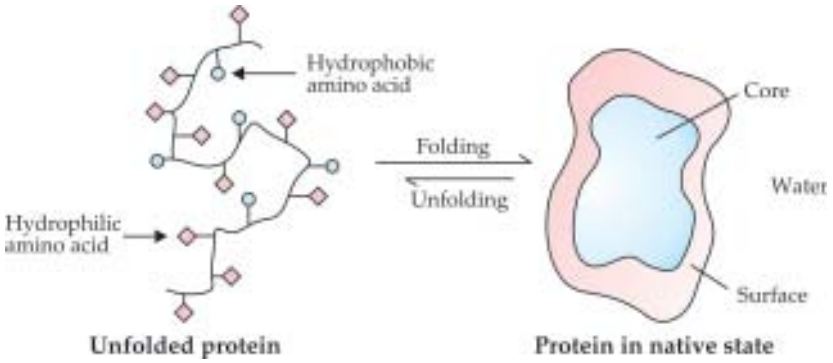

Fig. 1.5. Folding of a polypeptide due to interaction with water.

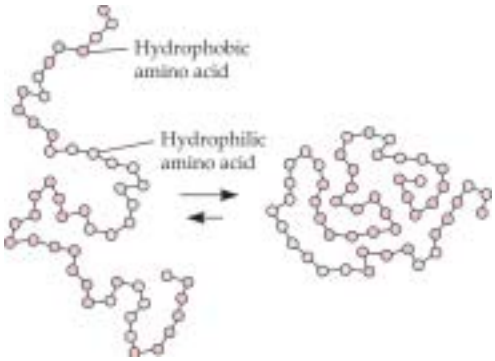

Fig. 1.6. Protein folding: Hydrophilic amino acids are on the surface, while hydrophobic amino acids get buried in the core.

- While hydrolysis is a thermodynamically favoured reaction, the amide and phosphoester bonds of polypeptides and oligonucleotides are still stable in the aqueous environment of the cell. This is because even though favourable, the rate of reaction is extremely slow making DNA stable for millions of years. Thermodynamics governing the equilibrium of a reaction do not determine the rate at which it will proceed. Breakdown rate is accelerated in presence of enzymes like proteases, nucleases, amylase which do not need any energy or ATP for action.
- The reverse process i.e. formation of peptide and phosphodiester bonds requires expenditure of ATP to occur.

Slight tendency to dissociate

- The ability of water to ionize, while slight, is of central importance for life. Water can act both as an acid and as a base. The removal and addition of hydrogen i.e. oxidation and reduction are a central concept of many metabolic reactions, e.g. shuttling of $NADH^+$/NADPH and NAD/NADP serves to transfer energy released from catabolism for ATP synthesis and anabolism.

Q. 2. What is pH and pKa? What is their physiological and clinical significance?

Ans. In pure water, molecules exist in equilibrium with hydrogen ions and hydroxide ions.

$$H_2O \leftrightarrow H^+ + OH^-$$

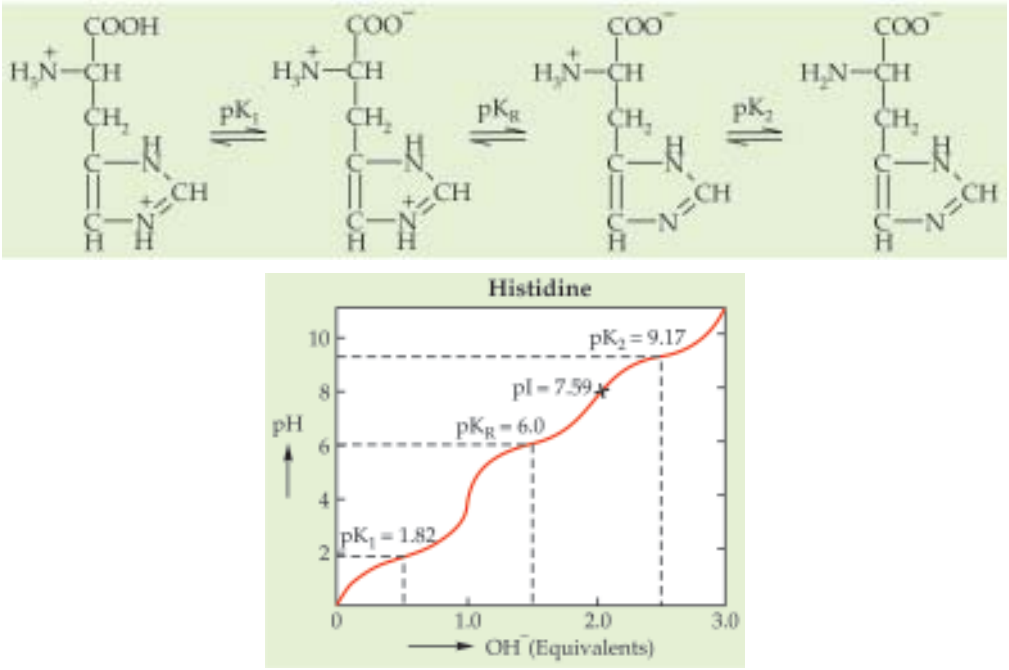

Fig. 1.12. Titration curve of histidine.

