Semester – I
MCB-103: Biophysical & Biochemical Principles
Gr. B : Fundamental Biochemistry

Chemical Modification of Proteins

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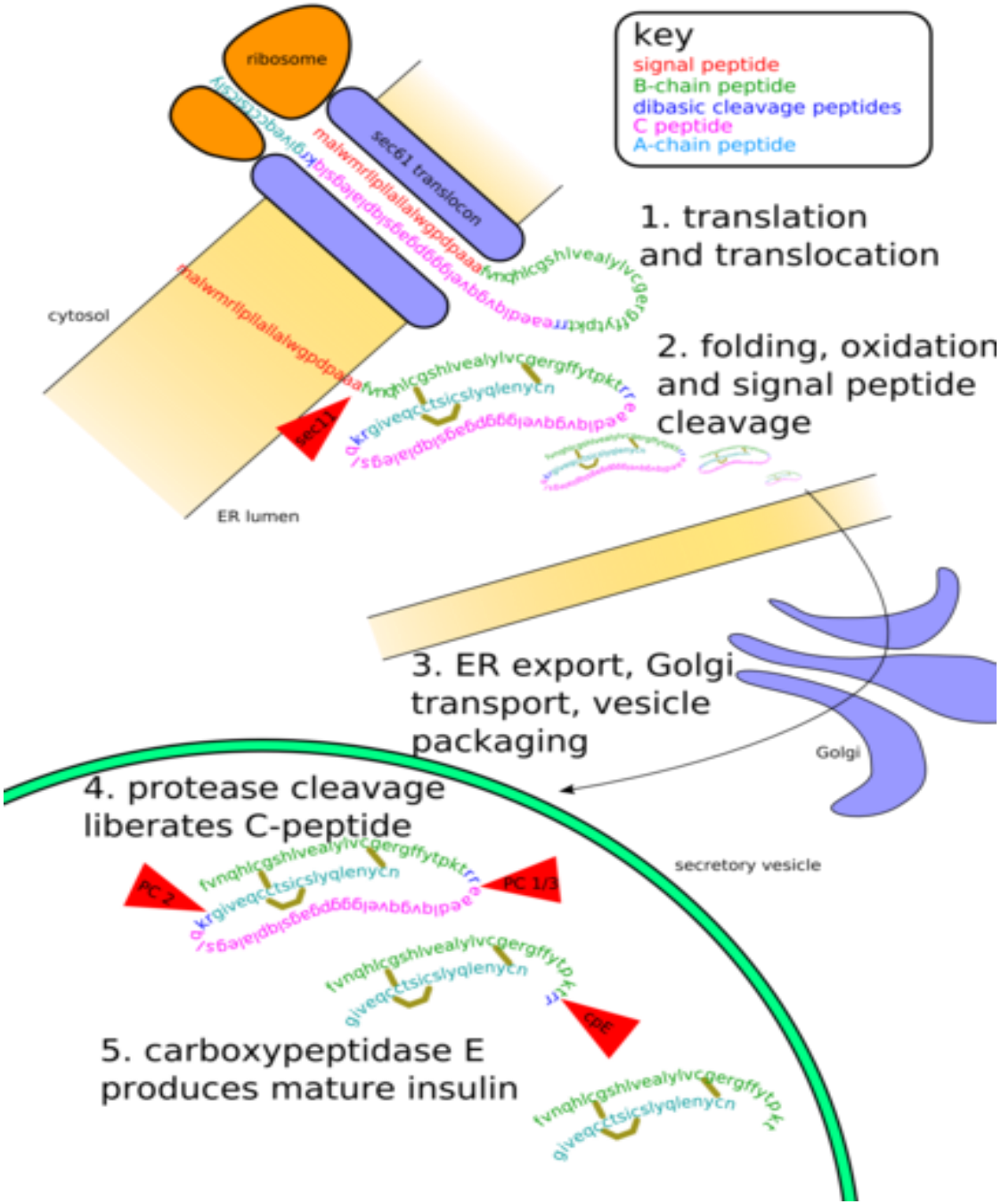
Modification of Proteins structure

