In eukaryotes, two isozymes (I and II) of methionine aminopeptidase (MetAP) catalyze the removal of the initiator methionine if the penultimate residue has a small radius of gyration (glycine, alanine, serine, threonine, proline, valine, and cysteine). Using site-directed mutagenesis, recombinant yeast MetAP I derivatives that are able to cleave N-terminal methionine from substrates that have larger penultimate residues have been expressed. A Met to Ala change at 329 (Met206 in Escherichia coli enzyme) produces an average catalytic efficiency 1.5-fold higher than the native enzyme on normal substrates and cleaves substrates containing penultimate asparagine, glutamine, isoleucine, leucine, methionine, and phenylalanine. Interestingly, the native enzyme also has significant activity with the asparagine peptide not previously identified as a substrate. Mutation of Gin356 (Gin233 in E. coli MetAP) to alanine results in a catalytic efficiency about one-third that of native with normal substrates but which can cleave methionine from substrates with penultimate histidine, asparagine, glutamine, leucine, methionine, phenylalanine, and tryptophan. Mutation of Ser195 to alanine had no effect on substrate specificity. None of the altered enzymes produced cleaved substrates with a fully charged residue (lysine, arginine, aspartic acid, or glutamic acid) or tyrosine in the penultimate position.

Proteins synthesized in eukaryotic cells undergo two common types of co/post-translational modifications at their N termini: initiator methionine cleavage and N\(^\text{a}\)-acyetylation. These reactions are catalyzed by two classes of enzymes, the methionine aminopeptidases (MetAP)\(^1\) and N\(^\text{a}\)-acyetyltransferases (1). In combination, they produce four distinct types of N termini: those with and without initiator methionine, and those with and without N\(^\text{a}\)-acyetylation. The penultimate residue adjacent to the initiator methionine is the principal factor that determines enzyme specificity and hence which of these four types of N termini the mature protein will possess. Proteins that have signal peptides removed during translocation steps do not retain these modifications. As predicted from mutant cytochrome sequences (2), the seven penultimate amino acid residues with the smallest side chain radii of gyration (glycine, alanine, serine, threonine, proline, valine, and cysteine) direct MetAP to cleave the initiator methionine. In addition, N-terminal glycine, alanine, serine, and threonine residues are usually N\(^\text{a}\)-acyetylated. This modification also occurs on the retained methionine of proteins with penultimate asparatic acid, glutamic acid, and asparagine (3–5). Prokaryotes have an initiator methionine cleavage pattern identical to that of the eukaryotes (5, 6); however, they N\(^\text{a}\)-acyetyl very few proteins and apparently have individual acetyltransferases for each substrate (1).

There are two major types of MetAPs that have been identified with this substrate cleavage pattern and both are expressed in eukaryotes. In addition, the type I enzymes are found in EuBacteria, while the type II enzymes are found in Archaea (8). Comparison of the Escherichia coli structure (9) with that of Pyrococcus furiosis (10) reveals that despite their low overall sequence similarity, the type I and type II enzymes possess a very similar fold in the catalytic domain. The most significant difference between these enzymes is a large helical domain insertion on the surface of the protein characteristic of the type II isozymes. The eukaryotic MetAP isozymes are differentiated from their prokaryotic counterparts by an additional N-terminal domain. The eukaryotic type I MetAP has two putative zinc finger motifs in this ~12-kDa region (11, 12), and the eukaryotic type II enzyme has a highly charged N terminus with alternating polyacidic and polybasic stretches in a similarly sized segment (8). Although it has not yet been demonstrated, these N-terminal extensions may be involved in the association of eukaryotic MetAP isozymes with intracellular structures/organelles such as the ribosome.

Historically, it has been reported that both types of MetAP are Co\(^2+\)-dependent metalloproteases, having two metal ions per catalytic unit (13–15). However, recent experiments have determined that Saccharomyces cerevisiae MetAP I containing Zn\(^2+\) in place of Co\(^2+\) has substantially higher activity under in vivo conditions than the Co\(^2+\)-substituted enzyme, albeit zinc ions are inhibitory at higher (nonphysiological) concentrations (16). Furthermore, unlike the Zn\(^2+\) enzyme, the Co\(^2+\) enzyme is inactivated by glutathione, which is present in high concentrations in the cytosol, further supporting the view that yeast MetAP I is a Zn\(^2+\)-metalloprotease in situ. However, in reconstituted preparations, the Zn\(^2+\) and Co\(^2+\)-MetAP I preparations act essentially identically.

