Proc. Natl. Acad. Sci. USA
Vol. 77, No. 4, pp. 2005–2009, April 1980
Biochemistry

Amino-terminal sequence and processing of the precursor of the leucine-specific binding protein, and evidence for conformational differences between the precursor and the mature form

(*Escherichia coli* periplasmic binding protein/leucine transport protein/*in vitro* synthesis and processing/DNA sequence of signal peptide)

DALE L. OXENDER*, JAMES J. ANDERSON*, CHARLES J. DANIELS*, ROBERT LANDICK*, ROBERT P. GUNSALUS†, GERARD ZURAWSKI‡, AND CHARLES YANOFSKY†

*Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48109; and †Department of Biological Sciences, Stanford University, Stanford, California 94305

Contributed by Charles Yanofsky, January 28, 1980

ABSTRACT A 2.1-kilobase *Bgl* II DNA fragment from *Escherichia coli* containing *livK*, the gene coding for the leucine-specific binding protein, has been cloned into the *Bam*HI site of the plasmid vector pBR322. The DNA sequence of segments of the resulting plasmid, pOX7, established the location of the *livK* gene and the direction of its transcription. *In vitro* protein synthesis directed by pOX7 DNA yielded the M_r 42,000 precursor of the leucine-specific binding protein and a small amount of the M_r 39,000 mature protein. Continued incubation of the *in vitro* reaction mixture after DNase and RNase treatment resulted in additional processing. The DNA sequence of the beginning of *livK* suggested that 23 additional amino acid residues are present as an extension of the NH_2 terminus of the mature protein. Amino acid sequence analysis established that the precursor has the predicted 23-residue extension. Proteolytic digestion studies with the precursor and mature forms of the leucine-specific binding protein indicate that there are conformational differences between the two. This suggests a possible role for the signal sequence in determining the conformation of the binding protein precursor that is recognized by the membrane.

The leucine-specific binding protein of *Escherichria coli*, a periplasmic component specified by the *livK* gene, serves as a high-affinity receptor in the transport of L- and D-leucine (1). *livK* is one of several genes affecting branched-chain amino acid transport that has been cloned into a plasmid vector (2). The availability of the cloned leucine transport genes has permitted us to investigate the *in vitro* synthesis of their polypeptide products. Initial studies showed that, *in vitro*, the periplasmic leucine-specific binding protein is synthesized in a precursor form that can undergo maturation to the mature form (2). This report describes the recloning of *livK* into the plasmid vector pBR322 (3), the determination of the sequence of the processed segment of the precursor, and an analysis of the *in vitro* synthesis and maturation of the leucine-specific binding protein.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. *E. coli* strain AE168 (F^- *arg his leu trp thy livP livH lstR*) was constructed by standard genetic techniques (4). The parental plasmid, pOX1, containing the cloned leucine transport genes is described elsewhere (2).

Restriction Endonuclease Digestion and Ligation of DNA Fragments. Endonuclease digestions were performed as described (5). *Bam*HI, *Hpa* II, *Hin*cII, *Hin*f, *Bgl* II, and other endonucleases were purified by using either the method of Greene *et al.* (6) or the heparin-agarose affinity chromatography procedure of Bickle *et al.* (7). Plasmid restriction fragments were analyzed by horizontal electrophoresis on 0.8% agarose gels or by vertical electrophoresis on 5% acrylamide gels as described (8, 9). Ligation procedures were performed as described (10–12); the ligation mixture was used directly for transformation (12).

Transformation. Cells were transformed as described (2, 12).

***In Vitro* Synthesis.** A coupled transcription/translation system was developed similar to that of Zubay *et al.* (13) as modified by Gunsalus *et al.* (12). Plasmid DNA extracted from transformed cells (12) was further purified by CsCl gradient ultracentrifugation (14) and used as template for protein synthesis. The reaction mixture was incubated for 45 min, and synthesis was terminated by adding DNase (250 μg/ml), RNase (250 μg/ml), and excess unlabeled methionine (1 mg/ml). Samples were boiled for 5 min in $NaDodSO_4$ sample buffer prior to electrophoresis on 12.5% $NaDodSO_4$/polyacrylamide gels according to Laemmli (15). Some samples were immunoprecipitated with a specific antiserum against the LIV binding protein (16). This antiserum is crossreactive with the mature leucine-specific binding protein. The precipitate was dissolved in $NaDodSO_4$ sample buffer for electrophoresis as described above.