The pKa values and the isoelectronic point, pI, are given below for the 20 α-amino acids.

pKa values and the isoelectronic point, pI, for the 20 α-amino acids

Amino acid	pKa_1	pKa_2	pKa_3	*pI*
Glycine	2.34	9.60	—	5.97
Alanine	2.34	9.69	—	6.00
Valine	2.32	9.62	—	5.96
Leucine	2.36	9.60	—	5.98
Isoleucine	2.36	9.60	—	6.02
Methionine	2.28	9.21	—	5.74
Proline	1.99	10.6	—	6.30
Phenylalanine	1.83	9.13	—	5.48
Tryptophan	2.83	9.39	—	5.89
Asparagine	2.02	8.80	—	5.41
Glutamine	2.17	9.13	—	5.65
Serine	2.21	9.15	—	5.68
Threonine	2.09	9.10	—	5.60
Tyrosine	2.2	9.11	—	5.66
Cysteine	1.96	8.18	—	5.07
Aspartic acid	1.88	9.60	3.65	2.77
Glutamic acid	2.19	9.67	4.25	3.22
Lysine	2.18	8.95	10.53	9.74
Arginine	2.17	9.04	12.48	10.76
Histidine	1.82	9.17	6.00	7.59

- Some IUPs act to inhibit other proteins by an unusual mechanism (Figs. 2.4 and 2.6) by wrapping around multiple target protein. Example: IUP p27, which controls cell cycle, lacks a definable structure in solution but inhibits action of multiple cyclin-dependent kinases (CDKs) by wrapping around them. Tumor cells have decreased p27. Lower levels of p27 imply poorer prognosis.
- Disorder in the bound state (fuzzy complexes): Intrinsically disordered proteins can retain their conformational freedom even when they bind specifically to other proteins. This can be further modified by post-translational and covalent modifications. Also, alternative splicing can alter the length of fuzzy/unstructured regions altering the specificity of binding of certain DNA binding proteins.

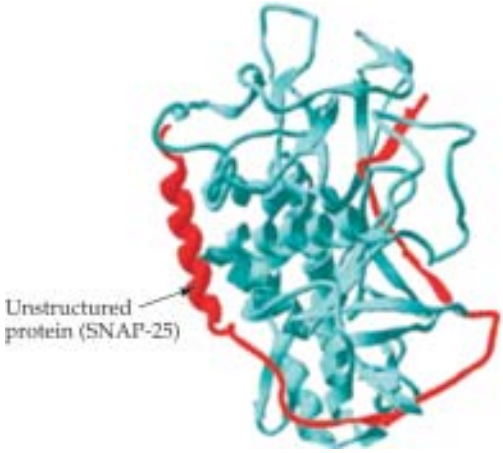

Fig. 2.6. SNAP-25 bound to BoNT/A. The unstructured proteins (SNAP-25) binds to the target (BoNT/A) by wrapping around it. Then the botulinum neurotoxin (BoNT/A) cleaves SNAP-25 by protease action. As SNAP-25 is required for vesicle fusion and neurotransmitter exocytosis, BoNT/A blocks release of neurotransmitters particularly acetylcholine causing toxicity.

The IUPs and PUFs include scaffold proteins, hormones, activation domains of transcription factors, cyclin-dependent kinases and their inhibitors, proteins in cellular signal transduction, and amino terminal segments of histone proteins.

Significance of IUPs

- Act as flexible linkers: Unstructured or unfolded regions in proteins act as flexible linkers allowing the connected domains to freely twist and rotate through space to recruit their ligands and allow large scale interdomain conformation changes on binding of ligands.
- Because of structural disorder and high charge density, function as spacers, insulators or linkers in larger structures.
- Help limit cell, genome and protein size: Compared with compact, folded proteins, disordered segments in proteins have larger intermolecular interfaces to which ligands, such as other proteins, could bind. Folded proteins will have to be two to three times larger to produce similar sized binding surface area as of a disordered protein. Such large proteins would increase cellular crowding or could increase cell size by 15% to 30%. The flexibility of disordered proteins may thus reduce protein, genome, and cell sizes.

Production of eukaryotic proteins in bacteria by recombinant DNA technology: Eukaryotic proteins may have disulfide bonds which are not usually formed in bacterial cytoplasm. Modulation of the redox environment in the bacterial cytoplasm, co-production of a disulfide isomerase and Ero1p in endoplasmic reticulum or DsbA and DsbB (involved in disulfide bond production) in the periplasm allows the formation of disulfide bonds in the proteins made by recombinant DNA technology.

Q. 6. Distinguish between peptides and proteins. Give examples of some important peptides.

Ans. Both are polymers of amino acids, i.e. chains of amino acids linked by peptide bonds. Peptides contain less than 40 amino acids, while proteins contain > 40 amino acids. 40 residues appear to be near the minimum required for a polypeptide chain to fold into a discrete and stable 3-dimensional shape that allows it to carry out particular functions. The largest protein is titin having 34,350 amino acids. Most proteins have 100 to 1000 amino acids.

When a few (2–20) amino acids are joined by peptide bonds, the structure is called an oligopeptide. Although the terms "protein" and "polypeptide" are sometimes used interchangeably, molecules referred to as polypeptides generally have molecular weights below 10,000, and those called proteins have higher molecular weights.

Peptides are short polymers of amino acids (< 40 amino acids). Shorter a peptide, greater are the chances that it exists as a linear unfolded polypeptide. However, they still serve many important biological functions. For example:

1. Peptide hormones – β-Corticotropin (ACTH), β-MSH, Gastrin, Glucagon, Secretin, Oxytocin, Vasopressin, TRH.
2. Antibiotics – Bacitracins, Penicillin, Polymyxins, Gramicidin S, Chloramphenicol.
3. Anticancer drug – Bleomycin.
4. Muscle relaxants – Kallidin, Bradykinin.
5. Vasoconstrictors – Angiotensins (Angiotensin I, Angiotensin II, Angiotensin III), Vasopressin.
6. Neuromodulators – Endorphins, Enkephalin.
7. Toxic peptides – