THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 269, No. 18, Issue of May 6, pp. 13609-13613, 1994
Printed in U.S.A.

β-Turn Formation in the Processing Region Is Important for Efficient Maturation of *Escherichia coli* Maltose-binding Protein by Signal Peptidase I *in Vivo**

(Received for publication, September 29, 1993, and in revised form, March 1, 1994)

Genevieve A. Barkocy-Gallagher‡, Janne G. Cannon, and Philip J. Bassford, Jr.§

From the Department of Microbiology and Immunology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599-7290

Signal peptidase I (also called leader peptidase) is the endopeptidase that removes the signal peptides of most secreted proteins during or after translocation in *Escherichia coli*. Precursor recognition is contingent in part on the presence of small, uncharged residues in the −3 and −1 positions relative to the cleavage site, and may also depend on the structure of the processing region. Most precursor processing regions include residues likely to form a β-turn. Mutations were introduced into the processing region of maltose-binding protein (MBP) that altered the prediction of β-turn formation in this region. MBP species with a decreased probability of β-turn formation were processed slowly or not at all, whereas MBP species with an increased probability of β-turn formation were processed efficiently. Mutations altering the prediction of β-turn formation in the MBP processing region were also made in *cis* to a proline in the +1 position. Cleavage at the normal processing site is blocked by proline in the +1 position; this MBP species, MBP27-P, inhibits processing of other proteins by signal peptidase I. Decreasing the probability of β-turn formation in the processing region of MBP27-P eliminated the inhibition of signal peptidase I, and these MBP27-P derivatives remained unprocessed, suggesting that the formation of a β-turn in the MBP processing region was necessary for recognition by signal peptidase I. Increasing the probability of β-turn formation in *cis* to proline at +1 in MBP did not alter recognition of the protein by the processing enzyme. The results presented here are consistent with the hypothesis that the efficiency of recognition and processing by signal peptidase I is increased by the formation of a β-turn in the processing region of the MBP signal peptide.

An N-terminal signal peptide is commonly present on proteins that traverse the cytoplasmic membrane of bacterial cells or the rough endoplasmic reticulum membrane of eukaryotic cells. Most of the time, the signal peptide is removed at the distal surface of the membrane after irreversible initiation of translocation. In *Escherichia coli*, signal peptidase II removes the signal peptides of lipoproteins and signal peptidase I (or leader peptidase) removes the signal peptides of other precursors (1). If a signal peptide is not removed, the protein usually remains membrane-bound but can be released into the periplasmic space in *E. coli* (2–5).

Signal peptides share little primary sequence homology but do have three common structural features (6, 7). The hydrophilic N-terminal region includes 1–3 positively charged residues, and the central core is composed of 9–15 hydrophobic residues postulated to form an α-helix. These two structures are involved in the initiation of export and translocation of the precursor across the membrane (8). The signal peptide C-terminal processing region consists of 4–6 amino acids and is usually more polar than the hydrophobic core. Positions −3 and −1 relative to the cleavage site are important for recognition and processing by signal peptidase I (9–13).

In addition, most precursor processing regions include residues likely to form a β-turn (11). Some reports suggest that substitutions resulting in structural changes around the signal peptide cleavage site affect translocation (14, 15), but the majority of studies report that alterations in the processing region directly affect processing. Substitutions changing the proline residue in the processing region of M13 procoat or pre-β-lactamase decrease or eliminate processing but not export (4, 5, 10, 16, 17). Proline frequently occupies position −6, −5, or −4 relative to the cleavage site (13); proline residues break α-helical structures and are often found in β-turns (18). Alterations in the early mature region of a chimeric OmpA-staphylococcal nuclease A protein that decrease the probability of β-turn formation at the processing site decrease processing (19). Kendall and co-workers (20) found that substitution of leucine for the proline residue at position −5 in the PhoA signal peptide dramatically decreases processing. Subsequently, Laforet and Kendall (21) postulated that the structure of the processing region is not a determining factor for maturation, but that the relative hydrophobicity of the amino acids preceding the cleavage site is important.

The probability of β-turn formation in the MBP[1] signal peptide processing region is strongest for the Ser-Ala-Ser-Ala sequence at positions −6 through −3 (see Fig. 1). Since the propensity for β-turn formation in this case does not rely strongly on a single residue as it does for proline-containing processing regions, MBP provides a good model to examine the effects of different substitutions near the processing site. In addition, the secretion and processing of MBP have been studied in detail (for review, see Ref. 22). In this study, changes designed to alter the probability of β-turn formation were introduced into the MBP signal peptide processing region. Alterations decreasing the probability of β-turn formation slowed or eliminated proc-

* This research was supported by Grant AI17292 from NIAID, National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This manuscript is dedicated to the memory of Philip J. Bassford, Jr.

