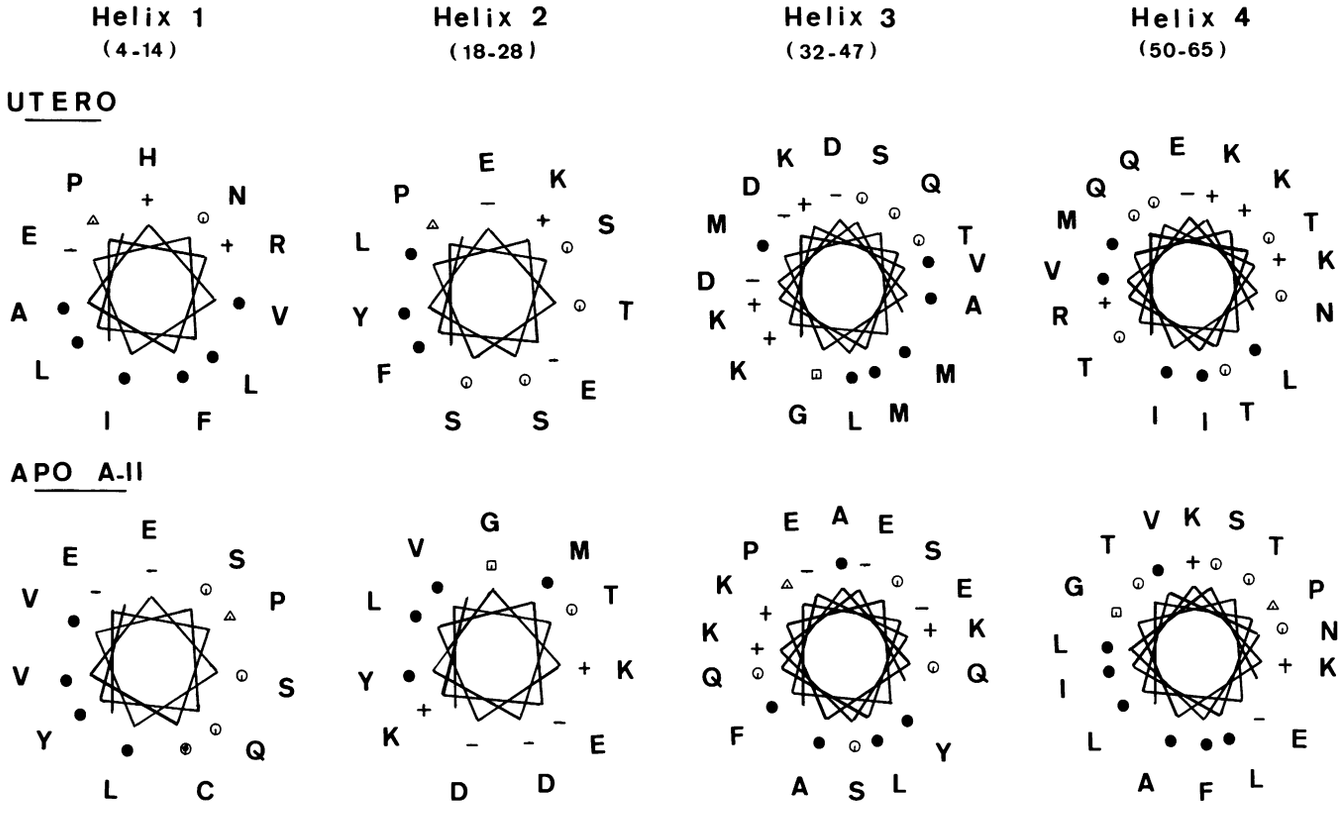

FIG. 3. Comparison between the amino acid sequences of the α-helical regions observed in uteroglobin (*Upper*) with the corresponding ones assumed in apolipoprotein A-II (*Lower*) presented as Edmunson helical wheels. It is noteworthy that hydrophobic arcs (●●●) are located at similar positions on the wheels.

