THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1991 by The American Society for Biochemistry and Molecular Biology, Inc.
Vol. 266, No. 24, Issue of August 25, pp. 16120-16127, 1991
Printed in U.S.A.

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Differentiation of Lipid-associating Helices by Use of Three-dimensional Molecular Hydrophobicity Potential Calculations*

(Received for publication, October 24, 1990)

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Several types of lipid-associating helices exist: transmembrane helices such as in receptor proteins, pore-forming helices in ion channel proteins, fusion-inducing peptides in viral proteins, and amphipathic helices such as in plasma apolipoproteins. In order to propose a classification of these helices according to their molecular properties, we introduce the concept of molecular hydrophobicity potential for such helical segments. The calculation of this parameter for α-helices enables the visualization of the hydrophobic and hydrophilic envelopes around the peptide and their three-dimensional representation by molecular graphics.

We have used this parameter to differentiate between pore-forming helices with a hydrophobic envelope larger than the hydrophilic component, membrane-spanning helices surrounded almost entirely by an hydrophobic envelope, fusiogenic peptides with an hydrophobicity gradient both around the helix and along the axis, and finally, amphipathic helices with a predominantly hydrophilic envelope. The structure of the lipid-protein complexes is determined by a number of different interactions: the hydrophobic interaction of the apolar faces of the helices with lipids, the polar interaction of the hydrophilic sides of different helices with each other, and the interaction of hydrophilic residues with the aqueous solvent. The relative magnitude of the hydrophobic and hydrophilic envelopes accounts for the differences in the structure of the lipid-protein complexes. Purely hydrophobic interactions stabilize transmembrane helical segments, while hydrophobic interactions with the lipid phase and with each other are involved in the stabilization of the pore-forming helices. In contrast, both hydrophobic interactions with the lipids and hydrophilic interactions with the aqueous phase contribute to the arrangement of amphipathic helices around the edges of the discoidal lipid-apoprotein complexes.

The interaction of protein segments with lipid structures such as phospholipid mono- or bilayers, which are the constituents of cellular membranes and plasma lipoproteins, involves different types of forces. Besides the electrostatic and van der Waals interactions, hydrophobic forces also play an important role in the stabilization of such complexes.

The hydrophobicity of a molecule is mostly represented by a single number, either log P, corresponding to its partition coefficient between water and octanol, or the Hansch's π constant derived from log P data (1). However, this representation of hydrophobicity becomes quite inadequate in the analysis of complex interactions between proteins and lipids (2). The "hydrophobic moment" of Eisenberg and McLachlan (3) attempted to elaborate on this concept. The hydrophobic moment has been extensively applied to the definition of amino acid amphiphilicity, reflecting the degree of hydrophobic polarization in a molecule consisting of several more or less hydrophobic moieties.

An additional step in the three-dimensional molecular representation of the hydrophobicity profile of a molecule was proposed by Cohen (4) and by Fauchère (5), in order to visualize the lipophilic and hydrophilic regions on the van der Waals surface. For this purpose, the concept of "molecular hydrophobicity potential," based either on the fractionation of the molecule into chemical fragments (6) or on the summation of hydrophobic atomic parameters proposed by Ghose and Crippen (7), was used. This concept has enabled the representation of molecular hydrophobicity profiles for 20 amino acids (4).

In this paper we extend this approach to the calculation of the hydrophobicity potential around lipid-associating helices, allowing for the prediction of a three-dimensional representation of this potential around the protein segment, by molecular graphics.

The lipid-associating helices belong to several types and have various modes of association with phospholipid bilayers (8), yielding complexes with different structural and functional properties. Based upon the structure of these lipid-protein complexes, the helices can be classified in different groups. One can differentiate between transmembrane helices anchoring the receptor proteins in cellular membranes (9–11), pore-forming helices such as in the ion channel proteins (12), fusion-inducing helices present in the virus proteins (13, 14), and amphipathic helices, occurring in plasma apolipoproteins and able to generate discoidal particles upon interaction with phospholipid liposomes (15–17). This classification relies primarily on the structure of the complexes, while the other properties of these helices, such as composition, sequence, or physicochemical behavior, do not help explain their mode of association with lipids.

The aim of this paper is therefore to propose a classification of the lipid-associating helices, based upon their molecular hydrophobic potential (MHP)[1] and to define the mean hydrophobic and hydrophilic angles of contours that represent the isopotential lines around the helices. The definition and calculation of the molecular hydrophobic potential will be dem-

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‡ Maître de Recherche of the Belgian Fonds National de la Recherche Scientifique.

[1] The abbreviations used are: MHP, molecular hydrophobic potential; apoA-I, apolipoprotein A-I; NDV, Newcastle disease virus.