‡ To whom correspondence should be addressed. Present address: Dept. of Biology, Jordan Hall, Box 7, Indiana University, Bloomington, IN 47405. Tel.: 812-855-3200; Fax: 812-855-6705.

§ Deceased.

[1] The abbreviations used are: MBP, maltose-binding protein (the prefix "pre" indicates the precursor form, whereas "m" indicates the mature form); RBP, ribose-binding protein; IPTG, isopropyl-1-thio-β-D-galactopyranoside; PAGE, polyacrylamide gel electrophoresis.

essing but did not affect translocation. In general, substitutions that increased the probability of β-turn formation did not alter processing efficiency.

MATERIALS AND METHODS

Strains and Plasmids—*E. coli* K12 strain BAR1091 (23) is a derivative of MC4100 (24) with a large, in-frame deletion in the MBP coding region. This strain was used as the host strain for plasmids encoding the various MBP species. Strain CJ236 (*dut⁻ung⁻*; Ref. 25) was used to construct templates for oligonucleotide site-directed mutagenesis.

Plasmid pJF2 is a derivative of pBR322 carrying the M13 intragenic region (2). This plasmid carries the *malE*⁺ gene under regulatory control of the *lacUV5* promoter and all of the plasmids used in this study were derived from it. Plasmid pGG25 harbors the *malE27-P* gene encoding a MBP species with proline in the +1 position relative to the normal cleavage site (26). Plasmid pJF8 encodes an amber mutation at position −4 relative to the cleavage site (2). Plasmid pJW4 carries the *malEΔ116* allele (27). This gene encodes a proteinase K-sensitive MBP species deleted for residues 168–176 of the mature moiety. This change does not alter export of the protein. To introduce the *malEΔ116* mutation into plasmids encoding MBP species with processing region alterations, the small *Eco*RI-*Bgl*II fragments of the latter were individually ligated to the large *Eco*RI-*Bgl*II fragment from pJW4. Plasmids were packaged as M13 phage particles using M13K07 helper phage (25).

Reagents—Minimal medium M63 supplemented with 0.2% carbon source and thiamine (2 μg/ml) and tryptone yeast agar were prepared as described previously (28). When required, ampicillin was added to minimal and complex media at concentrations of 25 and 50 μg/ml, respectively. To induce expression from plasmid-encoded *malE* genes, IPTG was added to agar plates and liquid media at 1 and 5 mM, respectively. [^{35}S]Methionine (1154 Ci/mmol) was obtained from DuPont NEN. Electrophoresis reagents were obtained from Sigma. Rabbit anti-MBP and anti-RBP sera have been described previously (29, 30). IPTG, T4 ligase, and Klenow fragment polymerase were obtained from Boehringer Mannheim or Life Technologies, Inc. XAR film was purchased from the Eastman Kodak Co.

Oligonucleotide Site-directed Mutagenesis of malE—The method of Zoller and Smith (31) was used with modifications to introduce mutations into the plasmid-encoded *malE* gene (9). Single-stranded DNA templates were prepared from cells of *E. coli* strain CJ236. Oligonucleotides complementary to the packaged, single-stranded DNA of plasmid pJF2 were prepared with an Applied Biosystems model 380A DNA synthesizer and purified as described previously (32). The primers repaired the amber codon of plasmid pJF8, so mutagenized plasmids restored a Mal+ phenotype to BAR1091 cells. Some of the mutagenesis reactions were performed using plasmid pMBPΔ-4/5/6 as the template DNA, to avoid any bias of nucleotide pairing during the reaction. Plasmid pGG25 provided the template DNA for construction of mutations in *cis* to the *malE27-P* mutation. All mutagenized plasmids were transformed into cells of strain BAR1091 and subsequently reintroduced into BAR1091 cells by phage M13-mediated transduction. Mutations were confirmed by DNA sequencing as described previously (32).