Deletion of the MetAP gene in prokaryotes is a lethal event (17); in yeast, both the type I and type II genes must be disrupted for lethality, indicating some redundancy in function, at least in that simple eukaryote (18). However, the specific inactivation of MetAP II by the antiangiogenic compounds, fumagillin and ovalicin, indicates some uniqueness in function (19, 20). The selectivity of fumagillin for the type II enzymes appears to be a matter of dose-response, since it has recently been shown that E. coli and yeast MetAP I can be inactivated by these reagents (21). Since antiangiogenic compounds have excellent potential in the treatment of cancer, the ability of

---

* This work was supported by National Institutes of Health (NIH) Grant DK32465, a research contract with Baxter-Hyland Division, and NIH postdoctoral fellowship GM18940 (to K. W. W.). 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.

† To whom correspondence should be addressed. Tel.: 949-824-6236; Fax: 949-824-8036; E-mail: rralab@uci.edu.

§ The abbreviation used is: MetAP, methionine aminopeptidase.
FIG. 1. Stereo representation of the E. coli MetAP I proposed binding pocket for the penultimate residue. S. cerevisiae MetAP I residue numbers are shown with the E. coli MetAP locations in parenthesis. The two active site metal ions are represented as cyan spheres (Co1 and Co2). The residues mutated to alanine in this study include serine 72, methionine 329, and glutamine 356 based on Ref. 9. This figure was generated using MOLSCRIPT (42).

fumagillin and ovalicin to differentiate between the type I and type II isozymes is of major interest currently.

The substrate specificity of the MetAP isozymes suggests a high degree of selectivity for methionine in the S1 site with an S1′ site that primarily limits the side chain length of the substrate to <3.68 Å (6). Although the S1′ pocket is not yet defined in molecular terms, the structure of the E. coli MetAP (9) is a useful guide to predict the catalytic site residues that define the cavity. Fig. 1 depicts the residues that presumably form the penultimate residue-binding site, of which Gln356 and Met329 are two such candidates.

In this study, data are presented for mutant forms of S. cerevisiae MetAP I, in which these two residues individually and together have been converted to alanine. A third site, Ser195, that is conserved in all of the MetAP isozymes was also mutated to alanine to determine if it has a significant catalytic function. Detailed kinetic analysis has established that the methionine and glutamine-substituted enzymes have an expanded substrate profile, while the serine substitution was without effect on specificity. In addition, evidence is presented that the current MetAP specificity profile, as determined from in situ measurements, although generally correct, should be modified to indicate that the midsized penultimate residues, such as threonine and valine, may not always allow initiator methionine processing and that asparagine in a limited number of cases may direct processing.

EXPERIMENTAL PROCEDURES

Cloning of Yeast MetAP I—MetAP I was cloned from S. cerevisiae total genomic DNA, based on the sequence of Chang et al. (11), using polymerase chain reaction with the following oligonucleotides: ACAGAATTCGACTGCACTACAAACAGTT and ACAGAATTCCTATTATA-ATTCTCTGTCTTGG (Genset, San Diego, CA). Polymerase chain reaction products were restricted with EcoRI, purified on a 1.2% agarose gel, and subcloned into pBluescript II (Stratagene, La Jolla, CA). Several clones were then sequenced using the Sequenase 2.0 protocol and an ABI 377 automated DNA sequencer (Applied Biosystems Inc., Foster City, CA).

Mutagenesis of Yeast MetAP I—Mutations in S. cerevisiae MetAP I were made using the Transformer site-directed mutagenesis system (CLONTECH Laboratories, Palo Alto, CA) and the following oligonucleotides: selection primer, CCGGGTCGACTTACATCGGCGCGCATC; S195A primer, CTAAAATCGTTTGTACGCGGGGAATGAATTTATTTG; and M329A primer, GTTATCACCATCGAGCTGCGGTAATTGAAGG-TACTTG; and Q356A primer, GTGAAACTGATGTCCTGATTGAGTTGACACAT-ATACACTG. All mutations were verified by DNA sequencing using the Sequenase 2.0 protocol and an ABI 377 automated DNA sequencer (Applied Biosystems Inc., Foster City, CA).

