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Biochemistry

N-terminal methionine-specific peptidase in *Salmonella typhimurium*

(N-terminal processing/aminopeptidase/protein maturation)

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ABSTRACT **Crude extracts of a multiply peptidase-deficient strain of *Salmonella typhimurium* contain an aminopeptidase that specifically removes N-terminal methionine from peptides. This activity shows pronounced specificity for the peptide's second amino acid. Methionine is removed from peptides with alanine, threonine, or glycine in this position but not when the second amino acid is leucine or methionine. The activity is stimulated by Co^{2+} and is inhibited by EDTA. Mutations that lead to overproduction (up to 30-fold) of the activity have been obtained by selecting for growth on Met-Gly-Gly as a methionine source. These mutations map at ≈3 map units, phage P22 cotransducible with *leu*. The overproducer mutations are dominant to wild type, and duplication of the wild-type allele of the locus leads to a gene dosage effect on peptidase levels. This suggests that the locus of the overproducer mutations may be the structural gene for the peptidase. NaDodSO$_4$/PAGE shows an increased level of a single protein (34 kDa) in the overproducer mutant. This protein is highly enriched in a purified preparation of the peptidase. The specificity of this enzyme suggests that it is involved in the cleavage of methionine from newly synthesized peptide chains. This activity can specifically remove methionine from the N terminus of a completed protein. Treatment of purified, unprocessed (N-terminal methionine) interleukin 1$_\beta$ with the purified peptidase results in removal of N-terminal methionine with no additional alterations. N-terminal processing of at least this protein can occur after translation is complete. We propose to call this enzyme peptidase M (methionine-specific aminopeptidase).**

Although all protein synthesis in bacteria is thought to initiate with *N*-formylmethionine, many mature proteins do not contain either *N*-formylmethionine or methionine at their N termini. Waller (1) showed that approximately half of the N-terminal amino acid from bulk *Escherichia coli* protein was not methionine. He noted that the distribution of N-terminal amino acids was highly nonrandom with alanine, serine, and threonine as the most frequent N termini after methionine. Sarimo and Pine (2) surveyed the distribution of N-terminal amino acids in the bulk protein of a variety of microbial species and concluded again that alanine, serine, and threonine compose most of the nonmethionine N termini. These experiments involved studies of bulk protein, and the results could have been skewed by the fact that only a few protein species make up a large fraction of the bulk protein of the cell (3). More recent data allow the direct comparison of DNA sequences with protein amino acid sequences. Tsunasawa *et al.* (4) have surveyed the available sequences and concluded that N-terminal methionine may be removed when the second amino acid is alanine, glycine, proline, serine, threonine, or valine but never when it is arginine, asparagine, aspartic acid, glutamic acid, glutamine, isoleucine, leucine, lysine, or methionine. These data are consistent with proposals by the earlier authors that the nature of the second amino acid determines whether or not methionine will be removed. The available data suggest that the rules for methionine removal are the same in microorganisms and in higher cells (4).

The pathway by which N termini are modified was defined by Adams (5). He showed that *E. coli* extracts contain an enzyme that deformylates almost any *N*-formylmethionine peptide. No activity capable of directly cleaving *N*-formylmethionine from an *N*-formylmethionine peptide was found. He concluded that N-terminal modification is a stepwise process, with deformylation preceding the removal of methionine by an aminopeptidase. Attempts to isolate this methionine-specific aminopeptidase have not been successful, however. Vogt (6) purified and characterized an activity capable of rapid cleavage of Met-Ala-Ser. He concluded that the specificity of this enzyme is not consistent with its involvement in N-terminal methionine removal. In addition, mutants of *E. coli* or *Salmonella* lacking this enzyme grow normally (7, 8). Earlier reports of a ribosomal peptidase activity (9) almost certainly are based on the artifactual association of the enzyme described by Vogt with the ribosome fraction (6). Another attempt to identify a methionine-specific aminopeptidase led to the isolation of an enzyme that appears to have specificity for methionine but hydrolyzes no substrates larger than dipeptides (10).