Specific cleavage of polypeptide chain :

Reagent	Cleavage site
Cyanogen bromide	Met (C)
O-iodosobenzoate	Trp (C)
Hydroxylamine	Aspargine-glycine bond
2-nitro-5-thiocyanobenzoate	Cys (N)
Trypsine	Lys (C), Arg (C)
Chymotrypsine	Phe (C), Trp (C), Tyr (C)
Pepsine	Phe (N), Trp (N), Tyr (C)
Submaxillarous protease	Arg (C)
Staphylococcal protease	Asp (C), Glu (C)
Asp – N – protease	Asp (N), Glu (N)
Thrombin	Carboxyl side of arginine

(C) – Carboxyl site (N) – Amino site


key
signal peptide
B-chain peptide
dibasic cleavage peptides
C peptide
A-chain peptide
ribosome
sec61 translocon
cytosol
ER lumen
sec11
1. translation and translocation
2. folding, oxidation and signal peptide cleavage
3. ER export, Golgi transport, vesicle packaging
Golgi
4. protease cleavage liberates C-peptide
PC 2
PC 1/3
secretory vesicle
CPE
5. carboxypeptidase E produces mature insulin

Acetylation

Acetylation describes a reaction that introduces an acetyl functional group (CH3 CO) into an organic compound. **Deacetylation** is the removal of the acetyl group. A reaction involving the replacement of the hydrogen atom of an hydroxyl group with an acetyl group yields a specific ester, the acetate.

Histone Acetylation and Deacetylation

Histones are acetylated and deacetylated on **lysine** residues in the N-terminal tail as part of gene regulation. Typically, these reactions are catalyzed by enzymes with "histone acetyltransferase" (HAt) or "histone deacetylase" (HDAc) activity.

Acetylated histones and nucleosomes represent a type of epigenetic tag within chromatin. Acetylation brings in a negative charge and neutralizes the interaction of the N termini of histones with the phosphate groups of DNA. As a consequence, the condensed chromatin is transformed into a transiently relaxed structure which allows genes to be transcribed. Acetylated chromatin is thought to be more "relaxed" and is called euchromatin. Methylated chromatin is more condensed (tightly packed), and referred to as heterochromatin.

Tubulin Acetylation and Deacetylation

Tubulin Acetylation and Deacetylation system is well worked out in Chlamydomonas. A Tubulin acetyltransferase located in the axoneme acetylates a specific lysine residue in the α-tubulin subunit in assembled microtubule. Once dissembled, this acetylation can be removed by another specific deacetylase which is cytosolic. Thus the axonemal microtubules (long half life) carry this signature acetylation absent from cytosolic microtubules (short half life).

Formylation reaction

A **Formylation reaction** in organic chemistry is the catch-all name for any organic reaction in which an organic compound is functionalized with a formyl group -CH=O.

Myristoylation

Myristoylation is an irreversible, post-translational protein modification. In this protein modification a myristoyl group (derived from myristic acid, $CH_3(CH_2)_{12}COOH$) is covalently attached via an amide bond to the alpha-amino group of an **N-terminal glycine** residue of a nascent polypeptide. The modification is catalyzed by the enzyme N-myristoyltransferase. Myristoylation also occurs post-translationally, for example when previously internal glycine residues become exposed by caspase cleavage during apoptosis. Myristoylation plays a vital role in membrane targeting and signal transduction in plant responses to environmental stress.

Methylation

In biological systems, methylation refers to the replacement of a hydrogen atom with the methyl group is catalyzed by enzymes; such methylation can be involved in modification of heavy metals, regulation of gene expression, regulation of protein function, and RNA metabolism.

Protein methylation typically takes place on arginine or lysine amino acid residues in the protein sequence. Arginine can be methylated once (monomethylated arginine) or twice (asymmetric dimethylated arginine) or one on both nitrogens (symmetric dimethylated arginine) by peptidylarginine methyltransferases (PRMTs). Lysine can be methylated once, twice or three times by lysine methyltransferases.

