FATTY ACID ACYLATION OF MEMBRANE PROTEINS

MILTON J. SCHLESINGER AND ANTHONY I. MAGEE
Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri 63110 U.S.A.

Recent studies in our laboratory (1) have shown that a substantial number of membrane-associated proteins become acylated with fatty acid during their transit from the rough endoplasmic reticulum (their site of synthesis) through intracellular organelles to the cell surface (their site of function). Much of the data showing the covalent binding of fatty acid to protein were obtained from analyses of tissue culture cells infected with either vesicular stomatitis virus (VSV) or Sindbis virus (SbV). The G glycoprotein of VSV and the E1 and E2 glycoproteins of SbV contain from 1–6 mol of acylated fatty acid/mol protein (2, 3); attachment of the fatty acid is a post-translational event occurring in the Golgi organelle (4). Temperature-sensitive mutants blocked in this event do not transport glycoprotein to the cell surface (5).

RESULTS AND DISCUSSION

Current efforts are directed toward identifying the precise hydroxy-amino acid(s) in the polypeptide chain that are esterified to fatty acid. Experiments in our laboratory as well as others[1] (6–8) have shown that fatty acids are localized to amino acid residues near the carboxy-terminal regions of the VSV G and SbV E1 and E2 glycoproteins. This is the portion of the protein that is embedded in the membrane (see Fig. 1). To isolate small peptides containing acylated fatty acids, we have been isolating these glycoproteins free of virions and membranes by SDS gel electrophoresis and subjecting them to proteases (i.e., V-8) or chemical reagents (i.e., CNBr or iodosobenzoate). The fragments have been separated by gel filtration on hydrophobic matrixes (LH20, LH60) in organic solvents and by high performance liquid chromatography using a C-8 reverse-phase column eluted with gradients of 2-propanol in 0.1% TFA. A small fragment (mol wt, ~600–800) has been obtained from VSV G and a larger fragment (mol wt, ~2000–3000) from SbV E2. Automated Edman degradation of these peptides has been complicated by severe washout, although amino acid analysis detects the present of large amount of serine and smaller amounts of threonine.

To test for possible functions of the fatty acid, we are preparing deacylated forms of these glycoproteins. We find that fatty acid can be released as the corresponding hydroxamic acid from the protein after 6–8 h treatments with 1M hydroxylamine at pH 7.0, 23°C, without drastic damage to the protein. These proteins are to be compared with the native forms for their ability to be reconstituted into liposomes and cell membranes. Protein-bound fatty acid could also lead to a selective binding of certain membrane lipids around the protein. Analysis of ^{32}P-labeled lipid associated with these glycoproteins is being carried out. These studies would detect only a "tight" binding of lipid; more subtle changes in "annular lipid" would have to be detected by cross-linking or spectroscopic methods.

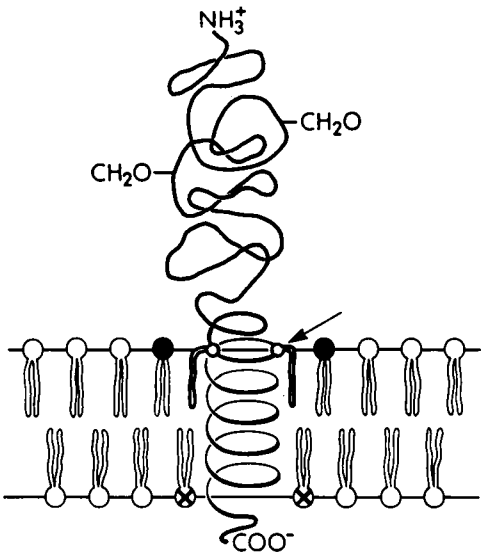

FIGURE 1 Schematic arrangement of VSV G protein in the membrane. ⊗ and ● indicate different phospholipids. Arrows indicate possible site of fatty acid binding to the protein.