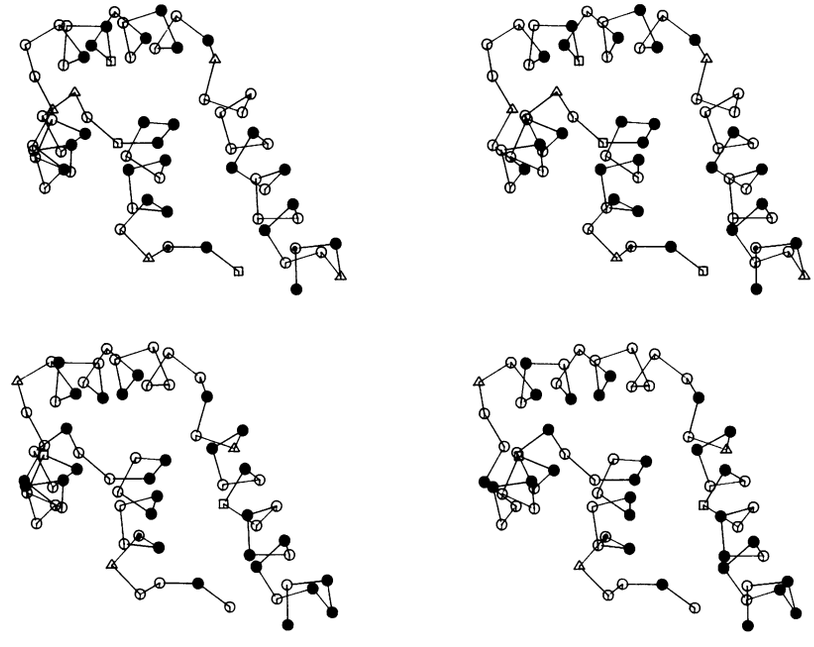

FIG. 4. Comparison between the folding pattern observed for uteroglobin (*Upper*) and apolipoprotein A-II (*Lower*) when the two sequences are aligned on the tertiary structure observed for uteroglobin in the crystal.

protein A-II. The existence of many common structural features between apolipoprotein A-II and other apolipoproteins (A-I, C-I, C-II, C-III, etc.) (8) prompt the possibility that the same scheme of folding and interaction might be useful for these additional molecules also. If such is the case, a major step towards the elucidation of the factors governing the stability of lipoproteins and the exchange of particles between them might be achieved.

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1. Osborne, J. C. & Brewer, H. B., Jr. (1977) *Adv. Protein Chem.* **31,** 253–327.
2. Pownall, H. J. & Gotto, A. M., Jr. (1983) in *Atherosclerosis: Mechanisms and Approaches to Therapy*, ed. Miller, N. E. (Raven, New York), pp. 125–141.
3. Segrest, J. P., Jackson, R. L., Morrisett, J. D. & Gotto, A. M., Jr. (1974) *FEBS Lett.* **38,** 247–253.
4. Pownall, H. J., Pao, Q., Hickson, D., Sparrow, J. T. & Gotto, A. M., Jr. (1982) *Biophys. J.* **37,** 175–177.
5. Morize, I., Surcouf, E., Vaney, M. C., Epelboin, Y., Buehner, M., Fridlansky, F., Milcrom, E. & Mornon, J.-P. (1987) *J. Mol. Biol.* **194,** 725–739.
6. Gaboriaud, C., Bissery, V., Benchetit, T. & Mornon, J.-P. (1987) *FEBS Lett.* **224,** 149–155.
7. Vaney, M. C., Surcouf, E., Morize, I., Cherfils, J. & Mornon, J.-P. (1985) *J. Mol. Graphics* **3,** 123–124.
8. Luo, C.-C., Li, W.-H., Moore, M. N. & Chan, L. (1986) *J. Mol. Biol.* **187,** 325–340.