Purification and characterization of aminopeptidase M from muscle and mucosa of the pig intestine

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Abstract: The aim of this investigation was to purify aminopeptidase M (APM) from the muscle layer of the small intestine, to compare it with APM of the mucosa and kidney, and to examine the degradation of gastrointestinal neural and hormonal peptides by muscle APM. APM was purified from the muscle and mucosa of the pig small intestine by DEAE-Sepharose and immuno-affinity chromatography. The specific activity of APM from muscle, mucosa, and kidney was 3900, 3000, and 3800 nmol/min per mg protein, respectively (substrate [LeuS]enkephalin). Muscle and mucosa APM contained four protein bands with apparent molecular weights of 150, 110, 73, and 52 kDa. Kidney APM contained three protein bands of 150, 110, and 56 kDa. The 150, 110, and 52/56 kDa bands cross-reacted with an APM antiserum. APM from each tissue degraded [LeuS]enkephalin and [MetS]enkephalin, but not cholecystokinin-8, gastrin releasing peptide-10, somatostatin-14, substance P, and vasoactive intestinal peptide. The enzymes were identically inhibited by APM antiserum, amastatin, bestatin, actinonin, and 1, 10 phenanthroline. Non-mucosal APM may degrade enkephalins and terminate their biological actions.

Key words: neuropeptide degradation, enkephalin degradation, immunoaffinity chromatography, enteric neurons

Introduction

Aminopeptidase M (APM, EC 3.4.11.2), also known as aminopeptidase N and CD 13, is a cell surface enzyme originally isolated from a microsome fraction of the pig kidney. The amino acid sequences of APM from humans, pigs, and rats have been deduced by molecular cloning. The enzyme is anchored to the plasma membrane by a single hydrophobic domain near the N-terminus and projects a long C-terminal portion, containing the active site, extracellularly. APM catalyzes the removal of unsubstituted N-terminal amino acids from peptides in the extracellular fluid. Although neutral residues are preferred, acidic and basic amino acids are also removed.

APM is widely distributed throughout the body and has multiple possible functions, including the degradation of dietary peptides, and the inactivation of peptide neurotransmitters and hormones. High levels of APM are found in the brush border of the kidney and the small intestine, where APM degrades luminal peptides prior to absorption. APM is also found in the central nervous system, where it degrades and inactivates neuropeptides. Immunoreactive APM is primarily localized to microvessels in the brain of the rat and pig, although diffuse staining is apparent in the neuropil. In addition, APM activity is found in synaptic and cerebral membranes, suggesting localization in neural tissue. APM is one of several enzymes that degrade enkephalins in the brain, since inhibitors of APM enhance the depolarization-stimulated release of [MetS]enkephalin from brain slices and thereby potentiate the central actions of enkephalins. These observations support the hypothesis that APM participates in the post-secretory metabolism of enkephalins in the central nervous system.

Gastrointestinal function is regulated by biologically active peptides from enteric neurons and endocrine cells. The biological actions of these peptides are terminated by enzymatic degradation, but the peptidases involved have not been fully characterized. Aminopeptidases participate in the degradation and inactivation of enkephalins in gastrointestinal tissue, since
Aminopeptidase inhibitors suppress the degradation of enkephalins in the stomach wall and potentiate enkephalin-stimulated contraction of gastric smooth muscle cells. We have previously localized immunoreactive APM to a sub-population of myenteric neurons innervating the muscle layer of the stomach and intestine, suggesting that neuronal APM may participate in enkephalin degradation in gastrointestinal muscle. However, APM has not been isolated from gastrointestinal muscle tissue and enzymatically characterized. Therefore, the first aim of this investigation was to isolate APM from the muscle layer and myenteric plexus of the gastrointestinal tract, and to compare it to APM from the brush border of the intestine and kidney. The ability of APM to degrade a wide range of neural and hormonal peptides has also not been fully assessed. Consequently, the second objective was to examine the degradation by highly purified APM of peptides known to regulate gastrointestinal function. The results show that APM from intestinal muscle and myenteric neurons closely resembles APM from the brush border of the intestine and kidney. APM degrades enkephalins, but not cholecystokinin octapeptide (CCK-8), gastrin-releasing peptide decapeptide (GRP-10), somatostatin-14, substance P, or vasoactive intestinal peptide (VIP).

Materials and methods

Materials

APM (leucine aminopeptidase, microsomal, EC3.4.11.2, pig kidney), puromycin, phenylmethylsulfonylfluoride (PMSF), ethylenediaminetetraacetic acid (EDTA), 1,10-phenanthroline, potato carboxypeptidase inhibitor (PCI), 8-hydroxyquinoline (free base), and non-immune rabbit serum were obtained from Sigma Chemical Co. (St. Louis, Mo.). DEAE-Sepharose Fast Flow and CNBr-activated Sepharose 4B were from Pharmacia-LKB Biotechnology Inc. (Pleasant Hill, Calif.). CCK-8 (sulfated), GRP-10, [Leu5]enkephalin, [Met5]enkephalin, somatostatin-14, substance P, VIP, amastatin, bestatin, actinonin, phosphoramidon (N-(a-rhamnopyranosyl-oxyhydroxyphosphinyl)-L-Leu-L-Trp), leupeptin, and pepstatin A were from Peninsula Laboratories Inc. (Belmont, Calif.). Captorpl (SQ 4125) was a gift from the Squibb Institute for Medical Research (Princeton, N.J.). A silver stain kit and an enhanced colloidal gold immunoblot assay kit were obtained from Bio-Rad (Richmond, Calif.).

Enzymatic characterization of APM

The purification of APM was monitored by incubating aliquots of column eluant with 25 μM [Leu5]enkephalin...