Clearly, a major problem in identifying a methionine-specific aminopeptidase in cell extracts is the presence of several broad-specificity enzymes capable of hydrolyzing N-terminal methionine peptides. We have shown that at least four such enzymes are present in crude extracts of *Salmonella typhimurium* and *E. coli* (7, 8, 11). We have also isolated mutants that lack all of these activities and observed that these mutants, although greatly restricted in their capacity to use peptides as amino acid sources, still use certain N-terminal methionine peptides (8, 11). This paper makes use of these mutant strains to identify a methionine-specific aminopeptidase and to isolate mutants that substantially overproduce this activity. The substrate specificity of this enzyme is entirely consistent with the proposal that it is involved in the removal of N-terminal methionine from newly synthesized proteins.

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Abbreviation: IL-1$_\beta$, interleukin 1$_\beta$.

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MATERIALS AND METHODS

Bacterial Strains. The bacterial strains used in this work are all derivatives of *S. typhimurium* LT2 and are listed in Table 1.

Media and Growth Conditions. Bacteriological media have been described (11). When used as a methionine source, Met-Gly-Gly was present at 0.1 mM. Peptide utilization was tested as described (7).

Genetic Techniques. Transduction with phage P22 HT 12/4 *int-3* was carried out by using standard procedures (13). Mutants able to use Met-Gly-Gly as a methionine source were selected by plating 0.1 ml of an overnight culture of TN2183 on an appropriately supplemented minimal plate containing Met-Gly-Gly as a methionine source. In some experiments a filter paper disc saturated with the mutagen diethyl sulfate was placed on the plate to increase the frequency of mutation.

Peptides. All peptides were obtained from commercial sources and were checked for homogeneity by HPLC. The composition and sequence of substrate peptides were confirmed by HPLC analysis of peptidase reaction products.

Peptidase Assay. The reaction mixture for assaying peptide hydrolysis contained 0.6 μmol of substrate (usually Met-Ala-Ser), 0.03 μmol of $CoCl_2$, 6 μmol of potassium phosphate buffer (pH 7.5), and enzyme in a total volume of 30 μl. After incubation (30 min, 37°C) the reaction was stopped by adding 3 μl of 50% trichloroacetic acid, and precipitated protein was removed by centrifugation (5 min, Eppendorf centrifuge). An aliquot of the deproteinized reaction mixture was derivatized with trinitrobenzenesulfonic acid (Pierce) and analyzed by HPLC using an Altex Ultrasphere ODS column and appropriate gradients of 0.1% trifluoroacetic acid/H_2O–0.1% trifluoroacetic acid/acetonitrile. This procedure allows detection of all reaction products as well as the substrate peptide. One unit of activity hydrolyzes 1 μmol of substrate per min. The assay was linear in time and protein.

Enzyme Purification. Strain TN2270 was grown in minimal glucose medium containing 0.4 mM leucine and 0.4 mM methionine. The cell pellet was suspended in 0.01 M potassium phosphate buffer (pH 7.5) and disrupted by sonication. After centrifugation, the supernatant was applied to a DEAE-cellulose (Whatman DE-52) column equilibrated with the phosphate buffer (above) and eluted with a linear gradient of KCl to 0.4 M in the same buffer. The activity eluted at a salt concentration of 0.1 M. The active fractions were combined, concentrated over an ultrafiltration membrane (YM-10, Amicon), and passed through an Ultrogel AcA 54 column (LKB) equilibrated in 0.05 M potassium phosphate buffer (pH 7.5). The activity eluted at a volume of 242 ml from a column with a void volume of 140 ml. The Met-Ala-Ser hydrolyzing fractions were combined and concentrated as above. The specific activity of this material was ≈13-fold higher than that of the starting extract and ≈290-fold higher than that of a wild-type extract. This material was further purified by chromatofocusing (Pharmacia PBE 94 in 0.025 M imidazole·HCl, pH 7.4, eluted with Polybuffer 74·HCl, pH 4.0). The purified material from the peak fraction of this column was used for the experiment shown in Fig. 1.

Preparation of N-Terminal Methionylated Human Interleukin 1_β (IL-1_β). Recombinant-produced IL-1_β contains 20–25% unprocessed N-terminal methionine and was purified from *E. coli* as described (15). The N-terminal methionylated and nonmethionylated forms of IL-1_β were separated by chromatofocusing (22). The separated proteins were precipitated with 3.2 M ammonium sulfate, resuspended with 50 mM sodium phosphate (pH 7.0), and then dialyzed against the same buffer.

