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PROTEIN-LIPID INTERACTIONS

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Summary. - *The structural basis of lipid-protein interactions are outlined with emphasis on the different classes of proteins present on membranes. The different kinds of lipid requirements are discussed with relevant examples together with the important kinetic advantage for the rate of reactions occurring on membranes.*

Riassunto (Interazioni lipide-proteina). - *Le basi strutturali delle interazioni lipide-proteina sono descritte in relazione alle classi differenti di proteine presenti nelle membrane. Con esempi inerenti sono discusse le differenti modalità dei requisiti lipidici insieme al corrispondente guadagno cinetico per la velocità delle reazioni che avvengono nella membrana.*

Many cellular proteins are membrane proteins

The large fraction of cell proteins that is associated with membranes controls the exchange of ions and metabolites across the plasma membrane and organelles such as mitochondria, chloroplasts and sarcoplasmic reticulum. Moreover in this way specific functions such as recognition and trasmission of signals can be performed and localized in restricted areas of the cell. An important and often underevaluated aspect of the membrane localization of enzymes is the large increase in the local concentration and orientation factor with a consequent enhancement of the rate of enzymatic reaction when the two partners of the reaction are confined on the same membrane which is a two rather than a three dimensional solvent [1]. This is relevant to the function of chains of oxidoreductive reactions that occur on the mitochondrial, chloroplast and microsomal membranes. Nearly all aspects of membrane structure and function are covered in an extensive series of articles recently collected [2].

Depending on their function, we can distinguish different groups of membrane proteins.

Transport proteins. - This group includes proteins that translocate objects as different as sugars, amino acids, ions and electrons. Moreover in several cases transport is coupled to enzymatic or binding activities. Despite these different functions, the available evidence reveals several common structural features in their hydrophobic domain, that part of the protein embedded in the lipid bilayer which interacts directly with lipids. This sector is generally large comprising several peptide stretches trasversing the bilayer. These segments are generally organized as α-helices perpendicular or slightly tilted [3] with respect to the membrane plane as outlined in Fig. 1; a very common feature of these transmembrane segments is their lenght and structure that has to meet the thickness and spatial organization of the lipid bilayer [4, 5]. As a consequence they are formed by a central region of 20-25 residues that in an helical organization form a segment of around 30 Å designed to match the hydrophobic core of the lipid bilayer. The central part is frequently flanked at its two ends by short segments (5-6 polar residues) interacting with the polar head groups of phospholipids as depicted in Fig. 1 [4, 5].

The presence in the hydrophobic domain of helices both perpendicular and tilted with respect to the membrane plane responds to the need of a best fitting of the lateral residues between themselves to generate a highly compact structure.

The external surface of the helices is made of hydrophobic residues to meet the requirement of interacting with the hydrocarbon chain of lipids while the other faces involved in protein-protein contacts or in carrier functions may also be polar [3]. No detailed three-dimensional structure of any membrane protein channels is at the present available but there is a general consensus on the idea that they are formed by the proper alignement of hydrophilic residues. It is expected that these aminoacid residues and those involved in protein-protein contacts are strongly conserved in evolution while for those residues interacting with lipids the only costraint is to be hydrophobic and hence they show within this limit a large fluctuation [6].

Several primary structures of membrane transport proteins have been deduced from the DNA sequence of their cloned genes [7-15]. One feature which emerges from the analysis of the distribution of hydrophobic-

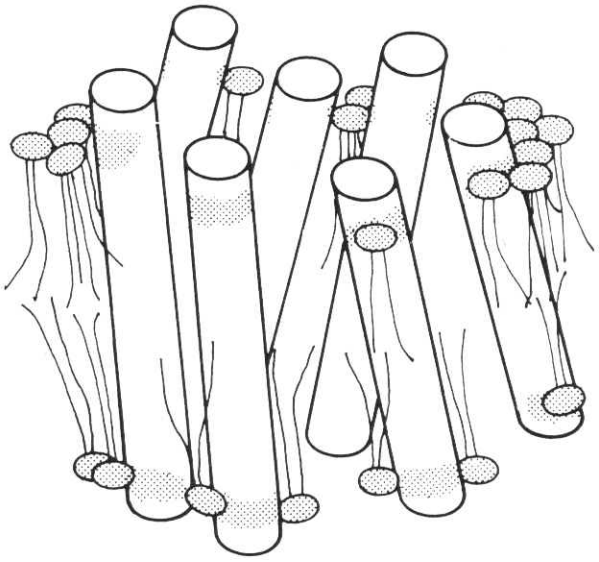

Fig. 1. - Schematic drawing of an integral membrane protein showing an array of helices crossing the lipid bilayer. The surface of these segments exposed to lipids is thought to be formed by a central hydrophobic part matching the hydrocarbon chains of lipids surrounded at both its ends by two shorts segments (dotted portions) of hydrophilic residues designed to meet the polar head groups of phospholipids (dotted in the figure). The water exposed part of the protein is not shown.

hydrophilic residues (hydropatic plots) is the above-mentioned presence of mainly apolar stretches of 20-25 residues. A second relevant aspect is that a large fraction of the protein mass of those transport protein possessing an enzymatic activity appears to be external to the lipid bilayer while this is not the case for some proteins involved only in ion transport such as bacteriorhodopsin, rhodopsin and partially the anion channel.