Kinetic Parameters of Yeast MetAP I with Normal Substrates—As seen in Fig. 2A, native yeast MetAP I has the
highest turnover rates on substrates with penultimate alanine or serine. The S195A mutant has a similar substrate preference (Fig. 2B), but its average turnover is only about two-thirds that of the native enzyme (Table I). In contrast, with the exception of the valine substrate, the M329A mutation produces turnover rates very similar to those of the native enzyme (Fig. 2C and Table I). The MetAP derivatives with Q356A and M329A/Q356A mutations have almost identical substrate preference patterns (Fig. 2, D and E, respectively) but with turnover rates that are only about one-third that of the native enzyme (Table I). Additionally, the $k_{\text{cat}}$ values for the threonine and valine substrates, whose side chains are somewhat larger, are exceptionally depressed, being less than 12 and 5% of the native enzyme, respectively.

For native MetAP I, substrates among the cleaved subset with larger penultimate residues (threonine, proline, and valine) have higher $K_m$ values than the smaller residues (glycine, alanine, and serine) (Fig. 3A). All mutant enzymes show a similar $K_m$ profile (Fig. 3, B–E) and have average $K_m$ values comparable with the native enzyme (Table I). However, the enzyme with the M329A/Q356A double substitution has a low $K_m$ for the threonine substrate (27% of native), mimicking the S195A enzyme (34% of native) much more than the enzyme with the Q356A substitution (149% of native) that it otherwise resembles (Fig. 3, E, B, and D, respectively).

All five enzymes have similar substrate specificity profiles with respect to catalytic efficiency ($k_{\text{cat}}/K_m$); the three smallest penultimate residues (glycine, alanine, and serine) having the highest values in every case (Fig. 4, A–E). This is due to a combination of higher $k_{\text{cat}}$ and lower $K_m$ values for these substrates. However, as shown in Table I, the S195A and M329A mutant enzymes have noticeably improved catalytic efficiencies (151 and 150% of native), while the mutant enzymes with Q356A and M329A/Q356A substitutions have substantially lower catalytic efficiencies (37 and 31% of native, respectively).

Velocity of Methionine Hydrolysis with Nonnormal Substrates—In agreement with previous reports (2–5), native MetAP I does not cleave N-terminal methionine from the nonnormal subset of peptides with the notable exception of the asparagine-containing substrate, which was cleaved at a low but significant rate (Fig. 5A). The S195A enzyme behaves almost identically to the native enzyme on the nonnormal substrates, including showing a significant activity with the asparagine peptide (Fig. 5B). The enzymes with M329A, Q356A, and M329A/Q356A mutations, however, are very effective at cleaving many of these nonnormal peptides showing significant activity toward all but substrates with fully charged penultimate residues (aspartic acid, glutamic acid, lysine, and arginine) and with tyrosine (Fig. 5, C–E). The M329A enzyme is more effective than the asparagine, leucine, isoleucine, and phenylalanine substrates than either of the enzymes with the Q356A or M329A/Q356A mutations, while the latter are better at cleaving histidine, glutamine, and tryptophan-containing peptides than the former. Consistent with the activities on the normal substrates, the enzyme with the Q356A substitution behaves almost identically to the one with the M329A/Q356A double mutation. Both of these enzymes cleave the MMSHRWDW double substrate with velocities that are comparable with the wild type enzyme acting on normal substrates and far exceed the velocity of other nonnormal substrates with any of the enzymes in this study.