Table 1. Bacterial strains

Strain	Genotype
TN2183	*leuBCD485 metA15 pepN90 pepA16 pepB11 pepP1 pepQ1 pepT7*::Mu *dl(X)*
TN2270*†	*pepM100 zae-3149*::Tn*10Δ16Δ17*
TN2501*†	*pepM100 zae1615*::Tn*10Δ16Δ17* Kan^r
TN2529*	*pepD3*
TN2547*†	*pepD3 pepM100 zae-3149*::Tn*10Δ16Δ17*
TN2563*‡	*pepD3* [*pyrA685*::Tn*10*/*pyrA*$^+$]
TN2565*†‡	*pepD3* [*pyrA685*::Tn*10 pepM*$^+$/*pyrA*$^+$ *pepM100 zae1614*::Tn*10Δ16Δ17* Kan^r]
TN2624*	*pepD3 dcp-1 zcf845*::Tn*10*
TN2626*†	*pepD3 dcp-1 zcf845*::Tn*10 pepM100 zae1614*::Tn*10Δ16Δ17* Kan^r
TT421	*panC540*::Tn*10*

*This strain carries all of the markers in TN2183 in addition to those indicated.

†Tn*10Δ16Δ17* and Tn*10Δ16Δ17* Kan^r (kanamycin resistant) are described in ref. 12.

‡Square brackets contain markers for which the chromosomal tandem duplications are heterozygous.

Table 2. Utilization of peptides as amino acid sources

Peptide	*pepM* allele	
	pepM$^+$	*pepM100*
Met-Ala	−	−
Met-Gly	−	−
Met-Ala-Ser	+	+
Met-Ala-Met	+	+
Met-Thr-Met	+	+
Met-Gly-Met	+	+
Met-Gly-Gly	−	+
Met-Gly-Met-Met	+	+
Met-Leu-Gly	−	−
Met-Met-Ala	−	−
Met-Met-Met	−	−
Leu-Gly	−	−
Leu-Gly-Gly	−	−
Leu-Leu-Leu	−	−

The methionine peptides were tested as methionine sources and the leucine peptides were tested as leucine sources.

Table 3. Peptide hydrolysis catalyzed by cell extracts

Substrate	Relative hydrolysis rate	
	TN2624 (*pepM*$^+$)	TN2626 (*pepM100*)
Met-Ala-Ser	1.0	1.0
Met-Ala-Met	1.0	1.0
Met-Thr-Met	0.2	0.2
Met-Gly-Met	0.5	0.5
Met-Gly-Gly	0.09	0.1
Met-Gly-Met-Met	0.8	0.7
Met-Leu-Gly	0*	0*
Met-Met-Ala	0	0
Met-Met-Met	0	0
Leu-Gly-Gly	0.02†	0.004†
Ala-Ala-Ala	0.004	0
Ala-Ala-Ala-Ala	0.04	0

The peptidase assay is described in the text; reaction time was 40 min. The hydrolysis rates for each extract are compared to the hydrolysis rate for Met-Ala-Ser for that extract. The specific activity for Met-Ala-Ser was 0.029 unit/mg of protein for TN2624 extract and 0.53 unit/mg of protein for TN2626 extract. The assay contained either 140 μg of protein (TN2624 extract) or 14 μg of protein (TN2626 extract).

*We estimate that a relative hydrolysis rate of 0.006 for the TN2624 extract assays or 0.003 for the TN2626 assays would have been easily detected.

†Very small amounts of glycine and Leu-Gly were produced. This is almost certainly the result of peptidases other than peptidase M.

Table 4. Effect of divalent cations on peptidase M activity

Addition	Relative hydrolysis rate
None	1
EDTA (1 mM)	<0.0002
Co^{2+}	9
Mn^{2+}	1
Mg^{2+}	0.9
Zn^{2+}	1

All divalent cations were added as chloride salts at a final concentration of 3 mM. Co^{2+} showed the same stimulation at 1 mM, the concentration used in the standard assay. The relative hydrolysis rate was assayed as described in the text using Met-Ala-Ser as substrate and crude extract of TN2626 (8 μg) as enzyme.

Analytical isoelectric focusing was carried out on thin-layer polyacrylamide gels (LKB Ampholine PAG plates; pH range, 3.5–9.5) according to the manufacturer's instruction.

