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Inhibition of Purified *Escherichia coli* Leader Peptidase by the Leader (Signal) Peptide of Bacteriophage M13 Procoat

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The leader peptide of bacteriophage M13 procoat inhibited the cleavage of M13 procoat or pre-maltose-binding protein by purified *Escherichia coli* leader peptidase. This finding confirms inferences that the leader is the primary site of enzyme recognition and suggests a rationale for the rapid hydrolysis of leader peptides in vivo.

Escherichia coli leader peptidase is an integral membrane protein of the plasma membrane (12) which cleaves presecretory and membrane proteins after they cross the membrane bilayer (1, 14). The released leader peptide (prepeptide or signal peptide) is rapidly degraded. The specificity of leader peptidase is somewhat unusual in that it cleaves a wide variety of preproteins which do not share a consensus sequence. However, leader peptides do possess common structural features (7, 9). Amino acid residues with small side chains are commonly found at positions −1 and −3 (with respect to the cleavage site), and a helix-breaking glycine or proline is found in residues −4 to −6. There is no apparent sequence conservation in the mature region of secreted proteins. Genetic studies have established that conserved residues of the leader are necessary for cleavage rather than for the process of membrane insertion (4, 5). Short peptides which span the cleavage site of bacteriophage M13 procoat, a membrane protein precursor (10), have been prepared by controlled proteolysis of the purified protein and were found to be accurately cleaved by leader peptidase (1a). These studies suggested that leader peptidase only recognizes the leader peptide.

To test this idea, we assayed the ability of chemically synthesized M13 procoat leader peptide to inhibit leader peptidase. This peptide has 23 residues from the initiator methionine to the alanine which immediately preceeds the procoat-cleavage site. The chemically synthesized M13 procoat leader peptide (purchased from Dennis Olshevsky, University of California, San Diego) was dissolved at 1 mg/ml in 0.05 M Tris chloride (pH 8.5)–0.1% (vol/vol) Triton X-100 (buffer A). Radiochemically pure ^{35}S-procoat (8), purified leader peptidase (11), and leader peptide, each at the indicated concentrations in 20-μl reaction mixtures, were incubated for 1 h at 37°C. Samples were then analyzed for cleavage by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography (3). While 10 ng of leader peptidase was sufficient to cleave 50% of the procoat to coat in our standard assay (Fig. 1A, lane 5), 10 μg was required for equivalent cleavage in the presence of 250 μg of leader peptide per ml, corresponding to a 1,000-fold inhibition of the enzyme. Titration of the leader peptide (Fig. 1B) showed inhibition at levels of 2 μg of leader peptide per ml. To confirm that the inhibition was indeed due to the leader peptide rather than to a contaminant in the synthesis reaction, the leader peptide was purified by high-pressure liquid chromatography. Fractions were collected across the region of the single peak of absorbance and were assayed for inhibition of leader peptidase. Inhibitory activity coincided with absorbance, confirming that the leader peptide per se was responsible for the inhibition (data not shown). The procoat leader peptide also inhibited the cleavage of pre-maltose-binding protein (Fig. 2), which showed that inhibition is not specific to one substrate and presumably occurs via interaction with the enzyme at its active site.

The in vitro cleavage of procoat by leader peptidase is neither stimulated nor inhibited by a soluble bacterial protein extract or by a wide variety of commercially available peptides (13). In the experiment shown in Fig. 1B, half-maximal inhibition occurred when the detergent was present

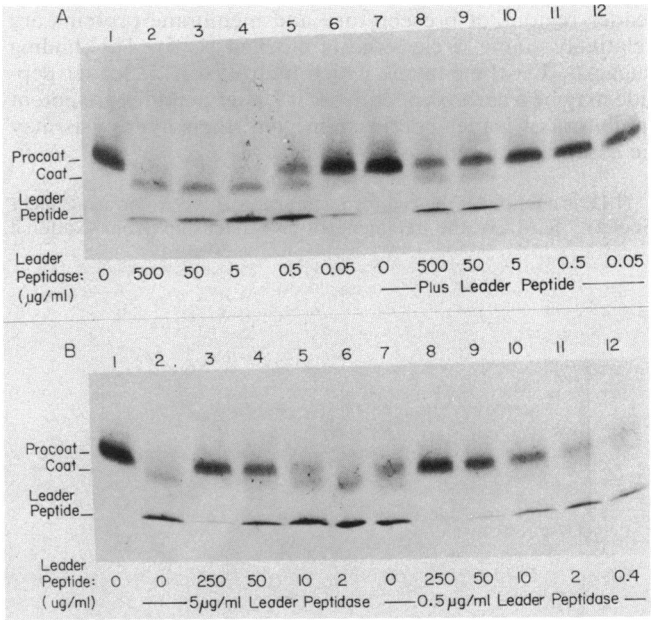

FIG. 1. Inhibition by synthetic leader peptide of the cleavage of M13 procoat by purified leader peptidase. (A) Lanes 7 to 12, 5 μg of leader peptide. (B) Lane 1, no leader peptidase; lanes 2 to 12, leader peptidase as indicated.

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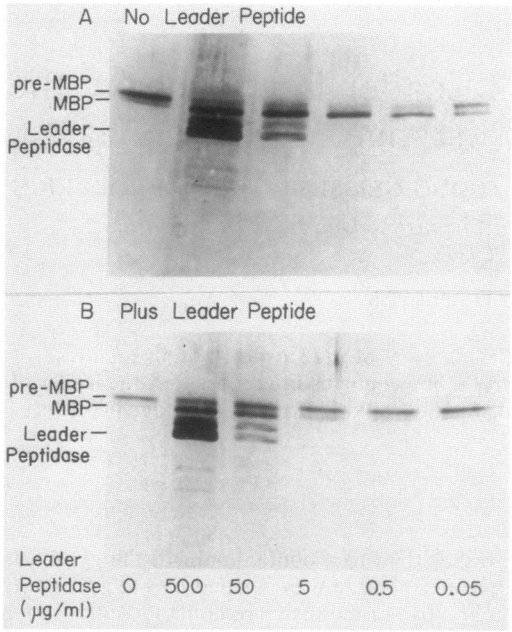

FIG. 2. Inhibition by leader peptide of the cleavage of pre-maltose-binding protein (pre-MBP) by purified leader peptidase. (A) Purified (2) pre-maltose-binding protein and leader peptidase were mixed in 10 μl of buffer A, incubated for 1 h at 37°C, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining (6). (B) Five micrograms of leader peptide was added to each cleavage reaction.

in a 500-fold weight excess over the leader peptide. Taken together, these data strongly suggest that the inhibition by leader peptide is specific and not due to any minor detergent effects of the peptide. Our results show that the leader peptide is indeed the primary region of substrate recognition by leader peptidase. In vivo, the leader peptide is rapidly degraded after its release from the preprotein (3), while the leader regions of presecretory and membrane proteins are relatively stable if cleavage is blocked (1, 5). This finding suggests that the enzyme which hydrolyzes the leader peptide may be a carboxypeptidase. If leader peptides are potent inhibitors of leader peptidase in vivo, their hydrolysis may be essential for cell growth.

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