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The EMBO Journal vol.10 no.10 pp.2747-2755, 1991

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Theoretical and functional analysis of the SIV fusion peptide

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Communicated by A.Burny

The fusion domain of simian immunodeficiency virus (SIV) envelope glycoproteins is a hydrophobic region located at the amino-terminal extremity of the transmembrane protein (gp32). Assuming an α helical structure for the SIV fusogenic domain of gp32 in a lipid environment, theoretical studies have predicted that the fusion peptide would insert obliquely in the lipid bilayer. This oblique insertion could be an initial step of the fusion process by disorganizing locally the structure of the lipid bilayer. We have tested this hypothesis by selectively mutagenizing the SIV gp160 expressed via a vaccinia virus vector, to alter the theoretical angle of insertion of the fusion peptide. The fusogenic activity of the wild-type and mutant glycoproteins was tested after infection of T4 lymphocytic cell lines by the recombinant vaccinia virus, and measure of syncytia formation. Mutations that modified the oblique orientation reduced the fusogenic activity. In contrast, mutations that conserve the oblique orientation did not alter the fusogenic properties. Our results support the hypothesis that oblique orientation is important for fusogenic activity.

Key words: fusion protein/fusogenic activity/gp32/SIV/ theoretical analysis

Introduction

The molecular mechanisms involved in cell recognition and penetration by enveloped viruses remain poorly understood. In some viral systems, specific receptors have been identified (Crowel and Lonberg-Holm, 1986; Marsh and Helenius, 1989). The CD4 molecule (Klatzman *et al.*, 1984; Dalgleish *et al.*, 1984) has been identified as the receptor for HIVs and SIVs. The interaction between CD4 and the external HIV glycoprotein (gp120) occurs with high affinity (affinity constant $=4\times10^{-9}$; Lasky *et al.*, 1987). As a consequence, T4 cells with their large numbers of CD4 molecules and cells of the monocyte−macrophage lineage which present smaller number of CD4 receptors are key targets of HIV infection *in vivo*. As demonstrated by transfection experiments, once entry has been provided to the viral genome, many cytoplasmic environments can accommodate viral replication. CD4 binding is a very rapid phenomenon, therefore fusion of the viral envelope with the plasma membrane is probably the major rate-limiting step among the initial events of HIV infection. A better understanding of the mechanisms of these early steps may provide insight for potential ways to interfere with cell−virus interaction and thus inhibit viral infection.

SIV glycoproteins, like those of other viruses, are synthesized as a 160 kDa polyprotein precursor, which is further cleaved into 2 subunits remaining non-covalently linked (gp120 and g41 for HIV, gp120 and gp32 for SIV). The endoproteolytic cleavage of HIV gp160 is essential for infectivity (McCune *et al.*, 1988). Mutagenesis studies have identified functional domains in HIV glycoproteins (Kolwalski *et al.*, 1987). The surface glycoprotein (gp120) contains the receptor-binding domain, while the transmembrane (gp41 for HIV, gp32 for SIV) protein contains a hydrophobic domain which anchors the surface−transmembrane protein complex in the viral envelope and host−cell membrane. Another highly hydrophobic domain conserved in all syncytia-inducing viruses has been located at the amino-terminal extremity of the transmembrane protein. By homology with fusion peptide of other fusogenic viruses (orthomyxoviruses and paramyxoviruses), this domain was proposed to be the fusion peptide of HIV and SIV (Gallaher, 1987). Mutational analysis of this region confirmed its importance in the fusion process—insertion mutations (Kowalski *et al.*, 1987) or points mutations (Felser *et al.*, 1989; Freed *et al.*, 1990) introduced in the amino-terminus extremity of HIV gp41 blocked the fusion event. Mutagenesis studies of SIV gp32 also supported a role for this hydrophobic region in cell fusion (Bosch *et al.*, 1989).

Interaction of viral fusion peptides with host−cell membranes was recently modeled by computer analysis (Brasseur *et al.*, 1990; Brasseur, 1990). For all syncytia-inducing paramyxo- and retro-viruses, the complete amino acid sequence of the envelope glycoproteins was analyzed by the Eisenberg procedure (Eisenberg *et al.*, 1982). In all cases, the region immediately upstream of the cleavage site consisted of a highly hydrophilic region immediately followed by a hydrophobic cluster, the so-called fusion peptide. The mode of insertion of the fusion peptide into the lipid layer was theoretically assessed assuming that the peptide under consideration adopted α helical structure in the lipid environment. The angle of insertion was calculated as the position in which the line joining the hydrophobic and hydrophilic centers in the assumed α-helical configuration is perpendicular to the interface.