RESULTS

Utilization of N-Terminal Methionine Peptides by Peptidase-Deficient Mutants. Table 2 shows that strains lacking peptidases N, A, B, D, P, Q, and T and dipeptidylcarboxypeptidase will use some small N-terminal methionine peptides as methionine sources. These strains will not utilize a wide variety of leucine peptides as leucine sources (ref. 16; C.G.M., unpublished observations). The specificity of methionine peptide use suggests that the second amino acid of the peptide determines whether it can be used. Examination of the peptides utilized shows that they are the same as those in which N-terminal methionine is cleaved from nascent proteins *in vivo*. Since wild-type (Pep^+) strains can grow on all of these peptides, the pattern of peptide use reflects the specificity of hydrolysis, not that of an uptake system.

Methionine Peptide Hydrolysis in Crude Cell Extracts. Extracts of a multiply peptidase-deficient strain (TN2624) hydrolyzed all of the N-terminal methionine peptides that support growth as methione sources (Table 3). In every case, only one peptide bond was hydrolyzed, yielding methionine as the only amino acid product. These extracts did not contain detectable activity toward Met-Leu-Gly, Met-Met-Ala, or Met-Met-Met but did hydrolyze Met-Gly-Gly at about 0.1 the rate of Met-Ala-Ser or Met-Ala-Met. The extracts also did not contain measurable activity toward several peptides with amino acids other than methionine at their N termini. Unlike other *Salmonella* and *E. coli* peptidases (7, 8), the activity could not be detected by activity stain after electrophoresis of crude extracts in nondenaturing PAGE gels (7). Peptide hydrolysis was stimulated by Co^{2+} but not by Mg^{2+}, Mn^{2+}, or Zn^{2+} and was inhibited by EDTA (Table 4). We propose to call this methionine-specific aminopeptidase peptidase M.

Isolation and Characterization of Mutants That Overproduce the Methionine-Specific Peptidase. The observation that Met-Gly-Gly does not support growth and is hydrolyzed by extracts at a slower rate than substrates with alanine as second amino acid suggested a method for isolating strains that overproduce peptidase M. When a methionine-requiring strain carrying nonreverting mutations in the genes specifying broad-specificity aminopeptidases (16) was plated on medium containing Met-Gly-Gly as a methionine source, mutants capable of using this peptide were obtained. Several of these mutants were purified and characterized. Although these mutant strains grew well on Met-Gly-Gly, they did not use Met-Leu-Gly, Met-Met-Ala, or Met-Met-Met nor did they grow on any of several N-terminal leucine peptides as leucine sources. Assays of peptide hydrolysis in an extract of one mutant, pepM100, showed a 20- to 30-fold increase in Met-Ala-Ser hydrolyzing activity. This strain was chosen for further characterization. The peptide use profile of a strain carrying *pepM100* is compared to its parent in Table 2. The data in Table 3 show that in an extract of the mutant strain, the levels of activity toward N-terminal methionine peptides with alanine, threonine, or glycine in the second position all show an ≈20-fold increase relative to the activity in a wild-type peptidase M strain. Met-Leu-Gly, Met-Met-Met, Met-Met-Ala, and several other peptides with N termini other than methionine are not hydrolyzed by either the mutant or parental extracts. The relative rates of hydrolysis for all substrate peptides are the same in the two extracts. These results suggest that the level of a single peptidase is increased by the *pepM100* mutation and that this peptidase is specific for N-terminal methionine as well as for the peptide's second amino acid. The removal of N-terminal methionine from Met-Gly-Met-Met shows that the enzyme is capable of hydrolyzing tetrapeptides and is not limited in specificity to tripeptides. The mutant strain will not grow on any of several N-terminal methionine dipeptides (for example, Met-Gly and Met-Ala), and extracts will not hydrolyze these dipeptides (data not shown). We propose to call the locus that leads to overproduction *pepM*.

The map position of the mutations leading to overproduction of peptidase M has been determined. We first isolated an insertion of the minitransposon Tn*10*Δ*16*Δ*17* (12) near the *pepM* locus (17). This insertion was used to target the formation of an Hfr strain with an origin of transfer at the site of the Tn*10*Δ*16*Δ*17* insertion (18). Conjugation crosses using Hfr strains constructed in this way suggested that *pepM* is located in the 98-7 map unit region of the *Salmonella* genome

Table 5. Mapping of *pepM* by P22 transduction

Donor	Recipient	Selected marker	Unselected marker	Cotransduction frequency, %
TN2695 *leu*$^+$ *pepM100*	TN2529 *pepM*$^+$	Leu^+	*pepM100**	7.5 (6/80)
LT2 *leu*$^+$ *pepM*$^+$	TN2547 *leu*$^-$ *pepM100*	Leu^+	*pepM*$^+$	8.3 (22/265)
TN2501 *pepM100 zae1615*:: Tn*10*Δ*16*Δ*17* Kan^r	TN2529 *pepM*$^+$	Kan^r	*pepM100**	17.0 (52/305)
TN2501 *zae1615*::Tn*10*Δ*16*Δ*17* Kan^r	TT421 *panC*::Tn*10*	Kan^r	Tet^s	11.8 (17/144)
TT421 *panC*::Tn*10*	TN2501 *zae1615*:: Tn*10*Δ*16*Δ*17* Kan^r	Tet^r	Kan^s	3.4 (6/176)

Tet^r, tetracycline resistant; Tet^s, tetracycline sensitive; Kan^s, kanamycin sensitive.
**pepM* alleles were scored by testing growth on Met-Gly-Gly as methionine source.

Table 6. Levels of peptidase M in duplication strains

Strain	Specific activity, μmol of methionine per min/mg
TN2529 (*pepM*$^+$)	0.025
TN2563 (*pepM*$^+$/*pepM*$^+$)	0.076
TN2547 (*pepM100*)	0.60
TN2565 (*pepM*$^+$/*pepM100*)	0.77

Standard assays using Met-Ala-Ser as substrate were performed.

(19). Phage P22-mediated transduction crosses with markers in this region showed that *pepM100* is linked ≈8% to *leu* at 3 map units. Other transposable elements linked to *pepM* were isolated and used as markers to further define the map position. Results of these crosses are shown in Table 5. These results establish precisely the location of *pepM* and the transposable elements. Since all mutations leading to Met-Gly-Gly utilization tested are linked by P22 transduction to *zae3149*::Tn*10*Δ*16*Δ*17*, it is likely that they are all alleles of the *pepM* locus.

A strain containing a duplication of the *pepM* locus was constructed by the method of Anderson and Roth (20) using a Tn*10* insertion in the *pyrA* gene. This duplication strain was used for dominance testing and to see if there is a gene dosage effect on the levels of peptidase M. The presence of *pepM* in the duplication was established by introducing *pepM100* (by cotransduction with a linked transposon) into one side of the putative duplication and showing that the expected pattern of haploid segregants was observed. Enzyme levels in extracts of several duplication strains are shown in Table 6. Clearly, the *pepM100* mutation is dominant to *pepM*$^+$. In addition, duplication of the wild-type *pepM* locus leads to increased levels of peptidase M. These results lead us to hypothesize that *pepM* is the structural gene for peptidase M. It seems reasonable to suppose that the mutations so far isolated affect a promoter or regulatory region of this gene.

Identification of the *pepM* Gene Product. Fig. 1 shows a NaDodSO$_4$/PAGE gel of extracts from a pepM$^+$ and a pepM100 strain. The level of a protein of molecular mass ≈34 kDa reproducibly appears to be increased in the pepM100 strain. A preparation of peptidase M purified from a pepM100 strain (see *Materials and Methods*) shows substantial enrichment for this protein (Fig. 1). It seems likely that this protein is peptidase M.

Enzymic Removal of N-Terminal Methionine from a Recombinant-Produced Protein. IL-1$_\beta$ is a monomeric protein (17.4 kDa) with the N-terminal sequence (Met)-Ala-Pro-Val-Arg-. The N-terminal methionylated and nonmethionylated forms of IL-1$_\beta$ (prepared as described in *Materials and Methods*) have estimated pI values of 6.45 and 6.70 (Fig. 2, lanes D and F). Incubation of N-terminal methionylated IL-1$_\beta$ with peptidase M (see legend to Fig. 2 for details) resulted in a 0.25 pH unit increase in pI, consistent with the removal of N-terminal methionine. There was no change in the gel appearance of the nonmethionylated IL-1$_\beta$ treated with enzyme apart from the expected disappearance of the small amount of contaminating methionylated IL-1$_\beta$ present (Fig. 2, lanes F and G). All of the preparations of IL-1$_\beta$, treated and nontreated with enzyme, had identical molecular masses, as estimated by NaDodSO$_4$/PAGE (data not shown), indicating that under the conditions used there had been no extensive N-terminal processing or internal cleavage of IL-1$_\beta$ proteins. N-terminal sequencing of proteins eluted from bands separated by isoelectric focusing indicated specific cleavage of methionine with no further processing of the authentic N terminus (alanine) (data not shown).

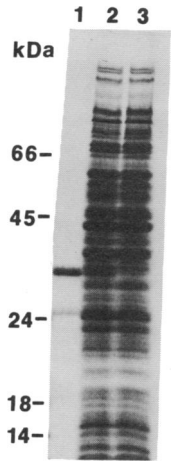

FIG. 1. Identification of peptidase M by NaDodSO$_4$/PAGE [NaDodSO$_4$/12.5% acrylamide gel prepared as described by Laemmli (14)]. Standards used for determination of the molecular mass of peptidase M were lysozyme (14.3 kDa), β-lactoglobulin (18.4 kDa), trypsinogen (24 kDa), ovalbumin (45 kDa), and albumin (66 kDa). Lanes 2 and 3 each contained 30 μg of protein from extracts of TN2529 (lane 3) and TN2547 (lane 2). Lane 1 contained purified peptidase M from the active peak of the chromatofocusing column. The peptidase M band in the pepM100 extract (lane 2) appears much darker than in the wild-type peptidase M extract (lane 3).

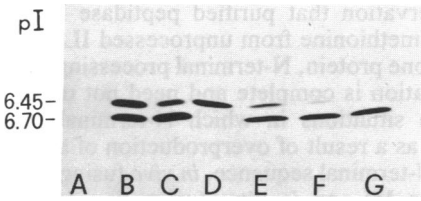

FIG. 2. Removal of N-terminal methionine from recombinant IL-1$_\beta$. IL-1$_\beta$, either N-terminal methionylated or nonmethionylated (0.64 mg·ml^{-1}) or an equal weight mixture of both (1.28 mg·ml^{-1}), was incubated in 50 mM sodium phosphate (pH 7.5) containing 0.5 mM Co^{2+} with purified peptidase M (6.4 μg·ml^{-1}) at 30°C for 60 min. Samples (20 μl) were applied directly to the isoelectric focusing gel. Lane A, peptidase M alone (0.26 μg); lane B, mixture of N-terminal methionylated and nonmethionylated IL-1$_\beta$ (28 μg); lane C, as lane B, treated with peptidase M; lane D, N-terminal methionylated IL-1$_\beta$ (14 μg); lane E, as lane D, treated with peptidase M; lane F, nonmethionylated IL-1$_\beta$ (14 μg); and lane G, as lane F, treated with peptidase M. The isoelectric points of N-terminal methionylated IL-1$_\beta$ (6.45) and nonmethionylated IL-1$_\beta$ (6.70) are indicated. Under the digestion conditions used, about 10% of N-terminal methionylated IL-1$_\beta$ remained undigested.

DISCUSSION

The properties of peptidase M are consistent with those expected for the aminopeptidase that removes N-terminal methionine from newly synthesized peptide chains. The enzyme is specific for N-terminal methionine, it shows a distinct preference for second amino acids that permit N-terminal methionine removal *in vivo*, and it is able to hydrolyze not only small peptides but also proteins with unprocessed N termini. Identification of this activity has been made possible by the previous isolation of mutants lacking other aminopeptidases that hydrolyze N-terminal methionine peptides in addition to other substrates. Peptidases A, N, B, and T will all hydrolyze Met-Ala-Ser, for example. Peptidase M appears to be a metallopeptidase, as are most of the other peptidases found in *E. coli* and *Salmonella* (21).