Radiolabeling of Proteins, Immune Precipitation, SDS-PAGE, Autoradiography, and Protein Localization—Cells of strain BAR1091 harboring derivatives of plasmid pJF2 were grown to mid-log phase in glycerol minimal medium, supplemented with ampicillin. Forty-five minutes after induction of MBP expression by the addition of IPTG to the cultures, cells were pulse-labeled with [^{35}S]methionine for 15 s, followed by a chase with excess cold methionine for specified times. Proteins were immunoprecipitated from solubilized cell extracts and analyzed by SDS-PAGE and autoradiography as described previously (33), except that MBP species with proline at the +1 position were resolved on a 7.5% polyacrylamide gel. Localization of protease-sensitive MBP species was performed by analysis of spheroplast association and proteinase K accessibility as described previously (2). Quantitation of proteins was carried out using an AMBIS Radioanalytic Imaging System; calculation of the ratio of precursor to mature species included adjustments for additional methionines in the precursor proteins.

RESULTS

Effects of Deletions and Substitutions in the Processing Region of PreMBP—The processing region of the MBP signal peptide is 6 amino acids long (Fig. 1) and, based on Chou and Fasman analysis (18), includes residues that could form a β-turn (Table I). We wanted to determine the significance of residues in the processing region of the MBP signal peptide

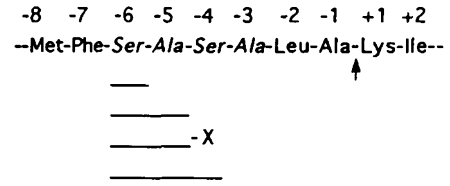

FIG. 1. **The processing region of the MBP signal peptide.** The residues encompassing the processing region of the MBP signal peptide are shown. The hydrophobic core region ends with the phenylalanine at position −7. The *arrow* indicates the normal processing site. The alternate processing site is between residues −3 and −2 (9). The amino acids involved in the predicted β-turn are *italicized*. *Lines* indicate the residues involved in deletions; *X* indicates substitutions made at position −4.

that are not part of the well established −3, −1 processing recognition site (9) but are potentially involved in the formation of a β-turn. Using oligonucleotide site-directed mutagenesis, derivatives of plasmid pJF2 were constructed encoding MBP species with deletions including the −6 to −4 residues of the processing region.

The kinetics of conversion from preMBP to mMBP for these MBP species are described in Table I. Mid-log phase cells induced for synthesis of MBP were pulse-labeled for 15 s with [^{35}S]methionine, and aliquots were removed at specific times during the chase period for analysis of MBP processing. Wild-type preMBP was completely processed to the mature form within 2 min (see Fig. 3). By deleting residues −6 and −5, the probability of a β-turn occurring in the processing region was decreased and this MBP species, MBPΔ−5/6, was processed very slowly (Table I, Fig. 2). To further explore the role of residues −6 through −4 in maturation, the −5 alanine was reinserted (MBPΔ−6) or substitutions were made in place of the serine residue at position −4 (MBPΔ−5/6.−4 series). The probability of a β-turn forming in the processing region remained low for these MBP species (Table I). Reintroduction of the −5 alanine (MBPΔ−6) did not restore efficient processing (Table I). The substitution of alanine at −4 in the shortened processing region (MBPΔ−5/6.−4A) slowed processing (Fig. 2). No processing was observed when leucine, isoleucine, or valine were substituted at the same position (Table I, Fig. 2) or when the −4 residue was also deleted (MBPΔ−4/5/6). Only the substitution of the α-helix-breaker proline at the −4 position restored efficient processing (MBPΔ−5/6.−4P; Table I, Fig. 2).

The cells containing plasmids expressing each of these shortened MBP species were Mal+, indicating that the MBP species must have been translocated across the membrane to some extent. To confirm that these MBP species were rapidly translocated and remained tethered at the outer surface of the membrane, the locations of precursors MBPΔ−6, MBPΔ−5/6.−4I, MBPΔ−5/6.−4A, and MBPΔ−4/5/6 in the cells were determined as described previously (2). MBP normally is protease-resistant; a deletion of 9 residues in the mature moiety of MBP renders the protein protease-sensitive without affecting secretion (27). The mutation encoding this deletion (Δ116) was introduced into plasmids encoding the aforementioned MBP species. The location of each MBP species was then determined by proteinase K accessibility in fractionated spheroplasts after a 1-min pulse-chase labeling. The precursor form of each of these MBP species was spheroplast-associated and digested by externally added proteinase K, indicating that they were rapidly translocated and located at the outer surface of the inner membrane (data not shown). Therefore, these deletions and substitutions decreasing the probability of β-turn formation in the MBP processing region affected maturation but did not detectably affect translocation.

