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N-Terminal Methionine Processing

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Abstract

Protein synthesis is initiated by methionine in eukaryotes and formylmethionine in prokaryotes. N-terminal methionine can be co-translationally cleaved by the enzyme methionine aminopeptidase (MAP). When recombinant proteins are expressed in bacterial and mammalian expression systems there is a simple universal rule which predict whether the initiating methionine will be processed by MAP and is based on the size of the residue adjacent (penultimate) to the N-methionine. In general, if the side-chains of the penultimate residues have a radius of gyration of 1.29 Å or less methionine is cleaved. This rule was originally suggested by Sherman et al., (1985) based on their pioneering studies with yeast.

Keywords

Protein synthesis; N-terminal methionine; methioinine aminopepidases; exopeptidase activity

Background

Protein synthesis is initiated by either methionine in eukaryotes or formylmethionine in prokaryotes, mitochondria, and chloroplasts. The N-terminal methionine can be co-translationally cleaved by the enzyme methionine aminopeptidase (MAP). In eukaryotes, methionine is removed either by cleavage of N-terminal signal peptide used for secretion etc., or by MAP. In prokaryotes, formylmethionine is first removed by formylmethionine deformylase resulting in N-terminal methionine which is then processed by MAP. In *E. coli* there is only one MAP (MAP1) and in humans and yeast, there are two: MAP1 and MAP2. The Yeast MAP1 has a 40% sequence similarity to *E. coli* MAP1. The MAP2 is highly conserved between *S. cerevisiae* and humans. Both MAP1 and MAP2 are metalloenzymes and share similar overall structures as indicated by X-ray crystallography of MAP1 from *E. coli* (Lowther, et al., 1999) and for MAP2 from human (Liu, et al, 1998). Approximately two-thirds of the proteins in any proteome are potential substrates for N-terminal methionine processing which appears to be essential for cell function. In bacteria and yeast, knockout or inhibition of MAP is lethal. In humans, MAP's are overexpressed in cancer cells and their inhibition is targeted for drug development. There are over 100 MAP protein structures in the Protein Data Bank (PDB) reflecting the importance of targeting this class of enzymes (Helgren et al, 2016).

Another major N-terminal processing event is N-terminal acylation and in eukaryotes this is catalyzed by N-terminal acetyltransferase (NAT). After removal of methionine by MAP, the

new N-terminal residue is acetylated by NAT and in the case of proteins which retain methionine, directly acetylated (Aresen, 2011).

Specificity

The sequence of the protein substrate can be designated: P1 - P1'- P2' - P3' – etc., where P1 is N-terminal methionine and processing occurs between P1 - P1' (nomenclature: Schecter & Berger, 1967). The specificity of MAP is determined by the size of the side chain of P1' (Sherman et al., 1985) Hence, Met is usually cleaved when P1' is glycine, alanine, serine threonine, cysteine, proline or valine. These P1' residues have side chains with a radius of gyration (refers to the overall spread of the molecule) of 1.29Å or less and those residues with a larger radius, result in non-cleavage and retention of methionine. The predictions of N-terminal processing are given in Table 1. Proteomic studies with data sets from bacteria, mammalian and yeast (Frottin et al., 2006; Bonnissone et al., 2013) confirm the universality of this simple rule. Also, using peptide libraries (Xiao et al, 2010), it was shown that the primary difference between human MAP1 and MAP2 is that the latter processes P1' Thr and Val more efficiently suggesting that this may be this enzymes role in-vivo.

Recombinant Protein Expression

When recombinant proteins are expressed, especially in E.coli, the N-terminal methionine can be retained regardless of the nature of P1', presumably due to saturation of MAP or depletion of required metal cofactors. In *E.coli*, N-formylmethione can also be retained (Rose et al., 1992). Removal of cleavable N-terminal methionine can be carried out by in-vitro digestion with purified methionine aminopeptidase (Miller et al., 1987) or by co-expression of MAP (Van Valkenburgh & Kahn, 2002). Liao et al. (2004) have described an engineered *E. coli* MAP that was able to process bulky P1' residues not normally cleaved by the wild-type enzyme. In *E.coli*, the need to remove noncleavable methionines can be circumvented by incorporating an N-terminal secretion leader sequence that localize the protein, minus the leader, to the periplasmic space or by incorporating cleavable N-terminal protein or peptide fusion tags such GST or a His-tag, etc.

It is worth mentioning that N-terminal methionine appears especially vulnerable to oxidation to methionine sulfoxide which can be detected by mass spectrometry and which can effect protein stability and antigencity for biologics (Rose, et al. 1992; Wang et al., 2001).It should be noted that it is sometimes possible to resolve proteins containing N-terminal methionine from those lacking it by chromatographic methods (Wingfield et al., 1987). Finally, it is not uncommon, for example in E.coli expression, to observe so-called ragged N-termini resulting from additional processing beyond N-methionine by other proteases. So regardless of the simple rule presented here, the actually N-terminal sequence should be directly determined to fully characterize the protein.

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Table 1
Predictions of N-Terminal Processing by Methionine Aminopeptidase

The cleavage between N-terminal Met (P) and adjacent residue (P1') is indicated using cleavage site nomenclature of Schechter and Berger (1967). The predicted processing (or removal) of N-terminal Met by E.coli MAP1 depends on the size of the P1' residue. When Thr and Val are P1', cleavage is variable depending on downstream sequence of P2'-P3'-P4'-P5' and expression levels etc. When proline is at the P2' position, methionine is normally not removed regardless of P1'. In mammalian cells, N-terminal Met is processed by either or both MAP-1 and MAP-2 and is similar to E.coli except for Thr and Val. When P1' is Thr or Val, Met is removed by MAP-2 and the extent of cleavage depends on P2' – when P2' is not Asp, Glu or Pro processing is complete but if consists of one of these residues processing does not occur (Xiao et al., 2010). The radius of gyration of amino acids is given and the units are in Angstroms (Å). The accessible surface area of the residue X in Gly-X-Gly is also given and the units are in square Angstroms ($Å^2$).

P1'	Cleavage	P2' Pro	Radius	Area
Glycine	Yes	No	0	0
Alanine	Yes	No	0.77	67
Serine	Yes	No	1.08	80
Cysteine	Yes	No	1.22	104
Theonine	Variable	No	1.24	102
Proline	Yes	No	1.25	105
Valine	Variable	No	1.29	117
Aspartic acid	No		1.43	106
Asparagine	No		1.45	113
Leucine	No		1.54	137
Isoleucine	No		1.56	140
Glutamine	No		1.75	144
Glutamic acid	No		1.77	138
Histidine	No		1.78	151
Methionine	No		1.80	160
Phenylanine	No		1.90	175
Lysine	No		2.08	167
Tyrosine	No		2.13	187
Trptophan	No		2.21	217
Arginine	No		2.38	196