The basis for selection of overproducer mutations is the observation that Met-Gly-Gly, although a substrate for the enzyme, is apparently not hydrolyzed sufficiently rapidly to allow its use as a methionine source. The availability of an overproducer of peptidase M provides a convenient source for purification of the enzyme. Overproduction of peptidase M does not seem to be deleterious to cell growth since strains containing the *pepM100* overproducer mutation grow normally under all conditions we have tested. The enzyme seems to have very pronounced specificity for the second amino acid, making it unlikely that overproduction would result in removal of methionine from proteins that normally remain unmodified.

The observation that purified peptidase M will remove N-terminal methionine from unprocessed IL-1_β shows that, for at least one protein, N-terminal processing can take place after translation is complete and need not occur cotranslationally. In situations in which N-terminal processing is incomplete as a result of overproduction of a protein with a cleavable N-terminal sequence, *in vivo* (using overproducers of peptidase M) and *in vitro* (using the purified enzyme) approaches to generating cleaved product can be contemplated. However, the apparent rigid specificity of peptidase M for the second amino acid would seem to make this enzyme useless for removing N-terminal methionine from intrinsically resistant N termini.

It should be possible also to use the *pepM* mutations to isolate strains that produce less peptidase M than wild-type cells. It will be interesting to see what the phenotypic consequences of such mutations will be.

Note Added in Proof. We have now cloned the structural gene for peptidase M from *S. typhimurium*. This gene codes for a 264 amino acid protein that can be expressed in large amounts in both *S. typhimurium* and *E. coli*. We have shown that peptidase M will remove N-terminal methionine from two other recombinant proteins, interleukin 2 and granulocyte/macrophage colony-stimulating factor. An enzyme activity from *E. coli* similar to peptidase M has recently been reported by Ben-Bassat *et al.* (23).

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1. Waller, J. P. (1963) *J. Mol. Biol.* **7,** 483–496.
2. Sarimo, S. S. & Pine, M. J. (1969) *J. Bacteriol.* **98,** 368–374.
3. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
4. Tsunasawa, S., Stewart, J. W. & Sherman, F. (1985) *J. Biol. Chem.* **260,** 5382–5391.
5. Adams, J. M. (1968) *J. Mol. Biol.* **33,** 571–589.
6. Vogt, V. M. (1970) *J. Biol. Chem.* **245,** 4760–4769.
7. Miller, C. G. & MacKinnon, K. (1974) *J. Bacteriol.* **120,** 355–363.
8. Miller, C. G. & Schwartz, G. (1978) *J. Bacteriol.* **135,** 603–611.
9. Tsai, C. S. & Matheson, A. T. (1965) *Can. J. Biochem.* **43,** 1643–1652.
10. Brown, J. L. (1973) *J. Biol. Chem.* **248,** 409–416.
11. Strauch, K. L. & Miller, C. G. (1983) *J. Bacteriol.* **154,** 763–771.
12. Foster, T. J., Davis, M. A., Roberts, D. E., Takeshita, K. & Kleckner, N. (1981) *Cell* **23,** 201–213.
13. Davis, R. W., Roth, J. R. & Botstein, D. (1980) *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 78.
14. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
15. Wingfield, P. T., Payton, M., Tavernier, J., Barnes, M., Shaw, A., Rose, K., Simona, M. G., Demczuk, S., Williamson, K. & Dayer, J.-M. (1986) *Eur. J. Biochem.* **160,** 491–497.
16. Strauch, K. L., Carter, T. H. & Miller, C. G. (1983) *J. Bacteriol.* **156,** 743–751.
17. Strauch, K. L., Lenk, J. B., Gamble, B. & Miller, C. G. (1985) *J. Bacteriol.* **161,** 673–680.
18. Chumley, F. G., Menzel, R. & Roth, J. R. (1979) *Genetics* **91,** 639–655.
19. Sanderson, K. E. & Roth, J. R. (1983) *Microbiol. Rev.* **47,** 410–453.
20. Anderson, R. P. & Roth, J. R. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43,** 1083–1087.
21. Miller, C. G. (1975) *Annu. Rev. Microbiol.* **29,** 485–504.
22. Wingfield, P. T., Graber, P., Rose, K., Simona, M. & Hughes, G. J. (1987) *J. Chromatogr.* **387,** 291–300.
23. Ben-Bassat, A., Bauer, K., Chang, S.-Y., Myambo, K., Boosman, A. & Chang, S. (1987) *J. Bacteriol.* **169,** 751–757.