Effects of Substitutions in the Processing Region of Full-

TABLE I
Processing and β-turn probability of preMBP species with deletions and substitutions in the processing region

MBP species	Alteration	β-Turn		Kinetics of processing[c]
		Probability[a]	Site[b]	
MBP (wild-type)	None	0.66	−6	+++++
MBPΔ−5/6.−4P	Deletion of −6 and −5, substitution −4P	0.44	−5	+++++*
MBPΔ−5/6	Deletion of −6S and −5A	0.38	−7	++
MBPΔ−6	Deletion of −6S	0.33	−6	++
MBPΔ−5/6.−4L	Deletion of −6 and −5, substitution −4L	0.25	−7	−
MBPΔ−5/6.−4A	Deletion of −6 and −5, substitution −4A	0.21	−7	+
MBPΔ−4/5/6	Deletion of −6 and −5 and −4	0.21	−6	−
MBPΔ−5/6.−4I	Deletion of −6 and −5, substitution −4I	0.20	−7	−
MBPΔ−5/6.−4V	Deletion of −6 and −5, substitution −4V	0.19	−7	−

[a] Highest probability of β-turn initiation × 10^4, from residues −7 to +1, as predicted by the Chou and Fasman method (18). Predictions were made using the PEPPLOT program of the University of Wisconsin Genetics Computer Group (38).
[b] Location of the N-terminal residue of the predicted β-turn. β-Turns involve 4 amino acids.
[c] +++++, completely processed within 2 min, * indicates that processing at times less than 2 min was somewhat delayed; ++++, approximately 90% mature protein within 2 min; +++, approximately 90% mature protein at 10 min; ++, approximately 40% mature protein at 10 min; +, approximately 10% mature protein at 10 min; −, no processing detected.

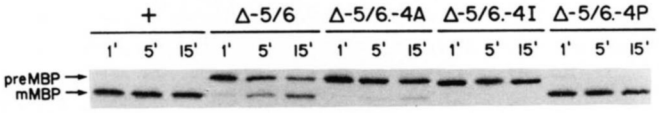

FIG. 2. **Kinetics of signal peptide processing in cells synthesizing various preMBP species with a deletion of residues −6 and −5 and substitutions at −4.** Glycerol-grown, IPTG-induced cells were pulse-labeled for 15 s with [^{35}S]methionine, and incubation was continued in the presence of excess cold methionine for the number of minutes indicated above each lane. The chase was terminated by the addition of ice-cold trichloroacetic acid. The resultant acid precipitates were solubilized, and the MBP was immunoprecipitated and analyzed by SDS-PAGE and autoradiography. The MBP species analyzed is indicated above each set of lanes. + denotes synthesis of wild-type MBP.

length PreMBP—The decrease in processing of MBP species with shortened processing regions implied that the structure of the region could indeed be important for maturation but left open the possibility that the distance between the hydrophobic core and the cleavage site was a significant factor. We also investigated the processing of preMBP species with similar changes in the full-length processing region, also expressed from derivatives of plasmid pJF2 constructed by oligonucleotide site-directed mutagenesis. The probability of β-turn formation in the processing region and the kinetics of processing of preMBP species with substitutions at positions −6, −5, or −4 are indicated in Table II. Unlike the shortened MBP species described above, all but one of these preMBP species were processed completely within a 5-min chase period in pulse-chase labeling experiments (Figs. 3 and 4, data not shown).

The substitution of glycine, leucine, valine, or isoleucine at position −6 decreased the probability of a β-turn occurring in the processing region; concomitantly, processing of these preMBP species was slower than processing of wild-type preMBP (Table II, Fig. 3). The substitution of alanine at position −6 also decreased the probability of a β-turn forming (Table II). Processing of preMBP-6A was complete within 2 min (Fig. 3) but was slightly slower than processing of wild-type preMBP when examined very soon after labeling (71% processed to mMBP-6A at 0 s of chase *versus* 85% mMBP (wild-type); data not shown). The remaining MBP species with substitutions at position −6, MBP-6P and MBP-6D, did not have a decreased probability of a β-turn occurring in the processing region; processing of these preMBP species was identical to that of wild-type preMBP (Table II, Fig. 3).

Substitutions were introduced at positions −5 or −4 of MBP that increased the probability of a β-turn occurring in the processing region. With one exception, processing of these preMBP species was identical to processing of the wild-type protein, even when examined immediately post-labeling (Table II, Fig. 4). Processing of preMBP with an aspartic acid substituted at position −5 (preMBP-5D) was slightly slower than that of wild-type preMBP (Fig. 4), but no preMBP-5D was evident at 2 min post-labeling (data not shown).