Computer analysis led to the conclusion that the fusogenic helices were obliquely oriented with respect to the lipid−water interface. This oblique orientation (found for every viral pH-independent fusion peptide analyzed to date) was proposed to locally disorganize the structure of the lipid bilayer and to generate new lipid phases which are thought to be associated with initial steps of membrane fusion. These theoretical assumptions about structure and orientation of the

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The hydrophobic effect in protein folding

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ABSTRACT In this review of protein folding we consider the noncovalent interactions existing between atoms or molecules at the molecular level. The electrostatic, Van der Waals, hydrogen bonding, and hydrophobic interactions are described and their contribution to protein conformation is discussed. The growing interest in the hydrophobic effect arises from its importance in the protein folding process, and a semiempirical simulation of the free energy of solvation is proposed. In most proteins, the different forces we describe contribute to the stability of the protein conformation in a complex way. However, in the case of the apolipoproteins and cytochrome C_{551}, the energetic contributions are easily distinguished. For this reason, these proteins are used to illustrate the importance of the different energy fields.— Lins, L., Brasseur, R. The hydrophobic effect in protein folding. *FASEB J.* 9, 535-540 (1995)

Key Words: *hydrophobicity · solvation energy*

BECAUSE THE 3-DIMENSIONAL STRUCTURE of a protein is determined by its amino acid sequence, the knowledge of this sequence alone should in principle enable prediction of the folded protein conformation. Such an ab initio calculation of the precise protein structure from amino acid sequence unfortunately is not yet feasible, although several attempts to develop such a method were published recently (1). A number of procedures, however, are available to predict the protein secondary structure, based on the statistical calculation of the tendency of individual amino acids to either promote or disrupt an α-helical, a β-sheet, a β-turn, or a random conformation (2, 3).

The side chains of polar amino acid residues can be considered as amphiphilic or biamphiphilic molecules consisting of a hydrophilic headgroup (backbone and/or polar extremity) and a hydrophobic segment of variable length (4). The amphiphilic character of the amino acid side chains is therefore similar in many respects to that of lipids and detergents (**Fig. 1**). Amphiphilic molecules can spontaneously organize into compact structures within the core of multimolecular systems. In this type of configuration, hydrophobic domains pack closely together to form the core of the structure and the hydrophilic headgroups point toward the polar aqueous phase. The segregation between hydrophobic and hydrophilic phases is the basic principle underlying the formation of biological membranes, lipid bilayers, and micelles (5). In this paper, we propose that the same principle can apply to the folding of soluble proteins (4). Indeed, the structure of these proteins can be visualized as hydrophobic core surrounded by a surface layer consisting of the polar headgroups of some of their amino acid constituents. In the course of its folding process, a protein tends toward a compact limit structure with a minimal area for the interface between hydrophobic core and hydrophilic surface. A new approach to the mechanisms underlying the folding process consists, therefore, of a stepwise analysis of the changes to this interface.

The physical nature of noncovalent interactions between atoms is fairly well understood for individual molecules in the gaseous or regular solid phase, but not in liquids because of the complex and variable interactions between molecular clusters in the liquid state. This is especially important for proteins, as the study of their folded conformations is performed mostly in an aqueous environment. In an aqueous solvent, intermolecular forces are determined mainly by the specific properties of the solvent molecule rather than by the intermolecular forces themselves. The strong interaction of water with ions, dipoles, and hydrogen bond donors or acceptors abolishes most of the intermolecular interactions that would exist between such groups, either in vacuum or in a nonpolar solvent. The hydrophobic interaction, the major noncovalent force in water, arises more from the strong interactions between water molecules than from direct interactions between solute molecules. Here we review the different energies involved in protein structuration. The "classical" energies (electrostatic, Van der Waals, and hydrogen bonding energies) are defined and quantified and particular emphasis is put on the hydrophobic effect. A new semiempirical equation for solvation energy is proposed and the importance of each energy field in protein structure is illustrated in three examples of lipid-interacting and soluble proteins.

ATOMIC INTERACTIONS INVOLVED IN PROTEIN STRUCTURATION

We propose to describe four noncovalent interactions, including electrostatic and Van der Waals interactions, hydrogen bonds, and hydrophobic force, that are thought to be involved in the folding and stabilization of protein structure as they are in lipid membrane organization.

Electrostatic forces

Electrostatic interactions take place between charged particles. The energy change as a function of the distance between two point charges that come close together is given by Coulomb's law:

$$Eelec_{ij} = \frac{q_i q_j}{\epsilon \, Dij}$$

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[2]Abbreviation: MHP, molecular hydrophobicity potential.

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Peptides in membranes: tipping the balance of membrane stability

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This review describes a class of peptides that associate with lipids in membranes and are commonly known as 'oblique-orientated peptides'. Owing to an asymmetric distribution of hydrophobic residues along the axis of the α-helix, such peptides can destabilize membranes or lipid cores, thereby facilitating such cellular processes as vesicular fusion or protein transport across subcellular compartments, as well as remodelling of lipid cores.