**Kinetic Constants of MetAP I for Nonnormal Substrates**—As seen in Fig. 6, A–C, native MetAP I has measurable activity with the nonnormal asparagine peptide. However, its $k_{\text{cat}}$ is 37-fold lower than the slowest normal substrate (valine); combined with a 2.8-fold lower $K_m$, its catalytic efficiency is over 13-fold lower than for the valine peptide. The enzyme with the S195A mutation behaves almost identically to the native enzyme having similar kinetic constants for the asparagine peptide. However, the enzymes with the M329A, Q356A, and M329A/Q356A mutations are much more effective than either the native or the S195A mutant enzymes on the asparagine substrate, having $k_{\text{cat}}$ values that are 8–20-fold higher. The improvement in catalytic efficiency on this substrate (7–12-fold) is principally manifested through higher $k_{\text{cat}}$ constants, since the $K_m$ values do not vary much between the enzymes.

As expected, native MetAP I has no measurable activity toward the nonnormal substrate MMSHRWDW, and the enzyme with the S195A mutation has only very low activity (Fig. 7). In contrast, enzymes with M329A, Q356A, and M329A/Q356A mutations display substantial activity, with $k_{\text{cat}}$ values (400–525 min$^{-1}$) as good as or better than three of the six normal MetAP substrates measured with the native enzyme (Fig. 4E). Importantly, these represent minimal $K_m$ values, since the enzyme produced two products during the reaction (MMSHRWDW and SHRWWD). These mutants have $K_m$ values ranging from 25 to 65 μM that are comparable with those of the native enzyme with normal substrates (23–254 μM) (Fig. 3A).
The combination of these constants yields catalytic efficiencies ranging from 8.5 to 15.7 μM−1 min−1, which is superior to the native enzyme with the normal substrates (1.3–7.6 μM−1 min−1) with the larger threonine, proline, and valine residues in the penultimate position.

DISCUSSION

An octapeptide substrate family with all 20 possible penultimate residues was used to detail the in vitro specificity of yeast recombinant MetAP I. Consistent with previous in situ studies of initiator methionine cleavage patterns, yeast MetAP I is capable of cleaving N-terminal methionine from substrates that have penultimate residues with small side chain radii of gyration (glycine, alanine, serine, threonine, proline, valine, and cysteine) in vitro (2–5). The enzyme cleaves methionine from substrates with penultimate serine and alanine much more efficiently than the other permissive substrates, due to a combination of higher kcat values and lower Km values for the smaller substrates. It is perhaps not surprising, therefore, that Met-Ala and Met-Ser N termini dominate the sequences found in the yeast genome, particularly among the metabolic or “housekeeping” enzymes that characterize the cytoplasmic protein population. Removal of initiator methionines from these proteins would return a significant amount of energetically expensive, free methionine to the amino acid pool, allowing other functions dependent on methionine, including new protein synthesis, to occur.

Although the kcat values measured are comparable with previous studies, the Km values determined for normal substrates range from 23 to 253 μM, which is orders of magnitude lower than previous studies (2680–6560 μM) (12, 23). This may be due to the use of longer, octapeptide substrates, compared with the three- and four-residue substrates used by others, possibly indicating that residues well downstream of the penultimate residue may contribute to substrate binding to the enzyme.
This may primarily operate through backbone interactions on the substrate and thus be largely sequence-independent.

It was also found that MetAP I can cleave substrates with penultimate asparagine, indicating that the established specificity pattern for co-/post-translational modification may have a greater degree of flexibility than originally detected (2, 7). Analysis of cytosolic proteins for whom the N terminus has been directly sequenced has shown that, although proteins with penultimate glycine, alanine, serine, cysteine, and proline are completely processed, threonine and valine result in retention of the initiator methionine residue 15 and 60% of the time, respectively (24). An additional study using recombinant methionyl-tRNA synthetase with 20 different penultimate residues, has shown that midsized penultimate residues such as asparagine, aspartic acid, leucine, and isoleucine are partially processed in *E. coli* (25). Taken together, this may indicate that midsize penultimate residues such as threonine, valine, and asparagine may only specify methionine removal part of the time, possibly dependent on other downstream determinants.

In this study, the *E. coli* MetAP structure (9) was instrumental in developing a mutagenesis strategy for the *S. cerevisiae* MetAP I enzyme although their catalytic domains are only 39% identical. However, sequence alignments with 12 other type I MetAPs also aided in developing the mutagenesis strategy. Residues in the active site cavity that were highly conserved and appeared to define cavity perimeters were considered good candidates for site-directed mutagenesis to alter the substrate specificity of the enzyme. Since the M329A and Q356A mutations allow yeast MetAP I to cleave six and seven of the 13 nonnormal substrates, respectively, it is clear that these residues have a significant role in determining if a substrate has the correct size penultimate residue. However, Met329 does not appear to have any other significant role in catalysis, since the M329A enzyme is at least as effective as the native enzyme in catalyzing N-terminal methionine removal from normal substrates. In contrast, Gln356 may serve a dual role with respect to substrate specificity and involvement in the normal catalytic reaction, since conversion of this residue to alanine results in a

---

**FIG. 6.** Catalytic constants for *S. cerevisiae* MetAP I on the nonnormal MNHRWDW substrate. A, turnover number; B, Michaelis constant; C, catalytic efficiency. Symbols under the bars indicate use of the following enzymes. Nat, native; S, S195A; M, M329A; Q, Q356A; MQ, M329A/Q356A. Assays were conducted as described under “Experimental Procedures.” Catalytic constants were determined using Lineweaver-Burk analysis. Three constants were determined for each sample, and the error bars were calculated as ± S.E.

**FIG. 7.** Catalytic constants for *S. cerevisiae* MetAP I on the nonnormal MMSHRWDW substrate. A, turnover number; B, Michaelis constant; C, catalytic efficiency. Symbols under the bars indicate use of the following enzymes. Nat, native; S, S195A; M, M329A; Q, Q356A; MQ, M329A/Q356A. Assays were conducted as described under “Experimental Procedures.” Catalytic constants were determined using Lineweaver-Burk analysis. Three constants were determined for each sample, and the error bars were calculated as ± S.E. Note that the turnover numbers for the MMSHRWDW peptide represent the lower limit of the *k* cat, since two products are made during the reaction (MHRWDW and SHRWDW).
Alteration of MetAP I Substrate Specificity

Table II

Sequence alignment of putative S₁ binding site residues

| S. cerevisiae type I | E. coli | Consensus of type I MetAPs | Other residues in type I MetAPs | Consensus of type II MetAPs | Other residues in type II MetAPs | Part of residue Lining S₁ pocket |
|---------------------|---------|----------------------------|--------------------------------|-----------------------------|--------------------------------|---------------------------------|
| Tyr²⁹¹ | Tyr¹⁶⁸ | 13/13 | Tyr | 8/8 | Leu | Side chain |
| Cys²⁹² | Cys¹⁶⁹ | 7/13 | Cys | 3/8 | Thr | 2 Asn, 1 Gly, 1 Side chain |
| Gly³⁰³ | Gly¹⁷⁰ | 13/13 | Gly | 8/8 | Gly | Side chain |
| His³⁰⁸ | His¹⁸⁵ | 8/13 | His | 5/8 | Asn | 3 Ie Side chain |
| Glu³²⁷ | Glu²⁰⁴ | 13/13 | Glu | 5/8 | Pro | 3 Thr Side chain |
| Met³²⁸⁴ | Met²⁰⁶ | 13/13 | Met | 8/8 | Phe | Side chain |
| Glu³⁵⁶ | Glu²³³ | 7/13 | Glu | 8/8 | Glu | Side chain |

loss of about two-thirds of the turnover rate. This may be due to an involvement of Gln³⁵⁶ in positioning of the substrate in the active site for optimum catalytic efficiency.

There are several features that distinguish the M329A and Q356A mutants. The Q356A mutant is able to cleave substrates with the very large tryptophan residues, while the M329A enzyme cannot. Although M329A and Q356A are positioned side by side in the enzyme, forming a common wall (Fig. 1), the replacement of Gln³⁵⁶ with the smaller side chain allows a large tryptophan to fit into the penultimate residue-binding site. In addition, the Q356A mutant cleaves histidine-containing substrates 30-fold more efficiently than the M329A mutant. It is possible that histidine also fits into the pocket formed by the missing glutamine, allowing reformation of the glutamine hydrogen bonding network. Consistent with this hypothesis, the M329A enzyme is much more efficient with phenylalanine and isoleucine substrates than is the Q356A mutant. These hydrophobic residues, which are about the same size as the missing methionine, may fill the cavity created by the M329A mutation. Gln³⁵⁶ has an important role in this hydrophobic interaction, since the M329A/Q356A mutant is not nearly as effective at cleaving the phenylalanine and isoleucine peptides as the M329A single mutant. This effect may be due to a hydrophobic interaction between the adjacent Met³²⁹ side chain (or the hydrophobic substrate in the M329A mutant) and the β- and γ-carbons of Gln³⁵⁶. Not surprisingly, Met³²⁹ does not play a significant role in the Gln³⁵⁶ hydrogen bonding network, since the double mutant is as effective as the Gln³⁵⁶ single mutant at cleaving the histidine substrate.

It is clear from these studies that size of the penultimate residue is not the only determinant of N-terminal methionine cleavage, since all enzymes tested are highly selective against residues expected to be fully charged at the pH of the reaction mixture. None of the five MetAP I enzymes tested has significant activity toward aspartic acid, glutamic acid, lysine, and arginine although aspartic acid and glutamic acid are not exceptionally large residues. Although the reason for this is uncertain, it may simply be due to charge repulsion. The shorter aspartic acid and glutamic acid residues may be repulsed by Glu³²⁷ on the wall of the pocket, and the longer lysine and arginine side chains may be repelled by His³⁰⁸ at the bottom of the pocket. Although mutation of Glu³²⁷ would likely be very detrimental to the enzyme due to its involvement in metal coordination, conversion of His³⁰⁸ to alanine could prove instructive in this regard.

The information gained from these site-directed mutagenesis studies has allowed a reexamination of the structure of E. coli MetAP I to identify other residues that may be involved in defining the substrate specificity of the enzyme. A cavity exists in the enzyme that is bordered by Met³²⁹ (Met²⁰⁶), Q356 (Gln²³³), Tyr²⁹¹ (Tyr¹⁶⁸), His³⁰⁸ (His¹⁸⁵), Glu³²⁷ (Glu²⁰⁴), Gly²⁹³ (Gly¹⁷⁰), and the backbones of Cys³⁵⁶ (Cys¹⁶⁹) and Pro³²⁸ (Pro²⁰⁵) (Fig. 1) (Table II). Tyr²⁹¹ forms a wall in this cavity and is conserved as tyrosine or phenylalanine in all 13 type I MetAP sequences known; all type II MetAPs have a conservative substitution of leucine in this position. His³⁰⁸ is at the bottom of this pocket and may define the maximum length of the penultimate residue that can be bound and, therefore, may be the residue responsible for discrimination between phenylalanine and tyrosine in the M329A mutant. His³⁰⁸ is conserved as either asparagine or histidine in all type I enzymes and as asparagine in all type II MetAPs. Glu³²⁷ serves a dual function as one of the metal coordinating residues as well as forming a wall in the putative penultimate residue binding pocket; Glu³²⁷ is completely conserved in all MetAPs. An absolutely conserved Gly³²⁷ is also part of the wall of this pocket. The absence of a side chain on Gly³²⁷ may be required to keep the cavity open, making it an excellent candidate for creating a MetAP mutant with a more limited specificity. The backbone of two residues, Cys³⁵⁶ and Pro³²⁸, also complete this pocket. The Pro³²⁸ residue is completely conserved in the type I enzymes and as proline or threonine in five and three of the type II enzymes, respectively. The Cys³⁵⁶ is less conserved, with 7 of 13 residues being cysteine in the type I enzymes with no particular pattern for this residue in the type II enzymes. Since the side chain of Cys³⁵⁶ is solvent-accessible, absolute conservation of this residue is not critical for maintaining the cavity shape. Alteration of the type I E. coli and type II P. furiosus enzymes reveals that all of these putative penultimate binding site residues occupy the same spatial geometry maintaining a similar cavity shape although these enzymes are highly divergent (16% identity) (data not shown).

The use of sequence alignments in conjunction with an enzyme crystal structure has proven to be a powerful tool in determining enzyme structure-function relationships. Two out of the three point mutants studied produced novel activities for the enzyme. One of the substrates, not normally cleaved by the native enzyme, was hydrolyzed with kinetic constants superior to three of the six normal substrates for MetAP I. Furthermore, one of these mutants (M329A) retained at least as much activity for the normal substrates as the native enzyme.

