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MEMBRANE BIOGENESIS

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

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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

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TABLE I
IN VITRO PROCESSING OF THE Y^r PRECURSOR

Cellular fractions added	Percent of polypeptide species lost after 60-min incubation at 37°C	
	β-Galactosidase	87 kd Precursor
None		
−ATP	<2	<2
+ATP	<2	5
Supernatant		
−ATP	<2	5
+ATP	<2	8
Membrane		
−ATP	<2	12
+ATP	<2	32
Membrane + Supernatant		
−ATP	<2	11
+ATP	<2	34

mechanism of precursor processing. These experiments suggest that the precursor can be modified by an ATP-dependent membrane component in vitro. Aliquots of a double-label Y^r supernatant fraction were incubated with supernatant and/or membrane fractions prepared from unlabeled cells. Incubations were performed in the absence or presence of 2 mM ATP since it has been recently demonstrated that several of the more interesting proteolytic enzymes are ATP dependent (Charette et al., 1981). In a typical experiment, incubations were at 37°C for 60 min and the reaction was terminated by heating at 50°C in SDS-gel sample buffer. The samples were fractionated by SDS-PAGE and analyzed as described (Fried, 1981). The effect of various cellular fractions and ATP on the recovery of β-galactosidase (an internal control) and the precursor are summarized in Table I. The various incubations did not affect β-galactosidase, a well-characterized stable protein. The precursor was not processed either by itself or when incubated with the supernatant fraction in the presence of ATP. In contrast, addition of membranes stimulated the loss of precursor and this was enhanced threefold by addition of ATP. No synergism was observed when supernatant and membrane fractions were both present. While these experiments demonstrated a specific loss of precursor, the appearance of the 30 kd membrane protein was not consistently observed. This may be due to the low sensitivity in the double-label analysis. On the other hand, material was found to accumulate at the top of the separating gel in a broad smear and could account for the loss of the precursor. The mature permease aggregates and runs in the same pattern when heated above 50°C (Fried, 1981). It is possible that intermediates in the processing of the precursor in vitro may be more susceptible to aggregation at 50°C. Experiments are in progress to determine the fate of the precursor in the in vitro system.

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FUSION AND DISASSEMBLY OF SENDAI VIRUS MEMBRANES WITH LIPOSOMES

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The mechanism of Sendai virus penetration (fusion of the viral membrane followed by disassembly) is being studied by using liposomes in place of the host cell. Fusion of Sendai virus membranes with liposomes after a 2-h incubation at 37°C was previously described (1). Sendai viruses were also previously shown to be enveloped by receptor-containing liposomes as in the ingestion step of endocytosis (2). Investigation of the initial steps of viral fusion with liposomes composed of phosphatidylcholine (PC), cholesterol, gangliosides, and phosphatidylethanolamine (PE) showed that the fusion of the viral membrane with the liposome occurs at the leading edge of the developing "endocytic vacuole," where there is a region of membrane with a small radius of curvature (3). Membrane fusion establishes the continuity of the viral and liposomal membranes. Viral disassembly with distribution of the viral glycoproteins on the surface of the liposome and deposition of the viral ribonucleoprotein

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