TABLE II
Processing and β-turn probability of preMBP species with substitutions in the processing region

MBP species	Substitution	β-Turn		Kinetics of processing[c]
		Probability[a]	Site[b]	
MBP (wild-type)	None	0.66	−6	+++++
MBP−5P	−5P for −5A	2.62	−6	+++++
MBP−5D	−5D for −5A	1.56	−7	+++++*
MBP−5S	−5S for −5A	1.21	−6	+++++
MBP−4N	−4N for −4S	1.01	−6	+++++
MBP−5Y	−5Y for −5A	0.99	−7	+++++
MBP−5T	−5T for −5A	0.94	−6	+++++
MBP−5H	−5H for −5A	0.81	−7	+++++
MBP−6D	−6D for −6S	0.81	−6	+++++
MBP−6P	−6P for −6S	0.66	−7	+++++
MBP−6G	−6G for −6S	0.56	−6	++++
MBP−6L	−6L for −6S	0.34	−6	+++
MBP−6V	−6V for −6S	0.34	−6	++++
MBP−6A	−6A for −6S	0.33	−6	+++++*
MBP−6I	−6I for −6S	0.24	−6	++++

[a] Highest probability of β-turn initiation × 10^4, from residues −7 to +1, as predicted by the Chou and Fasman method (18). Predictions were made using the PEPPLOT program of the University of Wisconsin Genetics Computer Group (38).
[b] Location of the N-terminal residue of the predicted β-turn. β-Turns involve 4 amino acids.
[c] +++++, completely processed within 2 min, * indicates that processing at times less than 2 min was somewhat delayed; ++++, approximately 90% mature protein within 2 min; +++, approximately 90% mature protein at 10 min.

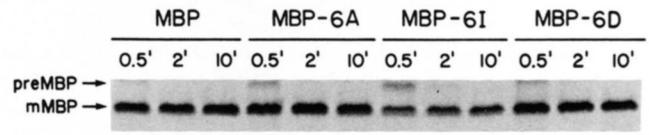

FIG. 3. **Kinetics of signal peptide processing in cells synthesizing various preMBP species altered at −6.** The experimental conditions are the same as those described in the legend to Fig. 2. The MBP species analyzed is indicated above each set of lanes.

Effect of Changes in cis to Proline in the +1 Position—MBP with a proline in the +1 position relative to the cleavage site, MBP27-P (there are 26 amino acids in the MBP signal peptide), is a competitive inhibitor of signal peptidase I (26). It is not processed, although an alternate processing site 2 amino acids upstream of the normal processing site is available. A chimeric protein with a proline in the +1 position, the RBP signal peptide, and the MBP mature moiety is slowly processed to completion, evidently at an alternate site that is also 2 residues N-

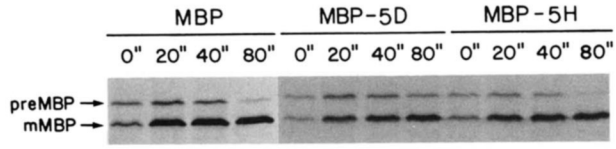

FIG. 4. **Kinetics of signal peptide processing in cells synthesizing various preMBP species altered at −5.** The experimental conditions are the same as those described in the legend to Fig. 2. The length of the chase period in seconds is indicated above each lane. The MBP species analyzed is indicated above each set of lanes.

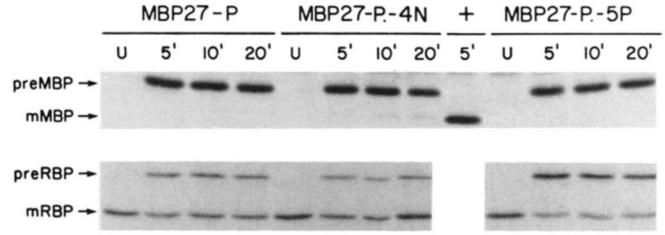

FIG. 5. **MBP and RBP maturation in cells producing various MBP species.** The experimental conditions are the same as those described in the legend to Fig. 2. The MBP species analyzed is indicated above each set of lanes. *U*, immunoprecipitates were obtained from cells uninduced for MBP synthesis and pulse-radiolabeled for 15 s, followed by an additional 5-min chase period. + denotes synthesis of wild-type MBP (RBP was not precipitated).

terminal to the normal processing site (26). This protein is a less effective inhibitor of signal peptidase I than MBP27-P. We postulated that processing of the chimeric protein was due to the higher probability of β-turn formation in the processing region of the RBP signal peptide relative to that of the MBP signal peptide. Therefore, increasing the probability of β-turn formation in the MBP27-P processing region might result in processing at the alternate site.

To test this hypothesis, mutations increasing the probability of β-turn formation were introduced into the processing region of the MBP27-P signal peptide; serine, proline, or threonine was substituted at position −5, or asparagine was substituted at position −4. The substitutions at position −5 changed the −3 residue of the alternate processing site, but each of these amino acids in the normal −3 position permits efficient MBP cleavage (9). Because proline is a strong α-helix breaker (18), we also substituted proline at position −6 of MBP27-P, even though this substitution does not change the prediction of β-turn formation. Only preMBP27-P with an asparagine at position −4 was processed, and just 3.4% of the protein was converted to the mature form within 20 min post-labeling (Fig. 5). This mMBP species migrated slightly slower that wild-type mMBP in a 7.5% polyacrylamide gel, suggesting that it resulted from processing at the alternate site (9, 26). The MBP27-P species with substitutions increasing the probability of β-turn formation in the processing region inhibited processing by signal peptidase I at least as well as unaltered MBP27-P (Fig. 5, data not shown). Thus, increasing the probability of β-turn formation in *cis* to proline at +1 in MBP did not affect recognition of the precursor by signal peptidase I and did not result in processing of the precursor MBP species.

If the various changes decreasing the probability of β-turn formation decreased recognition of MBP by signal peptidase I, then processing of RBP by signal peptidase I should not be inhibited by MBP27-P species with a lower probability of β-turn formation in the processing region. Deletion of the serine at position −6 or substitution of an alanine at −6 of MBP27-P relieved the block of RBP processing exerted by the proline residue at position +1. Both preMBP27-P species remained unprocessed (data not shown), so decreasing the probability of β-turn formation in MBP27-P resulted in MBP species that were not effectively recognized by signal peptidase I.

DISCUSSION

Two major steps in the export of proteins across the inner membrane of *E. coli* are translocation across the lipid bilayer and removal of the N-terminal signal peptide. It has been proposed that the signal peptide forms an α-helix to cross the lipid bilayer (34). Consensus analysis of known signal peptides suggests that residues in the processing region form a β-turn ending the α-helix (11), but there is disagreement whether this proposed structure is important for processing, translocation, or even if it is at all significant (4, 5, 10, 14–17, 19, 21). Based on studies with an idealized PhoA signal peptide, Laforet and Kendall (21) have postulated that the hydrophobicity of the residues in the processing region is important, rather than the propensity for β-turn formation. However, Dalbey and co-workers (10) found no correlation between processing and hydrophobicity of residues in this region of M13 procoat. We found no consistent correlation between hydrophobicity and processing for the MBP species described here. The signal peptide processing region of MBP involves residues −6 through −1; the highest probability of β-turn formation in this region, based on Chou and Fasman analysis (18), involves residues −6 through −3. Although Chou and Fasman rules may not apply to the signal peptide during translocation, our data are consistent with the hypothesis that formation of a β-turn in the processing region of the MBP signal peptide affects recognition of the precursor by signal peptidase I but does not significantly affect translocation.

Changes in the MBP signal peptide that decreased the probability of β-turn formation in the processing region without altering the −3, −1 cleavage recognition site (9) slowed or eliminated processing. Substitutions at the −6 position that decreased the probability of β-turn formation consistently resulted in slowed kinetics of MBP processing. Deletions involving the −6 through −4 residues (MBPΔ−6, MBPΔ−5/6, and MBPΔ−4/5/6) also decreased the probability of β-turn formation in the processing region and decreased processing. Several substitutions were made at position −4 of MBPΔ−5/−6, none of which restored a high probability of β-turn formation to the processing region. Of these, only proline restored efficient MBP processing (MBPΔ−5/6.−4P), almost to wild-type levels.