MetAP has also been studied for its role in the production of recombinant proteins, since incorrect processing of the N terminus can produce proteins that are inactive or immunogenic (40, 41). Overexpression of recombinant proteins can overload the ability of the host cell to process initiator methionine resi-
dues, resulting in a product that has a mixture of different N termini (7). In addition, cytosolic expression of a secreted protein can result in the presence of an initiator methionine that would not normally be found in the mature product. The mutant forms of MetAP presented here may prove useful as reagents for the in vitro or in vivo processing of recombinant proteins. The mature forms of enzymes with cleavable signal sequences often have N-terminal residues with large side chains. For cytoplasmic expression systems, the signal sequence can be removed during the cloning process, but all protein synthesis must be initiated with methionine (or N-formyl methionine); therefore, if the recombinant protein has a large penultimate residue, the methionine will be retained. The expanded specificity mutants of MetAP I could be used in these cases to remove these artificially retained initiator methionines. It may also be possible to create host cells that have MetAPs with contracted substrate specificity to allow protection of N termini from unwanted post-translational modifications. The initiator methionine could be retained in this system to prevent N-terminal post-translational modifications, and later in vivo treatment with the native MetAP could remove this protective methionine.

Acknowledgments—We thank Steve Disper for performing the mass spectroscopic analyses of the peptide substrates and Brian Matthews and Todd Lowther for rendering Fig. 1 and for the matrix-assisted laser desorption/ionization mass spectroscopic analysis of the yeast recombinant MetAP I. We would also like to thank Todd Lowther, Irwin Rose, and Roseann Cappel for constructive comments on the manuscript.