DNA Sequence Analysis. DNA fragments were 5′-labeled by using [γ-^{32}P]ATP and polynucleotide kinase. The labeled fragments were digested with a second endonuclease and separated by electrophoresis on 5% acrylamide gels. Each fragment was subjected to DNA sequence analysis by the procedure of Maxam and Gilbert (17). We used 17-inch (43.2 cm) 20% acrylamide and 26-inch (66 cm) 8% acrylamide gels [0.016-inch (0.4 mm) thick] for electrophoretic separation of the fragments (18).

RESULTS

Cloning of the *livK* Gene. We have described the isolation of a λ transducing phage containing the leucine transport genes from *E. coli* K-12 (2). Subsequent recloning of these genes, contained on a 13-kilobase (kb) *Eco*RI DNA fragment, gave the parental plasmid we designated pOX1 (2). The bacterial genes on this plasmid were shown to complement all four of the known high-affinity branched-chain amino acid transport genes, *livG*, *livH*, *livJ*, and *livK*. An endonuclease restriction map of the *Eco*RI DNA fragment of pOX1 is shown in Fig. 1.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: kb, kilobase(s).

‡ Present address: Division of Plant Industry, CSIRO, Canberra, A.C.T. 2601, Australia.

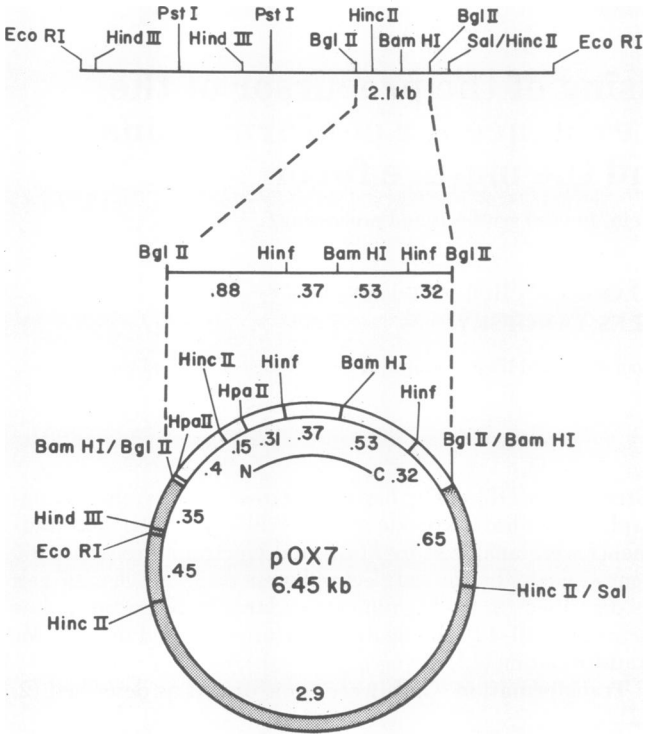

FIG. 1. Restriction map and construction scheme for plasmid pOX7. The 13-kb *Eco*RI fragment of pOX1 carrying the *liv* transport genes (see text) was further digested with *Bgl* II, and the 2.1-kb fragment (inside the dotted lines) inserted into the *Bam*HI site of the vector pBR322 (shaded area). The position and polarity of the *livK* gene product, the leucine-specific binding protein, are shown in pOX7 as an arc with NH_2 and COOH termini depicted (N and C, respectively).