The transfer of methyl groups from S-adenosyl methionine to histones is catalyzed by enzymes known as histone methyltransferases. **Histones which are methylated on certain residues can act epigenetically to repress or activate "gene" expression.** Protein methylation is one type of post-translational modification.

Glycation

Glycation (sometimes called non-enzymatic glycosylation) is the result of a sugar molecule, such as fructose or glucose, bonding to a protein or lipid molecule without the controlling action of an enzyme. Enzyme-controlled addition of sugars to protein or lipid molecules is termed glycosylation. Also, polysaccharides linked at the **amide nitrogen of asparagine** in the protein confer stability on some secreted glycoproteins. Experiments have shown that glycosylation in this case is not a strict requirement for proper folding, but the unglycosylated protein degrades quickly. Glycosylation may play a role in cell-cell adhesion (a mechanism employed by cells of the immune system), as well.

Ubiquitination

Ubiquitin is a small regulatory protein that is *ubiquitous* in eukaryotes. **Ubiquitination** (or **Ubiquitylation**) refers to the post-translational modification of a protein by the covalent attachment (via an isopeptide bond) of one or more ubiquitin monomers. Ubiquitin (originally, **Ubiquitous Immunopoietic Polypeptide**) was first identified in 1975 as an 8.5 kDa protein of unknown function expressed universally in living cells. Poly-ubiquitination, the process in which a chain of at least four ubiquitin peptides are attached to a lysine on a substrate protein, most commonly results in the degradation of the substrate protein via the proteasome.

Number of residues	76
Molecular mass	8564.47 Da
Isoelectric point (pI)	6.79
Gene names	RPS27A (UBA80, UBCEP1), UBA52 (UBCEP2), UBB, UBC

Hydroxylation

Hydroxylation is any chemical process that introduces one or more hydroxyl groups (-OH) into a compound (or radical) thereby oxidising it.

Proline is the principal residue to be hydroxylated in proteins, which occurs at the Cγ atom, forming hydroxyproline (Hyp), an essential element of collagen, in turn a necessary element of connective tissue. In some cases, proline may be hydroxylated instead on its Cβ atom. Lysine may also be hydroxylated on its Cδ atom, forming hydroxylysine (Hyl). These three reactions are catalyzed by very large, multi-subunit enzymes **prolyl 4-hydroxylase**, **prolyl 3-hydroxylase** and **lysyl 5-hydroxylase**, respectively. For both the enzymes activity required Ascorbic acid as co-enzyme.

Phosphorylation

Phosphorylation is the addition of a phosphate (PO_4) group to a protein or a small molecule or "the introduction of a phosphate group into an organic molecule". Phosphorylation is catalyzed by various specific **protein kinases**, whereas **phosphatases** *de*phosphorylate. Phosphorylation is observed on serine, threonine, tyrosine and histidine residues.

Serine/threonine protein kinases (EC 2.7.11.1) phosphorylate the OH group of serine or threonine. Activity of these protein kinases can be regulated by specific events (e.g. DNA damage), as well as numerous chemical signals, including cAMP/cGMP, Diacylglycerol, and Ca^{2+}/calmodulin.

Tyrosine-specific protein kinases (EC 2.7.10.1) phosphorylate tyrosine amino acid residues, and are used in signal transduction.

Histidine kinases are structurally distinct from most other protein kinases and are found mostly in prokaryotes as part of two-component signal transduction mechanisms.

Sulfation

Tyrosine sulfation is a posttranslational modification where a sulfate group is added to a tyrosine residue of a protein molecule. Secreted proteins and extracellular parts of membrane proteins that pass through the Golgi apparatus may be sulfated. Sulfation is known to happen in animals and plants. It is catalyzed by tyrosylprotein sulfotransferases (TPSTs) in Golgi apparatus. Types of human proteins known to undergo tyrosine sulfation include adhesion molecules, G-protein-coupled receptors, coagulation factors, serine protease inhibitors, extracellular matrix proteins, and hormones.