

Post-Translational Modification	Cellular Location of Modification	Enzyme	Motif	Example	Mass Change (Monoisotopic)	Mass Change (Average)
N-Acetylation <sup>[1]</sup>	Co-translational modification, Secretory granules	N-acetyltransferases	N-terminal Ser or Ala and other residues	Alpha-MSH, <sup>[16]</sup> beta-endorphin, rat growth hormones, LeuEnk	+42.0106	+42.0373
Amidation <sup>[2]</sup>	Secretory granules	peptidylglycine a-amidating monooxygenase	C-terminal Gly	secretin, glucagons, VIP, GIP-like peptide, Vasopressin, Alpha-MSH <sup>[16]</sup>	-58.0036	-58.0366
Bromination <sup>[3]</sup>	Unknown	Unknown	Trp	Neuropeptide B <sup>[17]</sup>	+77.9105	+78.8962
$\gamma$ -Carboxylation <sup>[4]</sup>	Unknown	Vitamin K Dependent Carboxylase and others unknown	Glu-X-X-X-Glu-X-Cys (for Bone Proteins)	Conus Neuotoxins <sup>[18]</sup>	+43.9898	+44.0098
Dipeptidase <sup>[5]</sup>	Golgi	Aminopeptidase	N-terminal X-Pro and X-Ala	Substance P <sup>[5]</sup>	Depends on X	Depends on X
Disulfide bond (not supported)	Rough ER	Various	Two Cys residues	Insulin, Orexin A <sup>[19]</sup>	-2.01566	-2.01588
Glycosylation N-linked <sup>[6]</sup>	ER/Golgi	Oligosaccharyl transferase (pH ~7)	Asn-X-Ser/Thr; X not proline, in N-terminal regions	Glycoprotein hormones, FSH, LH, choriogonadotropin, luteinizing hormone <sup>[20]</sup>	+162.0528	+162.1424
Glycosylation O-linked <sup>[7]</sup>	ER/Golgi	UDP-N-acetylgalactosamine	Ser/Thr-Pro-Pro-Pro	Proteins that are part of mucosal or the extracellular matrix fluid, Chromogranin A <sup>[7]</sup>	+162.0528	+162.1424
Hydroxylation of Pro <sup>[8]</sup>	ER	Prolyl-4-hydroxylase	X-Pro-Gly/Ala	Prodermorphin <sup>[8]</sup>	+15.9949	+15.9994
Methylation of Glu <sup>[9,10]</sup>	Unknown	Various Types	Occurs on O-atom of Glu, preferentially at an N-terminal Glu in insects	Pea-sulfakinin <sup>[9]</sup> , Corticotropin <sup>[21]</sup>	+14.0157	+14.0269

Phosphorylation <sup>[1,11]</sup> of Ser or Thr	ER/Golgi, follows glycosylation	Casein Kinases (CK)/ Protein Kinase A (PKA)	Ser/Thr-X-Asp/Glu/Asn/Gln; Ser/Thr-X <sub>1-2</sub> -Ser/Thr; Lys-Arg-X-X-Ser; and Arg-Arg-X-Ser	ACTH, CLIP, <sup>[16]</sup> Chromogranin A <sup>[7]</sup>	+79.9663	+79.9799
Pyroglutamylation <sup>[12]</sup>	Secretory granules (pH 8)/ chromaffin granules	Glutaminyl cyclase	N-terminal Glu or Gln residue	Alpha-MSH <sup>[22]</sup> , sulfakinin <sup>[9]</sup>	Glu: -18.0106 Gln: -17.0266	Glu: -18.02 Gln: -17.0306
Sulfation <sup>[1, 13-15]</sup> of Tyr	TGN/ follows glycosylation	Tyrosylprotein sulfotransferase	Asp/Glu-Tyr and >2 acidic residues in +5 to -5 region, 1 Pro or Gly or at least two Asp Ser or Asn in -7 to +7 region, also no disulfide bond or N-linked glycan within -7 to +7 region	Alpha-MSH <sup>[22]</sup> , CCK, gastrin, secretogranin I & II, Leu-Enk, sulfakinin <sup>[9]</sup>	+79.9568	+80.0642

## References

1. Mains, R. E.; Eipper, B. A.; Glembotski, C. C.; Dores, R. M., Strategies for the biosynthesis of bioactive peptides. *Trends in neurosciences* **1983**, 6, 229-235.
2. Eipper, B. A.; Milgram, S. L.; Husten, J. E.; Yun, H.-Y.; Mains, R. E., Peptidylglycine  $\alpha$ -amidating monooxygenase: A multifunctional protein with catalytic, processing, and routing domains. *Protein Sci.* **1993**, 2, 489-497.
3. Tanaka, H.; Yoshida, T.; Miyamoto, N.; Motoike, T.; Kurosu, H.; Shibata, K.; Yamanaka, A.; Williams, S. C.; Richardson, J. A.; Tsujino, N.; Garry, M. G.; Lerner, M. R.; King, D. S.; O'Dowd, B. F.; Sakurai, T.; Yanagisawa, M., Characterization of a family of endogenous neuropeptide ligands for the G protein-coupled receptors GPR7 and GPR8. *Proc. Natl. Acad. Sci. USA* **2003**, 100, 6251-6256.
4. Stanley, T. B.; Stafford, D. W.; Olivera B. M.; Bandyopadhyay, P. K., Identification of a vitamin K-dependent carboxylase in the venom duct of a Conus snail. *FEBS Lett.* **1997**, 407, 85-88.
5. R. Mentlein., Proline residues in the maturation and degradation of peptide hormones and neuropeptides. *FEBS Lett.* **1988**, 234, 251-256.