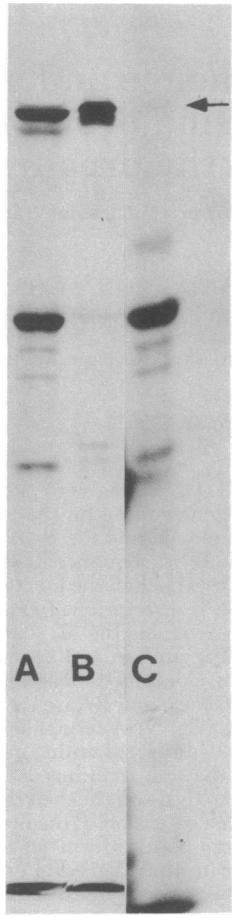

FIG. 2. *In vitro* synthesis of the leucine-specific binding protein. L-[^{35}S]Methionine-labeled products of an *in vitro* transcription/translation system were dissolved in $NaDodSO_4$ sample buffer, run on 12.5% $NaDodSO_4$/polyacrylamide gels, and autoradiographed. Lanes: A, pOX7 as the DNA source; B, material immunoprecipitated from the reaction mix of lane A when the mixture was incubated 30 min with anti-LIV-binding protein antibody at 37°C and then with anti-rabbit IgG for an additional 2 hr before centrifugation and washing; C, vector pBR322 as the DNA source. The arrow designates the location of the presumed M_r 42,000 precursor of the leucine-specific binding protein (see text).

By using the restriction map, various strategies were developed for subcloning portions of the *Eco*RI fragment with the intention of locating the individual transport genes. Advantage was taken of the finding that only two *Bgl* II restriction sites were present in the *Eco*RI fragment and no *Bgl* II sites were contained in the pACYC184 vector. Restriction of pOX1 DNA with *Bgl* II to remove the 2.1-kb fragment followed by ligation of the remaining DNA gave a new plasmid, pOX5. Genetic complementation analysis indicated that pOX5 lacked *livK*, the gene that codes for the leucine-specific binding protein. The 2.1-kb *Bgl* II DNA fragment from pOX1 was subcloned by taking advantage of the homology between the *Bgl* II and *Bam*HI restriction sites (*Bam*HI, G↓GATCC and *Bgl* II A↓GATCT). Therefore, we ligated the *Bgl* II fragment into the single *Bam*HI site of plasmid pBR322 (3). The resulting 6.5-kb plasmid, pOX7 (Fig. 1), was used in subsequent studies.

***In Vitro* Expression.** pOX7 DNA was used as a template in an *in vitro* coupled transcription/translation system similar to that developed by Zubay *et al.* (13). L-[^{35}S]methionine was used to label synthesized proteins. Labeled proteins were resolved by electrophoresis on 12.5% $NaDodSO_4$/polyacrylamide gels. Only two major polypeptides were produced with pOX7 template DNA (Fig. 2, lane A) other than those coded for by the plasmid vector (Fig. 2, lane C). These proteins had apparent M_r of 42,000 and 39,000. The smaller polypeptide corresponded to the leucine-specific binding protein released from cells during osmotic shock treatment. Treatment of the total S30 reaction mixture with antibodies to the mature LIV-binding protein (Fig. 2, lane B) precipitated both proteins, showing that the M_r 42,000 species is antigenically similar to the leucine-specific binding protein. Presumably, it is its precursor form. When the incubation was stopped after 30–40 min, almost all of the leucine-binding protein was found in the precursor form. Continued incubation of the transcription/translation mixture for an additional 25 min in the presence of DNase, RNase, and an excess of unlabeled methionine resulted in a significant decrease in the relative amount of the M_r 42,000 protein and a concomitant increase in the relative amount of the M_r 39,000 protein (Fig. 3). The addition of a nonionic detergent (Triton X-100), which has been reported to stimulate processing activity (19, 20), stimulated processing only slightly.

Other investigators have reported that processing activity is membrane associated (20, 21). An analysis of the S30 preparation for NADH oxidase activity, a membrane marker enzyme (22), indicated the presence of significant amounts of membranes. Therefore, membrane-associated components may be responsible for the processing activity in our *in vitro* system.

Identification of the Location and Direction of Transcription of the Leucine-Specific Binding Protein Gene, *livK*. Our *in vitro* studies indicated that *livK* (estimated to be about 1.1 kb) is entirely contained within the 2.1-kb *Bgl* II fragment. Because the *Bam*HI site divides this fragment into 0.8- and 1.2-kb segments, there is a high probability that the DNA sequence around the *Bam*HI site is within the structural gene. The 2.1-kb *Bgl* II fragment prepared from pOX1 DNA was digested with *Bam*HI and the 1.2- and 0.8-kb fragments were isolated. These two fragments were labeled at their 5′ termini by using γ-ATP[^{32}P] and polynucleotide kinase. Secondary cleavage of the end-labeled fragments was performed with *Hin*f to give singley labeled fragments. Each fragment was subjected to DNA sequencing analysis as described by Maxam and Gilbert (17). DNA sequences were obtained from the two