REFERENCES
1. Bradshaw, R. A., Brickey, W. W., and Walker, K. W. (1998) Trends Biochem. Sci. 23, 263–267
2. Sherman, F., Stewart, J. W., and Tsunasawa, S. (1985) BioEssays 3, 27–31
3. Huang, S., Elliott, R. C., Liu, P. S., Koduri, R. K., Weickmann, J. L., Lee, J. H., Blair, L. C., Ghosh-Dastidar, P., Bradshaw, R. A., Bryan, K. M., Einarsen, B., Kendall, R. L., Kolacz, K. H., and Saito, K. (1987) Biochemistry 26, 8242–8246
4. Biais, J. P., Kasper, T. J., and Bunn, H. F. (1988) J. Biol. Chem. 263, 8443–8449
5. Moerschell, R. P., Hosokawa, Y., Tsunasawa, S., and Sherman, F. (1990) J. Biol. Chem. 265, 19638–19643
6. Hirel, P. H., Schmitter, J. M., Dessen, P., Fayat, G., and Blanquet, S. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8247–8251
7. Tsunasawa, S., Stewart, J. W., and Sherman, F. (1985) J. Biol. Chem. 260, 5382–5391
8. Arfin, S. M., Kendall, R. L., Hall, L., Weaver, L. H., Stewart, A. E., Matthews, B. W., and Bradshaw, R. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7714–7718
9. Roderick, S. L., and Matthews, B. W. (1993) Biochemistry 32, 3907–3912
10. Tahiev, T. H., Oki, H., Tsukihara, T., Ogasahara, K., and Yutani, K. (1998) J. Struct. Biol. 121, 68–72
11. Chang, Y. H., Teichert, U., and Smith J. A. (1992) J. Biol. Chem. 267, 8007–8011
12. Zuo, S., Guo, Q., Ling, C., and Chang, Y. H. (1995) Mol. Gen. Genet. 246, 247–253
13. Ben-Basset, A., Bauer, K., Chang, S. Y., Myambo, K., Boosman, A., and Chang, S. (1987) J. Bacteriol. 169, 751–757
14. Chang, Y. H., Teichert, U., Smith, J. A. (1990) J. Biol. Chem. 265, 19992–19997
15. Kendall, R. L., and Bradshaw, R. A. (1992) J. Biol. Chem. 267, 20667–20673
16. Walker, K. W., and Bradshaw R. A. (1998) Protein Sci. 7, 2684–2687
17. Chang, S. Y. P., McGary, E. C., and Chang, S. (1989) J. Bacteriol. 171, 4071–4072
18. Li, X., and Chang, Y. H. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 12357–12361
19. Sin, N., Meng, L., Wang, M. Q. W., Wen, J. J., Bornmann, W. G., and Crews, C. M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6099–6103
20. Griffith, E. C., Su, Z., Turk, B. E., Chen, S., Chang, Y. H., Wu, Z., Biemann, K., and Liu, J. O. (1997) Chem. Biol. 4, 461–471
21. Lowther, W. T., McMillen, D. A., Ovville, A. M., and Matthews, B. W. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12153–12157
22. Walker, K. W., Yi, E., and Bradshaw, R. A. (1999) Biotechnol. Appl. Biochem. 29, 157–163
23. Klinkenberg, M., Ling, C., and Chang, Y. H. (1997) Arch. Biochem. Biophys. 347, 193–200
24. Flinta, C., Persson, B., Jornvall, H., and Heijne, G. (1986) Eur. J. Biochem. 154, 193–196
25. Hirel, P. H., Schmitter, J. M., Dessen, P., Fayat, G., and Blanquet, S. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8247–8251
26. Movva, N. R., Semon, D., Meyer, C., Kawashima, E., Wingfield, P., Miller, J. L., and Miller, C. G. (1990) Mol. Gen. Genet. 223, 345–348
27. Suh, J. W., Boylan, S. A., Oh, S. H., and Price, C. W. (1996) Gene (Amst.) 169, 17–23
28. Fleischmann, R. D., Adams, M. D., White, O., Clayton, R. A., Kirkness, E. F., Kerlavage, A. R., Bult, C. J., Tomb, J. F., Dougherty, B. A., Merrick, J. M., et al. (1995) Science 269, 496–512
29. Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., et al. (1997) Nature 388, 539–547
30. Eiglmeyer, K., Hone, N., Woods, S. A., Caudron, B., and Cole, S. T. (1993) Mol. Microbiol. 7, 197–206
31. Philipp, W. J., Poulet, S., Eiglmeyer, K., Pascopella, L., Balasubramanian, V., Heyn, B., Bergh, S., Bloom, B. R., Jacobs, W. R., Jr., and Cole, S. T. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 3132–3137
32. Fraser, C. M., Gocayne, J. D., White, O., Adams, M. D., Clayton, R. A., Fleischmann, R. D., Bult, C. J. (1995) Science 270, 397–403
33. Hilbert, H., Himmelreich, R., Plagens, H., and Herrmann, R. (1996) Nucleic Acids Res. 24, 628–639
34. Sensen, C. W., Klenk, H. P., Singh, R. K., Allard, O., Chan, C. C., Liu, Q. Y., Penny, S. L., Young, F., Schenk, M. E., Gaasterland, T., Doolittle, W. F., Ragan, M. A., and Charlebois, R. L. (1996) Mol. Microbiol. 22, 175–191
35. Smith, D. R., Doucette-Stamm, L. A., Deloughery, C., Lee, H. M., et al. (1997) J. Biol. Chem. 179, 7135–7155
36. Bult, C. J., White, O., Olsen, G. J., Zhou, L., et al. (1996) Science 275, 1058–1073
37. Wu, S., Gupta, S., Chatterjee, N., Hileman, T. G., Denslow, N. D., Merrick, W. C., Chakrabarti, D., Osterman, J. C., and Gupta, N. K. (1995) J. Biol. Chem. 268, 10796–10781
38. Klenk, H. P., Clayton, R. A., Tomb, J. B., White, O., Nelson, E. K. (1997) Nature 390, 364–370
39. Proudfoot, A. E., Power, C. A., Hoogewerf, A. J., Montjivet, M. O., Borlat, F., Offord, R. E., and Wells, T. N. C. (1997) J. Biol. Chem. 272, 2599–2603
40. Newton, D. L., Boque, L., Wlodawer, A., Huang, C. Y., and Rybak, S. M. (1998) Biochemistry 37, 5173–5183
41. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950