The proline residue in MBPΔ−5/6.−4P and the other amino acid substitutions described in this study could be interrupting the α-helical structure of the hydrophobic core other than by formation of a β-turn *per se* or could affect processing in an undetermined manner. However, we believe that our results are more consistent with the probability of β-turn formation in the region than with the α-helix breaking or other defined properties of the substituted amino acids. For example, even though glycine is described as a strong α-helix breaker (18), the substitution of glycine for serine at position −6 of MBP decreased the probability of β-turn formation and caused slower MBP processing. Glycine in place of proline in an idealized yeast signal peptide processing region or in M13 procoat does not support efficient processing, implying that glycine does not provide the necessary function in these circumstances, either (10, 35). Proline causes a kink in the peptide backbone and is commonly found in β-turns, so it is possible that a β-turn forms in MBPΔ−5/6.−4P despite the low probability determined by the Chou and Fasman algorithm (18).

While there was a consistent correlation between the decreased probability of β-turn formation in the processing region and decreased processing of preMBP species, the magnitude of the change in processing did not always correlate with the magnitude of the decrease in β-turn forming probability. The

processing defects for the truncated MBP species were more dramatic than for MBP species with simple substitutions in the processing region, suggesting that the length of the processing region in MBP is important. This is an intriguing result, since MBP species with shortened hydrophobic cores are efficiently translocated and processed, and it has been implied that the hydrophobic core and processing region of MBP can overlap (36, 37). Most prokaryotic signal peptide processing regions that have been identified are 6 amino acids in length (38). Our data suggests that for MBP, 6 amino acids *versus* 4 in the processing region contributed additional flexibility or propensity to turn.

Plasmids were also constructed encoding MBP species with substitutions at position −6, −5, or −4 that increased the probability of β-turn formation. These full-length MBP species had processing kinetics that were unaltered or only slightly altered (MBP-5D) from the kinetics of wt preMBP processing, even though the predicted β-turns of several of these MBP species initiated at residue −7, one position beyond the designated processing region. Similarly, Nothwehr and Gordon (39) found that processing of a eukaryotic protein in an *in vitro* translocation system occurred when the predicted β-turn initiated within 1 residue of the processing region.

MBP27-P has a proline in the +1 position relative to the normal processing site, is not processed, and is a competitive inhibitor of signal peptidase I (26). Both RBP with a proline in the normal +1 position and an RBP-MBP chimeric precursor with proline at +1 position are processed, probably at an alternate site 2 residues upstream of the normal processing site (26). We hypothesized that processing of the latter two proteins resulted from the higher probability of β-turn formation in the RBP signal peptide processing region, which is due to an asparagine residue at position −4 relative to the normal cleavage site. In this study, we examined processing of MBP27-P derivatives with substitutions resulting in a high β-turn probability. Only MBP27-P with asparagine at position −4 was processed, probably at the alternate processing site. However, processing of the RBP and RBP-MBP chimeric proteins with proline at +1 is much more rapid than processing of MBP27-P.-4N, and proceeds to completion (26).[2] In addition, the MBP27-P derivatives with an increased probability of β-turn formation were effective inhibitors of signal peptidase I, unlike the RBP and chimeric proteins with proline in the +1 position.[2] Therefore, increasing the probability of β-turn formation did not alter recognition of MBP27-P by signal peptidase I; processing did not occur at the alternate processing site.

It has been suggested that the presence of a β-turn in the processing region is important for protein translocation (14, 15). These reports are consistent with the loop model, which proposes that the signal peptide and early mature region insert into and through the membrane as a reverse hairpin structure (40). Perhaps the predicted β-turn early in the mature region of MBP plays this role. We found no evidence of a defect in translocation for MBP species with a decreased probability of β-turn formation, based on localization of unprocessed MBP species to the outer surface of the membrane and the Mal+ phenotype of the cells. However, it remains possible that we did not identify slight defects in translocation. The results of this study are consistent with the hypothesis that the β-turn in the processing region of the MBP signal peptide is important for the interaction of the protein with signal peptidase I, perhaps in presentation or binding of the processing site to the enzyme.

[2] G. A. Barkocy-Gallagher, unpublished data.

Acknowledgments—We thank Tom Silhavy for stimulating discussions and Ross Dalbey for critically reading the manuscript.