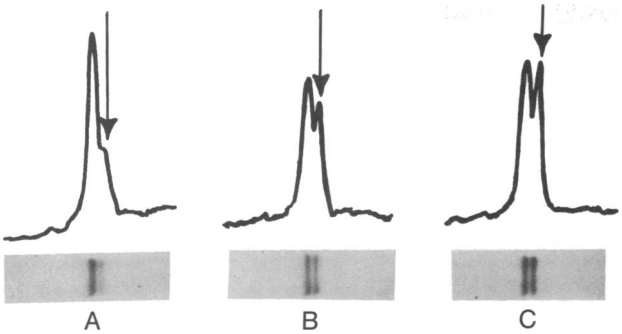

FIG. 3. Effect of incubation on the processing of the leucine-specific binding protein precursor made *in vitro*. Proteins made *in vitro* using pOX7 DNA as template in a coupled transcription/translation system were labeled for 45 min at 37°C with L-[^{35}S]methionine. DNase (250 μg/ml), RNase (250 μg/ml), and unlabeled L-methionine (1 mg/ml) were added to stop further labeling; samples were then incubated for 5 min (*A*) or for 25 min in the absence (*B*) or presence (*C*) of 2% (wt/vol) Triton X-100. After dissolving and heating (95°C, 5 min) in $NaDodSO_4$ sample buffer, the samples were electrophoresed in a 12.5% $NaDodSO_4$/polyacrylamide gel and autoradiographed. The portions of the autoradiograms shown were scanned in an Ortec densitometer. The arrow marks the mature form of the leucine-specific binding protein.

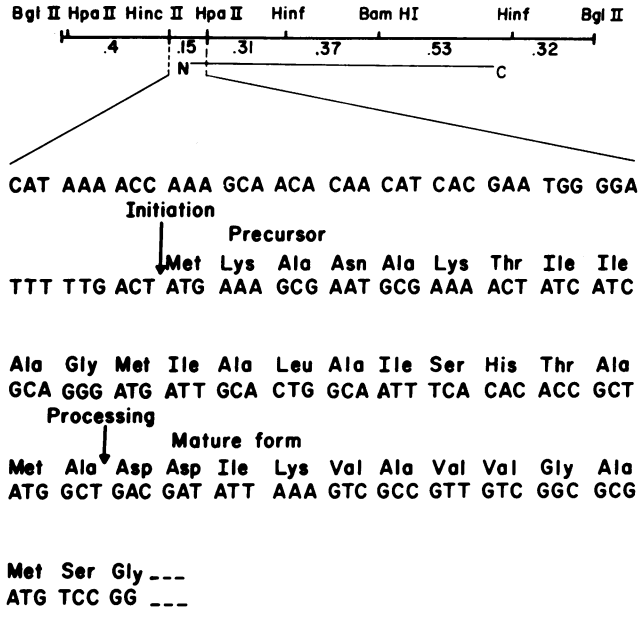

FIG. 4. DNA sequence of a restriction fragment carrying the region corresponding to the NH_2-terminal segment of the leucine-specific binding protein. The sequence of the 0.15-kb fragment shown was determined as described in *Materials and Methods*; the DNA sequence is shown along with the deduced amino acid sequence.

Bgl II fragment ends and the two *Bam*HI fragment ends. The DNA sequence proximal to the *Bam*HI fragment ends were converted into the corresponding amino acid sequence and compared with partial amino acid sequence data for the leucine-specific binding protein (23). The comparison indicates that the *Bam*HI site is located in the DNA region corresponding to amino acid residue 254 in the leucine-specific binding protein. This comparison also establishes that the direction of transcription of the *livK* gene is from left to right on the plasmid as drawn in Fig. 1.

NH_2-Terminal Sequence. The location of the *livK* gene and its direction of transcription provided us with the information necessary to identify the region of the 2.1-kb *Bgl* II fragment that codes for the NH_2-terminal amino acids. Additional restriction mapping identified a 650-nucleotide *Hpa* II fragment containing a *Hin*cII site 0.8 kb upstream from the *Bam*HI site relative to direction of transcription. This fragment should contain the DNA sequence coding for the NH_2 terminus of the leucine-specific binding protein which is located at a position equivalent to 254 amino acid residues from the *Bam*HI site. This *Hpa* II fragment was labeled, cut with *Hin*cII, and subjected to sequence analysis. The complete DNA sequence was determined for the 150-nucleotide-pair fragment and the sequence of the first 300 nucleotides of the 500-nucleotide-pair fragment. These sequences were converted into the corresponding amino acid sequences and compared to the previously determined NH_2-terminal amino acid sequence for the mature leucine-specific binding protein (Fig. 4).

