HYDROPHOBIZED PROTEINS PENETRATING LIPID MEMBRANES

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At present there is conclusive evidence[1,2] that proteins pass across biomembranes during such vital biological processes as secretion and targeting of proteins towards different cellular compartments, penetration of toxins into cells, etc. There exist numerous different mechanisms of protein translocation[1-5], but evidently their common feature is an interaction of the protein with membrane. It is well known[5,6] that in many cases such an interaction requires the presence of a signal peptide, i.e. of a N-terminal hydrophobic amino acid sequence in the protein molecule. Signal peptides can probably act[7,8] as "anchors" in protein interactions with membranes; the absence of these peptides makes proteins incapable[4] of binding and penetration. On the contrary, the introduction of a signal peptide (by gene manipulation) into a non-secretory protein can transform[9] the latter into secretory one.

It should be noted that the hydrophobic anchors in proteins are not only signal peptides but also post-translationally introduced non-protein groups, e.g. lipid moieties. Most recent data have shown that the process of protein hydrophobization by phospholipids[2,10] and fatty acids[11] is widespread in Nature and evidently plays an important role in protein transport[2,10,12]

Hence, it can be reasonably expected that a molecule of a water-soluble protein incapable of passing across the membrane barrier may be converted into a membrane-penetrating form if equipped with hydrophobic anchor. The key experimental problem of introducing a small number of hydrophobic anchor groups into a protein molecule, was recently solved[13] in our laboratory. For this purpose we used[13] a system of surfactant reversed micelles in organic solvent as a reaction medium[14] for protein modification by water-insoluble reagents (such as, e.g. acylation with fatty acid chlorides). In this system hydrophobized proteins containing 1-2 fatty acid residues per protein globule can be obtained[13]. As a matter of fact, proteins contain[11] in vivo exactly the same small quantity of anchor groups.

Next, we studied[15] the membrane activity of two classical water-soluble enzymes (trypsin and α-chymotrypsin) modified with stearoyl chloride, using multilamellar liposomes. The latter were prepared by direct dispersion of egg lecithin in solutions of specific substrates of trypsin and α-chymotrypsin (ethyl esters of N-benzoyl-L-arginine or N-acetyl-L-tyrosine, respectively). Naturally, only the free (not entrapped in liposomes) substrate was hydrolyzed when the native enzyme was added to the system: the substrate separated by the liposome membrane was not accessible to the enzyme. A different result was obtained when the hydrophobized enzyme was added instead of the native enzyme. In this case both the free and liposome-entrapped forms of the substrate were hydrolyzed. It was shown[15] in another experiment that the liposomes were stable in the presence of hydrophobized enzymes. These data provide conclusive evidence showing that the hydrophobized enzyme pass through the bilayer membrane in liposomes.

This postulate was confirmed[16] in experiments with a giant spherical membrane prepared from a water drop containing the substrate and released into the enzyme solution from a glass capillary wetted by a lecithin solution in octane. This provided a system in which the substrate drop fixed at the capillary became separated from the external enzyme solution by a lecithin layer. Unlike the native enzyme the stearoylated trypsin was translocated[16] from the external solution into the drop thus hydrolyzing the substrate.

The question remained to be answered whether the translocation of hydrophobized proteins as revealed in the above model experiments is equally valid for complex biological systems. To this end we investigated the effect of the ricin A chain on intact B-cells ("Namalva" line). The ricin molecule, a toxin capable of penetrating into the cell and blocking enzyme synthesis in 60 S ribosomes, consists[17] of two polypeptide subunits, A and B-chains. The B-chain is responsible for binding of the toxin to the cell membrane and for translocating the active A-chain into the cell. The toxin A fragment alone (in the absence of the B-chain) possesses weak, non-specific toxicity only: it cannot pass through the cell membrane and reach the ribosomes. This "defect" of the A-chain can be compensated when hydrophobic anchor groups are introduced into it: *we found the toxic effect of the A-chain acylated by stearic acid on B-cells to be close to that of native ricin.* In these experiments the effect of ricin on the cells was measured in terms of cell destruction as well inhibition of both protein (^{35}S-methionine inclusion) and DNA (^{3}H-thymidine inclusion) biosyntheses.

The mechanism of protein translocation through lipid membrane is not yet fully understood. However, literature data[18] permit us to assume that artificially hydrophobized proteins can induce the formation of other than bilayer structures in the membrane, such as intermembranous lipidic particles similar to reversed micelles or the hexagonal H_{II}-phase, which are included into

their inner polar cavity (Fig. 1). These intramembrane lipidic particles have a dynamic character, so that after being formed on one side of the lipid bilayer they can, in principle, dissociate on its opposite side. This means that they may be regarded as possible transmembrane carriers (Fig. 1).

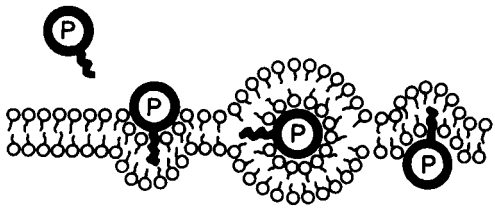

FIG. 1
Hypothetical mechanism of translocation of an artificially hydrophobized protein (P) through the lipid membrane.

The translocation effect of artificially hydrophobized proteins is not only of theoretical, but also of practical importance as it can be utilized for the design of essentially new drugs capable of penetrating into target-cells. Moreover, it is conceivable that this simple method[13] of chemical modification of proteins with hydrophobic anchor groups may provide an alternative to modification of proteins by gene engineering[9].

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