NOTE ADDED IN PROOF

Rates of release by *M* Hydroxylamine (pH 8.0; 23°C) of fatty acid from G, E1, E2, and PE2 (the cellular precursor of E2) in the denatured state (reduced in SDS gels), in the native state in cellular membranes in detergent micelles, and in virons indicate the following: (*a*) folding these glycoproteins can alter stability of the fatty acid bond or mask the bond from reagent, (*b*) El has two different kinds of fatty acid bonds, (*c*) conversion of PE2 to E2 stabilizes fatty acid bonds. Reconstitution of G into liposomes shows that deacylation can affect interaction of G with membrane lipid.

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MEMBRANE BIOGENESIS

A SOLUBLE PRECURSOR OF A MUTANT *lac* PERMEASE IN *ESCHERICHIA COLI*

VICTOR A. FRIED
Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260 U.S.A.

The molecular mechanisms by which membrane proteins are assembled into the lipid milieu are unknown. The *lac* permease of *Escherichia coli* is an intrinsic inner membrane protein with an apparent subunit M_r = 30,000. A mutant of the *lac* permease, called Y^f, has been isolated which is defective in the biogenesis of the permease (Fried, 1977). By studying this mutant, I hope to gain insight into one possible mechanism of membrane protein biogenesis.

RESULTS AND DISCUSSION

Evidence for defective biogenesis of the *lac* Y^f permease came from the unusual induction and deinduction kinetics of permease activity in this mutant (Fried, 1977). It was found that upon induction of *de novo* synthesis, the Y^f permease activity appeared slowly, and upon deinduction, when *de novo* synthesis was stopped, permease activity continued to appear. These results suggested that the permease was synthesized in a relatively stable precursor form which was slowly processed into the active permease. The precursor form of the Y^f permease appears to be an 87,000 dalton (87 kd) polypeptide localized in the bacterial cytoplasm (Fried, 1981). This polypeptide was detected by double-label analysis on sodium dodecyl sulfate gels (SDS-PAGE) and identified as a *lac*-specific polypeptide on a two-dimensional gel system. In vivo pulse-chase experiments were consistent with the notion that the 87-kd soluble polypeptide was a precursor of the 30-kd membrane protein. The precursor appears to be a chimera of the *lac* Y and *lac* A gene products since it is immunoprecipitated by antibody raised to the *lac* thiogalactoside transacetylase (the *lac* A gene product) and its size is consistent with that of a read-through translation product of the *lac* Y-A genes (Buchel et al., 1980). It is likely that the solubility of the precursor and its slow processing into the membrane form are due to the structural perturbation of the permease (NH_2-terminal) end of the molecule caused by its fusion to the soluble *lac* A gene product at the COOH-terminal end. This study reports the partial purification and characterization of the *lac* Y^f precursor and initial characterization of the processing system.

The precursor of the Y^f permease has been purified 30-fold. Since the precursor has no biological activity, purification was followed by double-label analysis, SDS-PAGE, and immunoprecipitation with antithiogalactoside transacetylase antibody as described (Fried, 1981). Fractionation by gel filtration on Bio-Gel A 0.5 m (Bio-Rad Laboratories, Richmond, CA) gave a threefold purification. Fractions containing the precursor were pooled and fractionated by anion exchange chromatography on DEAE Sephadex A-50 (Pharmacia Fine Chemicals, Uppsala, Sweden). The precursor was eluted with 0.175 M NaCl in 0.05 M Tris/Cl, pH 7.5 and the peak fraction was ~ 40% pure as determined by isotope ratio and SDS-PAGE.

The apparent "native" size of the precursor was determined by gel filtration on a calibrated Bio-Gel A 0.5 m column. The precursor eluted with an apparent M_r = 110 ± 8 kd. Dimeric or higher aggregates of the precursor were not detected. This result makes it unlikely that the aqueous solubility of the precursor is due to self-association through the hydrophobic permease part of the molecule. On the other hand, the apparent native molecular weight is ~ 25 kd greater than that determined by SDS-PAGE, a difference that could be resolved by the gel filtration analysis. This difference may mean either that the precursor has a larger Stoke's radius than predicted for the globular protein of equivalent mass due to unique folding at the permease end of molecule, or that it is tightly associated with smaller peptides which preserve its soluble state. Material of higher purity will be required to distinguish between these alternatives.

Experiments have been initiated to determine the

Dr. Fried's present address is Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee, 38101.