Membrane anchored proteins. - Under this title we can group an heterogeneous set of proteins with the common feature of possessing a single transmembrane segment arranged in an α-helical conformation. These proteins, localized on the plasma membrane, are involved in the binding of hormones, growth factors, antigens, connective tissue proteins and other cells [16-18]. Binding may be followed by a conformational change that tranfers a message inside the cell. It is difficult to envisage how this can occur through such a highly rigid structure as an α-helix in an hydrophobic milieu. This opens the possibility that the message may be transmitted via a clustering of receptors induced by the binding of the ligand.

In other cases these proteins serve as a mean to bring a ligand or an ion inside the cell through the endosome-lysosome or endosome-Golgi pathways as in the case of lipoproteins (cholesterol) and transferrin (iron) [19].

Membrane anchoring serves sometimes the need to localize a certain enzymatic activity to a particular intracellular membrane or organelle such as in the case of the cytochrome P-450 family bound to microsomes or of cytochrome c1 bound to the inner mitochondrial membrane [20, 21].

In some cases fatty acids are conjugated to the Cys, Ser and Thr residues of the membrane sector [22]. Their role is unclear; however the presence of additional hydrocarbon chains surely increases the hydrophobicity and hence the membrane anchoring capability of the membrane embedded protein segment. This is less likely for the bulky hydrophobic sectors of transport proteins such as the acetylcholine receptor.

Periplasmic proteins. - These proteins are localized at the membrane surface where from they can be solubized by addition of appropriate salts or chelating agents or pH treatments [23]. They vary for the modality of attachment to the membrane. A first group, including hydroxy butirate dehydrogenase, interacts with the polar heads of lipids and they may display different selectivity for the various lipid classes [24]. A second group is bound to the membrane via interactions with integral proteins as in the case of spectrin [25]. Recently it has been shown that a large group of proteins, including acetyl choline esterase and 5'-nucleotidase, is covalently bound to the inositol ring of phosphatidylinositol via a Cys residue and a glycan and glucosamine moieties [26]. This peculiar mode of membrane binding may offer a rapid and simple method of releasing the protein when needed by the cell. This process may be relevant for the antigenic variation of Trypanosoma whose major surface antigen is also bound to the membrane via phosphatidylinositol.

Transient lipid protein interaction. - A particular case of lipid-protein interaction is represented by complement, perforins, several viruses and proteic toxins that are water soluble and yet under certain conditions become able to penetrate into the hydrophobic core of the lipid bilayer [27-29]. This phenomenon involves a gross conformational rearrangement of their structure that can be triggered in different ways such as proteolysis, reduction or pH changes [28-32]. This kind of process may be at the basis of the import of nuclear coded proteins into the matrix of mitochondria and chloroplasts [33, 34]. In fact it has been recently found that several signal sequences directing nuclear coded proteins to mitochondria and chloroplasts are surface active and it was suggested that their ability to perturb the lipid bilayer organization is functional to their role in the actual process of protein translocation across the organelle membranes [35, 36].

This transient lipid interaction of proteins has been studied in details in the case of influenza and Semliki Forest viruses and of certain microbial proteic toxins such as diphtheria toxin. It has been shown that the acidic pH reached in the endosomes, where these proteins are collected via receptor-mediated endocytosis, induces a structural change with exposure of hydrophobic surfaces. Thus the protein penetrates into the endosomal

lipid bilayer and, in the case of the viruses, induces the fusion of the viral and endosomal membranes.

A particular group of lipid interacting proteins is esemplified by melittin, the major component of the bee venom, and the toxin of *Staphylococcus aureus* [37]. These are small polypeptides (around 25 residues) arranged as a single helix characterized by a C-terminal cluster of few charged residues linked to an amphipatic helix i.e. a helix with an hydrophilic and hydrophobic face. Figure 2 shows a possible arrangement of a cluster of melittin molecules with the hydrophobic face oriented towards lipids and the hydrophilic face forming a water filled region [38-40]. The perturbation of the lipid bilayer structure that is effected in this way is thought to be the cause of the cell lysis.