From these results we determined that the 150-base pair *Hpa* II/*Hin*cII fragment contains the DNA sequence that codes for the first 13 amino acid residues of the mature leucine-specific binding protein and includes the region immediately preceding the NH_2-terminal region of the mature form. These results allowed us to predict the amino acid sequence of the "signal" peptide region of the precursor form. The DNA sequence of the NH_2-terminal extension contained three potential ATG initiation codons at positions 2, 12, and 23 with reference to the NH_2-terminal aspartate residue of the mature form of the binding protein. Because the molecular weight of the precursor form was ≈2500 greater than that of the mature form, we tentatively assumed that the methionine residue at 23 is the NH_2-terminal residue of the precursor and that the signal peptide consists of 23 amino acid residues attached to the NH_2 terminus of the mature protein.

Sequence Analysis of the Precursor of the Leucine-Specific Binding Protein. Microtechniques using automated Edman degradation were used to determine the NH_2-terminal sequence of the precursor form of the leucine-specific binding protein. The leucine-specific binding protein precursor was synthesized *in vitro* with L-[^{35}S]methionine, L-[^{3}H]lysine, or L-[^{3}H]threonine in separate reactions. The labeled proteins were separated by $NaDodSO_4$/gel electrophoresis, cut out of the gel, and eluted by electrophoresis. The labeled protein was mixed with unlabeled myoglobin and subjected to automated Edman sequence analysis. The sample from each round of degradation was collected in a vial, the radioactivity was measured, and the myoglobin residue released was determined. The results are presented in Table 1. Methionine was found at positions 1 and

Table 1. Edman degradation of leucine-specific binding protein precursor synthesized *in vitro*

Edman cycle	Labeled amino acid released, relative cpm in %*		
	Met	Lys	Thr
1	100	2	15
2	3	52	23
3	2	5	12
4	2	8	14
5	1	4	16
6	1	100	38
7	2	7	100
8	1	3	27
9	3	4	15
10	3	9	23
11	4	—	—
12	19	—	—
13	8	—	—

Deduced sequence: Met-Lys-X-X-X-Lys-Thr-X-X-X-X-Met-X.
* cpm in cycle sample as a percentage of the cpm released in the cycle with the highest yield.

12, lysine at positions 2 and 6, and threonine at position 7. These data indicate an NH_2-terminal partial amino acid sequence of Met-Lys-X-X-X-Lys-Thr-X-X-X-X-Met-X. These results confirm the predicted translation initiation site for the precursor form of the leucine-specific binding protein (Fig. 4).

Proteolytic Digestion of Leucine-Specific Binding Protein Synthesized *in Vitro* and *in Vivo*. Several proteolytic enzymes were used to test the susceptibility of the *in vitro* synthesized leucine-specific binding protein to proteolysis. The leucine-specific binding protein was significantly more resistant to digestion by trypsin and chymotrypsin than by papain (Fig. 5 *Left*). Only the mature form of the leucine-specific binding protein was resistant to trypsin and chymotrypsin digestion. Similar results were obtained when purified leucine-specific binding protein was treated under similar conditions (Fig. 5 *Right*).

DISCUSSION

The leucine-specific binding protein specified by gene *livK* is one of four previously identified genes involved in high-affinity branched chain amino acid transport in *E. coli* (24, 25). These four genes form a cluster that maps near minute 74 on the *E. coli* chromosome (1). This gene cluster has been cloned into a plasmid vector (see Fig. 1) and the resulting plasmid, pOX1, has been used for *in vitro* study of expression of leucine transport genes (2). The subcloning of the 2.1-kb *Bgl* II restriction fragment from pOX1 into another plasmid vector provided us with a plasmid, pOX7, containing only *livK*, the structural gene for the leucine-specific binding protein. With this plasmid we studied expression of the *livK* gene *in vitro* and were able to determine the translation initiation site and the direction of transcription. As shown in Fig. 4, a *Bam*HI site was located at a position corresponding to amino acid residue 254 of the leucine-specific binding protein. This information established the direction of transcription and the location of the region on the DNA fragment that codes for the NH_2-terminal amino acid sequence of the binding protein. Additional mapping identified a 650-base *Hpa* II fragment that spans the *Hin*cII site which is very near the predicted NH_2-terminal coding region. The DNA sequence of one end of the *Hpa* II fragment contained a segment that codes for the first 13 amino acid residues of the mature leucine-specific binding protein. Reading the amino acid sequence upstream in the same reading frame provided us with an NH_2-terminal extension of the amino acid sequence.