MEMBRANE FUSION mediates a range of intra- and intercellular functions, in addition to playing a role in lipid metabolism. These functions include cell signalling events, protein translocation, exocytosis and cell–cell fusions (e.g. spermatozoid–egg fusion)[1,2]. Fusion events, such as the entry of enveloped viruses into host cells, or virally induced cell–cell fusions that lead to cell death, have also been implicated in infectious diseases[3–5]. The destabilization of neuronal membranes in Alzheimer's disease[6,7] and in prion diseases[8] might also be promoted by fibril-forming peptides and proteins. Furthermore, the remodelling of lipoproteins that takes place during the transport of cholesterol and triglycerides is mediated by fusion-like events and by the destabilization of their neutral lipid core[9–11].

A common feature of the above processes is the active participation of a hydrophobic peptide, which helps destabilize either the lipid bilayer of a cell membrane or the lipid core in a lipoprotein[12]. A definition and a classification of these peptides according to their properties and biological targets, and their proposed modes of action are discussed in this review.

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Hydrophobicity of lipid-associating peptides

The interaction of peptides with lipids involves a number of non-covalent interactions[13]: hydrophobic interactions occur between lipid acyl chains and hydrophobic residues of the peptide, while electrostatic interactions take place between the polar residues of the peptide, the phospholipid head groups and the solvent molecules.

Hydrophobic peptides are characterized by the mean hydrophobicity of the entire peptide (Ho), the mean hydrophobicity of the residues entering the lipid phase (Ho_{pho}), the hydrophobic moment (μ_H)[14] and the molecular hydrophobicity potential (MHP)[11]. The molecular hydrophobicity potential represents contours of isopotential hydrophobic lines around the peptide, depicting both the hydrophobicity gradient along the peptide sequence and the magnitude of the hydrophobic envelope around the peptide. As shown by infra-red spectroscopic measurements[15], most lipid-associating peptides adopt an α-helical conformation when associated with the lipids, although some peptides can exist as β-sheets[16], especially under a self-associated or aggregated form.

According to these criteria, lipid-associating peptides can be classified as either: (1) peptides displaying a constant hydrophobicity along the axis of an α-helix (Fig. 1a–d); or (2) peptides with a hydrophobicity gradient along the axis of the helical peptide (Fig. 1e).

A hydrophobicity gradient 'tilts' peptides in membranes

Oblique-orientated peptides have a hydrophobicity gradient that runs along the axis of the helical peptide (this can be calculated by the method of Jähnig[17]). The hydrophobicity gradient is calculated by moving a 13–15 residue window from the amino- to the carboxyl terminus of the protein sequence. The oblique-orientated peptides are predominantly hydrophobic, but show increasing hydrophobicity from one extremity of the peptide sequence to the other (Fig. 1e). As a consequence, such peptides cannot take an orientation parallel to the phospholipid acyl chains, and instead, they insert at an angle of between 30–60° at a hydrophobic–hydrophilic interface. This is illustrated in Fig. 2 for the carboxy-terminal peptide of the cholesteryl ester transfer protein, CETP. The oblique insertion of the peptide facilitates the formation of inverse micelles within the bilayer, thereby favouring membrane fusion[18,19]. Oblique-orientated peptides have a specific orientation at a lipid–water interface, when measured in a static state; however, their true, dynamic configuration probably consists of fluctuations around this mean. These fluctuations might amplify the destabilizing effect of the tilted peptides, as might peptide–peptide associations that lead to the formation of pores[1].

Oblique-orientated peptides typically comprise 12–18 residues, at least 8–10 of which are hydrophobic. The minimum length is set through a requirement for optimal conformation and stability of the peptide structure, while the maximum length limit is required for optimal orientation at the hydrophobic–hydrophilic interface. According to their mean hydrophobicity, they can be divided into two subclasses.

Class A peptides have a mean hydrophobicity of around 0.9 and can interact with lipid bilayers and cellular membranes. These peptides consist almost entirely of hydrophobic residues, have a low amphipathicity, low hydrophobic moment and include viral fusion peptides, protein signal peptides, the carboxy-terminal domain of the β-amyloid peptide and the 118–135 peptide of the human prion protein (Table I).

Class B peptides, by contrast, have an hydrophobicity between 0.2–0.6, a high amphipathicity and a hydrophobic

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Running Title: Main chain H-bonds in proteins

PEX, ANALYTICAL TOOLS FOR PDB FILES

II. H-PEX: non-canonical H-bonds in alpha helices

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Key words: Statistical analysis, Main chain geometry, alpha helix, beta sheet, CH···O bonds, NH···O bonds.