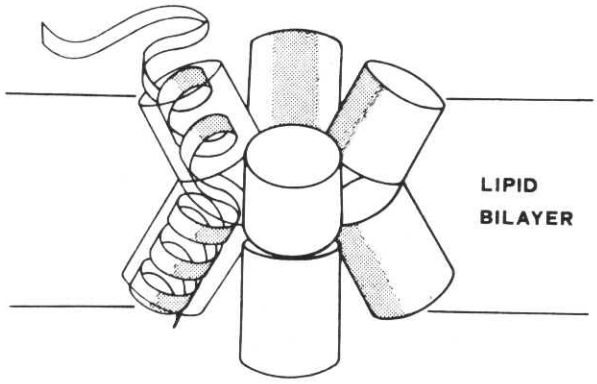

Fig. 2. - A possible arrangement of melittin molecules in the membrane with the hydrophobic face of the helices exposed to lipids and the hydrophilic central part (dotted) likely to be filled by water.

Lipid requirements

Integral proteins interact both with the polar head group of phospholipids and with their fatty acid chain while peripheral proteins are thought to be involved in a interaction with the polar head group only and are not expected to perturb significantly the hydrophobic region of the lipid bilayer [23]. Despite their large non homogeneity due to the presence of a variable number of double bonds in different positions, hydrocarbon chains are expected, for the very nature of the forces involved, not to show any particular specific interaction with any membrane protein. They simply provide a plastic, dynamic and water repelling structure and seal the membrane around integral proteins by hydrophobic interactions with the central surface of the hydrophobic sector formed by non polar amino acid lateral residues.

Figure 3 depicts schematically three extreme situations that can be defined for the interaction of a protein with the polar head groups of lipids. Case 1 refers to an aspecific binding of lipids to a protein mediated via the

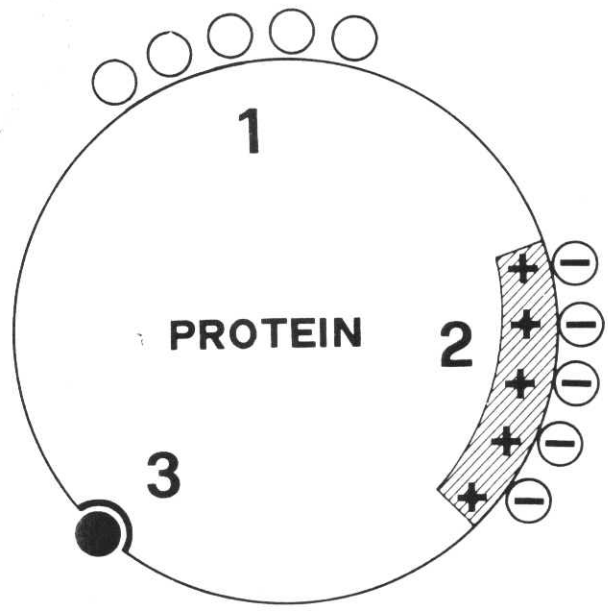

Fig. 3 - Schematic drawing of three prototype cases of lipid-protein interactions: 1) refers to an unspecific interaction in the sense that any lipid class can occupy site 1 on the protein; 2) refers to an interaction specific for negative lipids meaning that site 2 is most of the time occupied by a negative phospholipid; 3) illustrates the case of an absolutely specific interaction of a certain protein site for a defined lipid; the presence of the particular lipid is required for the physiological function of the protein. It should be noted that all the intermediate affinities and hence specificity situations are expected to occur in any biological membrane.

two collars of polar residues flanking the central hydrophobic region of the membrane embedded sector of an integral protein [4, 5, 41, 42]. This interaction is short-lived i.e. a single lipid molecule can be easily substituted by a similar one belonging to the same lipid class [43]. Phospholipids such as phosphatidylcholine, sfingo myelin and phosphatidylethanolamine constituting the larger share of membrane phospholipids are expected to fall in case 1.

Case 2 describes the situation of a protein showing a preference for a particular lipid class. Electrostatic forces appear to be involved in the well documented requirement of negative lipids for a maximal activation of the (Na^+, K^+)-ATPase. Hydrogen bonding could be involved in binding particular classes of lipids such as gangliosides. The stronger lipid-protein interaction of case 2 has two consequences. First, the mean residence time of such lipids around the protein is higher than that of type 1 lipids; they can be substituted only by lipids of the same class or charge and not by other lipids of the membrane [43]. Second, the proteins cause a lateral phase segregation of lipids enriching the first lipid shell around it of the preferred lipids.

Case 3 refers to the possibility of an absolute requirement of a certain specific lipid for the activity of the protein as shown by bovine heart cytochrome c oxidase for cardiolipin [44] and the adrenergic receptor for a glico-

lipid [45]. Presumably in this case the protein forms a specific binding site for the lipid and the residence time of the lipid in this site is very long. It should be noted that a specific lipid-protein binding in a membrane does not require necessarily affinities as high as those of most enzyme-substrate interactions because of the intrinsec concentrated nature of the two-dimensional membrane solvent [1].

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