The translation start codon was located in two ways. First, *in vitro* synthesis of the leucine-specific binding protein using pOX7 plasmid DNA as template produced a precursor form of the binding protein with a molecular weight that was approximately 2500 higher than that of the mature binding protein. This result suggested that the methionine codon at position −23 is the start codon. A second approach involved sequence determination, by microtechnique, of labeled *in vitro* synthesized precursor protein. The results of the sequence experiments allow us to align the protein with the predicted NH_2-terminal amino acid sequence and establish that the precursor form of the leucine-specific binding protein has a 23-amino acid residue extension at the NH_2 terminus of the mature form of the binding protein. The signal sequence of 23 amino acid residues contains several basic amino acids at the beginning with a series of hydrophobic amino acid residues in the middle. This sequence is similar to that of other prokaryotic signal sequences (26, 27).

The leucine-specific binding protein is located in the periplasmic space and therefore is a secretory protein. Current models for the synthesis and secretion of proteins (28, 29) predict that the leucine-specific binding protein would be synthesized in a precursor form containing a signal sequence which would be subsequently cleaved by a membrane-associated enzyme. The *in vitro* synthesis studies presented in Fig. 2 show that after 30–40 min of incubation most of the leucine-specific binding protein is present in a precursor form. The results in Fig. 3 show, however, that continued incubation at 37°C after DNase and RNase treatment produces significant processing of the precursor form to the mature form. It is possible that the residual processing activity observed in these experiments re-