6. Breuer, W.; Bause, E., Oligosaccharyl transferase is a constitutive component of an oligomeric protein complex from pig liver endoplasmic reticulum. *Eur. J. Biochem.* **1995**, 228, 689-696.
7. Strub, J. M.; Sorokine, O.; Dorsselaer, A. V.; Aunis, D.; Metz-Boutigue, M. H., Phosphorylation and *O*-glycosylation sites of bovine chromogranin A from adrenal medullary chromaffin granules and their relationship with biological activities, *J. Biol. Chem.* **1997**, 272, 11928–11936.
8. Seethaler, G.; Le Caer, J. P.; Rossier J.; Kreil, G., Frog prodermorphin expressed in mammalian cells is partly converted to the hydroxyproline containing precursor. *Neuropeptides* **1993**, 25, 61-64.
9. Predel, R.; Brandt, W.; Kellner, R.; Rapus, J.; Nachman, R. J.; Gäde, G., Post-translational modifications of the insect sulfakinins: sulfation, pyroglutamate-formation and O-methylation of glutamic acid. *Eur. J. Biochem.* **1999**, 263, 552-560.
10. Diliberto, E. J.; Axelrod, J., Characterization and substrate specificity of a protein carboxymethylase in the pituitary gland, *PNAS* **1974**, 71, 1701-1704.
11. Knippschild, U.; Gocht, A.; Wolff, S.; Huber, N.; Lohler, J.; Stoter, M., The casein kinase 1 family: participation in multiple cellular processes in eukaryotes. *Cell. Signal.* **2005**, 17, 675-689.
12. Fischer, W. H.; Spiess, J., Identification of a mammalian glutarminyl cyclase converting glutaminyl into pyroglutamyl peptides. *Proc. Natl. Acad. Sci. USA* **1987**, 84, 3628-3632.
13. Huttner, W. B., Tyrosine sulfation and the secretory pathway. *Ann. Rev. Physiol.* **1988**, 50, 363-376.
14. Hortin, G.; Folz, R.; Gordon, J. I.; Strauss, A. W., Characterization of sites of tyrosine sulfation in proteins and criteria for predicting their occurrence. *Biochem. Biophys. Res. Commun.* **1986**, 141, 326-333.
15. Huttner, W. B.; Baeuerle, P. A., *Modern Cell Biology*. Liss: New York, 1988; Vol. 6, p 97-140.
16. Che, F. Y.; Biswas, R.; Fricker, L. D., Relative quantitation of peptides in wild-and *Cpe<sup>fat/fat</sup>* mouse pituitary using stable isotopic tags and mass spectrometry. *J. Mass Spectrom.* **2005**, 40, 227–237.
17. Fujii, R.; Yoshida, H.; Fukusumi, S.; Habata, Y.; Hosoya, M.; Kawamata, Y.; Yano, T.; Hinuma, S.; Kitada, C.; Asami, T.; Mori, M.; Fujisawa, Y.; Fujino, M., Identification of a neuropeptide modified with bromine as an endogenous ligand for GPR7. *J. Biol. Chem.* **2002**, 277, 34010-34016.
18. Brown, M. A.; Begley, G. S.; Czerwiec, E.; Stenberg, L. M.; Jacobs, M.; Kalume, D. E.; Roepstorff, P.; Stenflo, J.; Furie, B. C.; Furie, B., Precursors of novel Gla-containing conotoxins contain a carboxy-terminal recognition site that directs  $\gamma$ -carboxylation. *Biochemistry* **2005**, 44, 9150-9159.
19. Soll, R.; Beck-Sickinger, A. G., On the synthesis of orexin A: a novel one-step procedure to obtain peptides with two intramolecular disulphide bonds. *J. Peptide Sci.* **2000**, 6, 387-397.
20. Maghuin-Rogister, G.; Hennen, G. P., Bovine luteinizing hormone: study of the primary structure around the carbohydrate attachment sites of the luteinizing hormone alpha-subunit. *Eur. J. Biochem.* **1971**, 21, 489-497.
21. Kim, S.; Li, C. H., Enzymatic methyl esterification of specific glutamyl residue in corticotropin. *Proc. Natl. Acad. Sci. USA* **1979**, 76, 4255-4257.
22. Bateman, A.; Solomon, S.; Bennett, H. P., Post-translational modification of bovine pro-opiomelanocortin: tyrosine sulfation and pyroglutamate formation, a mass spectrometric study. *J. Biol. Chem.* **1990**, 265, 22130-22136.