REFERENCES

1. Dev, I. K., and Ray, P. H. (1990) *J. Bioenerg. Biomembr.* **22,** 271–290
2. Fikes, J. D., and Bassford, P. J., Jr. (1987) *J. Bacteriol.* **169,** 2352–2359
3. Dalbey, R. E., and Wickner, W. (1985) *J. Biol. Chem.* **260,** 15925–15931
4. Kadonaga, J. T., Pluckthun, A., and Knowles, J. R. (1985) *J. Biol. Chem.* **260,** 16192–16199
5. Pluckthun, A., and Pfitzinger, I. (1988) *J. Biol. Chem.* **263,** 14315–14322
6. Duffaud, G. D., Lenhardt, S. K., March, P. E., and Inouye, M. (1985) *Curr. Top. Membr. Transp.* **24,** 65–104
7. Nothwehr, S. F., and Gordon, J. I. (1990) *BioEssays* **12,** 479–484
8. Randall, L. L., and Hardy, S. J. S. (1989) *Science* **243,** 1156–1159
9. Fikes, J. D., Barkocy-Gallagher, G. A., Klapper, D. G., and Bassford, P. J., Jr. (1990) *J. Biol. Chem.* **265,** 3417–3423
10. Shen, L. M., Lee, J.-I., Cheng, S., Jutte, H., Kuhn, A., and Dalbey, R. E. (1991) *Biochemistry* **30,** 11775–11781
11. Perlman, D., and Halvorson, H. O. (1983) *J. Mol. Biol.* **167,** 391–409
12. von Heijne, G. (1983) *Eur. J. Biochem.* **116,** 17–21
13. von Heijne, G. (1986) *Nucleic Acids Res.* **14,** 4683–4690
14. Iino, T., and Sako, T. (1988) *J. Biol. Chem.* **263,** 19077–19082
15. Adams, M. D., and Oxender, D. L. (1991) *J. Cell Biol.* **46,** 321–330
16. Kuhn, A., and Wickner, W. (1985) *J. Biol. Chem.* **260,** 15914–15918
17. Koshland, D., Sauer, R. T., and Botstein, D. (1982) *Cell* **30,** 903–914
18. Chou, P. Y., and Fasman, G. D. (1978) *Adv. Enzymol.* **47,** 45–148
19. Duffaud, G., and Inouye, M. (1988) *J. Biol. Chem.* **263,** 10224–10228
20. Kendall, D. A., Doud, S. K., and Kaiser, E. T. (1990) *Biopolymers* **29,** 139–147
21. Laforet, G. A., and Kendall, D. A. (1991) *J. Biol. Chem.* **266,** 1326–1334
22. Bassford, P. J., Jr. (1990) *J. Bioenerg. Biomembr.* **22,** 401–439
23. Rasmussen, B. A., MacGregor, C. A., Ray, P. H., and Bassford, P. J., Jr. (1985) *J. Bacteriol.* **164,** 665–673
24. Casadaban, M. J. (1976) *J. Mol. Biol.* **104,** 541–555
25. Kunkel, T. A., Roberts, J. D., and Zakour, R. A. (1987) *Methods Enzymol.* **154,** 367–382
26. Barkocy-Gallagher, G. A., and Bassford, P. J., Jr. (1992) *J. Biol. Chem.* **267,** 1231–1238
27. Collier, D. N., Bankaitis, V. A., Weiss, J. B., and Bassford, P. J., Jr. (1988) *Cell* **53,** 273–283
28. Miller, J. H. (1972) *Experiments in Molecular Genetics*, pp. 431–435, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
29. Collier, D. N., Strobel, S. M., and Bassford, P. J., Jr. (1990) *J. Bacteriol.* **172,** 6875–6884
30. Bankaitis, V. A., and Bassford, P. J., Jr. (1984) *J. Biol. Chem.* **259,** 12193–12200
31. Zoller, M. J., and Smith, M. (1984) *DNA* **3,** 479–488
32. Hutchinson, C. A., III, Nordeen, S. K., Vogt, K., and Edgell, M. H. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 710–714
33. Emr, S. D., and Bassford, P. J., Jr. (1982) *J. Biol. Chem.* **257,** 5852–5860
34. Briggs, M. S., Cornell, D. G., Dluhy, R. A., and Gierasch, L. M. (1986) *Science* **233,** 206–208
35. Yamamoto, Y., Taniyama, Y., and Kikuchi, M. (1989) *Biochemistry* **28,** 2728–2732
36. Collier, D. N., and Bassford, P. J., Jr. (1989) *J. Bacteriol.* **171,** 4640–4647
37. Bankaitis, V. A., Rasmussen, B. A., and Bassford, P. J., Jr. (1984) *Cell* **37,** 243–252
38. von Heijne, G. (1985) *J. Mol. Biol.* **184,** 99–105
39. Nothwehr, S. F., and Gordon, J. I. (1990) *J. Biol. Chem.* **264,** 3979–3987
40. Inouye, M., and Halegoua, S. (1980) *Crit. Rev. Biochem.* **7,** 339–371