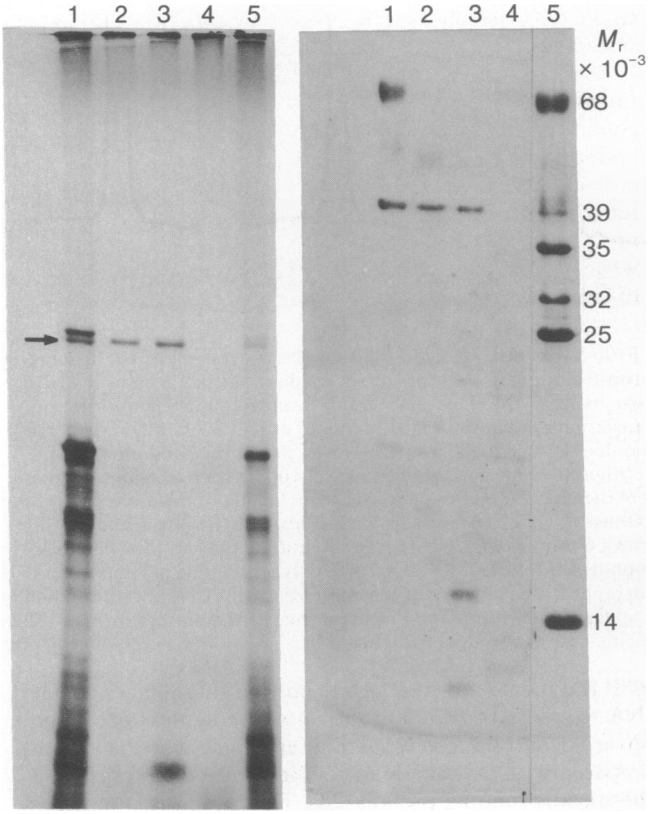

FIG. 5. (*Left*) Digestion of *in vitro* synthesized proteins with proteolytic enzymes. Proteins made *in vitro* by using pOX7 DNA as template were labeled for 45 min at 37°C with L-[^{35}S]methionine. The reaction was terminated by the addition of DNase (250 μg/ml), RNase (250 μg/ml), and unlabeled methionine (1 mg/ml). After 5 min at 37°C, aliquots of the reaction mixture were removed and incubated for 60 min at 37°C with or without proteolytic enzymes. Lanes: 1, before incubation with proteolytic enzymes; 2, trypsin (77 μg/ml); 3, chymotrypsin (77 μg/ml); 4, papain (77 μg/ml); 5, no addition. At the end of the incubation samples were combined with an equal volume of $NaDodSO_4$ sample buffer and heated for 5 min at 95°C. The mixture was subjected to 12.5% $NaDodSO_4$/polyacrylamide gel electrophoresis; the gel was stained, dried, and autoradiographed. The arrow indicates the position of mature leucine-specific binding protein. (*Right*) Digestion of the mature leucine-specific binding proteins with proteolytic enzymes. Purified leucine-specific binding protein (60 μg/ml) and bovine serum albumin (35 μg/ml) were incubated in 83 mM Tris·HCl buffer (pH 7.4) for 2 hr at 37°C with or without proteolytic enzyme. Lanes: 1, without enzyme; 2, trypsin (35 μg/ml); 3, chymotrypsin (35 μg/ml); 4, papain (35 μg/ml). Digestions were terminated by the addition of an equal volume of $NaDodSO_4$ sample buffer followed by heating for 5 min at 95°C. Samples were resolved by 12% $NaDodSO_4$/polyacrylamide gel electrophoresis. The gel was stained with Coomassie blue, destained, and dried on filter paper. Lane 5 contains molecular weight markers: bovine serum albumin (68,000), leucine-specific binding protein (39,000), galactose-binding protein (35,000), sulfate-binding protein (32,000), histidine-binding protein (25,000), and lysozyme (13,800). The galactose-, histidine-, and sulfate-binding proteins were a gift from Robert Hogg.

sults from traces of membrane material present in the *in vitro* system.

Previous studies have shown that the periplasmic binding proteins are remarkably stable under various protein denaturing conditions (30, 31). The LIV-binding protein was shown to undergo large reversible conformational changes in the presence of either urea or guanidine (31). In the present study we found that the mature form of the leucine-specific binding protein was resistant to digestion by trypsin and chymotrypsin whereas the *in vitro* synthesized precursor form was susceptible to these enzymes. The resistance to proteolytic digestion of the *in vitro* synthesized and processed leucine-specific binding protein suggests that the *in vitro* processing produces the mature form of the protein. It appears that the NH_2-terminal signal sequence of the precursor form prevents the leucine-specific binding protein from assuming its most stable conformation. It is possible that the conformational changes associated with the conversion of the precursor to the mature leucine-specific binding protein play a role in the secretion and maturation processes. Evidence for conformational differences between percursor and processed forms of thyroid-stimulating hormone β subunit have also been obtained by using antibodies to the mature and denatured forms of the hormone (32).

These results suggest an alternate role of the NH_2-terminal extension or signal sequence in protein transmembrane secretion to that suggested by the signal hypothesis (29). Perhaps the NH_2-terminal extension or signal peptide itself is not sufficient for recognition by the membrane, but it may induce a conformation in the precursor that allows the protein to be recognized and transported through the membrane. After the precursor is transported, the NH_2-terminal extension is cleaved, resulting in a conformational change that will not permit the mature form to be transported back across the membrane. This signal-induced conformation hypothesis more closely resembles the membrane trigger hypothesis (33) than the signal hypothesis (29). Which of these hypotheses is correct is not yet known.

Most of these studies were carried out while D.L.O. was a Macy Faculty Scholar on sabbatical leave at Stanford University supported by the Josiah Macy, Jr., Foundation. Additional support was provided by Grants GM 11024 (D.L.O.) and GM 09738 (C.Y.) from the U.S. Public Health Service, National Science Foundation Grant PCM 77-24333 (C.Y.), and American Heart Association Grant 69C-15 (C.Y.) and Grant in Aid (J.J.A.). J.J.A. is an Established Investigator of the American Heart Association. G.Z. is a Career Investigator Fellow of the American Heart Association. C.Y. is a Career Investigator of the American Heart Association. R.P.G. is a Postdoctoral Fellow of the U.S. Public Health Service. R.L. is supported by a Horace H. Rackham Predoctoral Fellowship.

1. Oxender, D. L. (1974) in *Biomembranes*, ed. Manson, L. A. (Plenum, New York), Vol. 5, pp. 25–79.
2. Oxender, D. L., Anderson, J. J., Daniels, C. J., Landick, R., Gunsalus, R. P., Zurawski, G., Selker, E. & Yanofsky, C. (1980) *Proc. Natl. Acad. Sci USA* **77**, 1412–1416.
3. Bolivar, F., Rodriguez, R. L., Greene, P. J., Betlach, M. C., Heyneker, J. L. & Boyer, J. W. (1977) *Gene* **2**, 95–113.
4. Anderson, J. J. & Oxender, D. L. (1978) *J. Bacteriol.* **136**, 168–174.
5. Philippson, P. & Davis, R. W. (1979) in *Bethesda Research Laboratories Catalog* (Bethesda Research Laboratories, Rockville, MD).
6. Greene, P. J., Heynecker, H. L., Bolivar, F., Rodriguez, R. L., Betlack, M. C., Corurrubias, A. A., Backman, K., Russel, D. J., Tait, R. & Boyer, H. W. (1978) *Nucleic Acids Res.* **5**, 2373–2380.
7. Bickle, T. A., Pirrotta, V. & Imber, R. (1977) *Nucleic Acids Res.* **4**, 2561–2572.
8. Mc Donnell, M. W., Simon, M. N. & Studier, F. W. (1977) *J. Mol. Biol.* **110**, 119–146.
9. Stauffer, G. V., Zurawski, G. & Yanofsky, C. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4833–4837.
10. Bahl, C. P., Marians, K. J., Wu, R., Stawinsky, J. & Narang, S. A. (1976) *Gene* **1**, 81–92.
11. Selker, E., Brown, K. & Yanofsky, C. (1977) *J. Bacteriol.* **129**, 388–394.
12. Gunsalus, R. P., Zurawski, G. & Yanofsky, C. (1979) *J. Bacteriol.* **140**, 106–113.
13. Zubay, G., Chambers, D. A. & Cheong, L. C. (1970) in *The Lactose Operon*, eds. Beckwith, J. R. & Zipser, D. A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 375–391.
14. Clewell, D. B. & Helinski, D. R. (1969) *Proc. Natl. Acad. Sci. USA* **62**, 1159–1166.
15. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
16. Anderson, J. J. & Oxender, D. L. (1977) *J. Bacteriol.* **130**, 384–392.
17. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–564.
18. Sanger, F. & Coulson, A. R. (1978) *FEBS Lett.* **87**, 107–110.
19. Randall, L. L., Josefsson, L. G. & Hardy, S. J. S. (1978) *Eur. J. Biochem.* **92**, 411–415.
20. Chang, C. N., Blobel, G. & Model, P. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 361–365.
21. Mandel, G. & Wickner, W. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 236–240.
22. Osborn, M. J., Gander, J. E., Parisi, E. & Carson, J. (1972) *J. Biol. Chem.* **247**, 3962–3972.
23. Quay, S. C. & Oxender, D. L. (1979) in *Regulation of Membrane Transport in Biological Regulation and Development*, ed. Goldberg, R. F. (Plenum, New York).
24. Oxender, D. L., Anderson, J. J., Mayo, M. M. & Quay, S. C. (1977) *J. Supramol. Struct.* **6**, 419–431.
25. Mayo, M. M. (1979) Dissertation (Univ. of Michigan, Ann Arbor, MI).
26. Inouye, M. & Halegoua, S. (1980) *CRC Crit. Rev. Biochem.*, in press.
27. Thibodeau, S. M., Palmiter, R. D. & Walsh, K. A. (1978) *J. Biol. Chem.* **253**, 9018–9023.
28. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67**, 835–851.
29. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67**, 852–862.
30. Oxender, D. L. & Quay, S. C. (1976) in *Methods in Membrane Biology*, ed. Korn, E. D. (Plenum, New York), **6**, 183–242.
31. Penrose, W. R., Zand, R. & Oxender, D. L. (1970) *J. Biol. Chem.* **245**, 1432–1437.
32. Giudice, L. C. & Weintraub, B. D. (1979) *J. Biol. Chem.* **254**, 12679–12683.
33. Wickner, W. (1979) *Annu. Rev. Biochem.* **48**, 23–45.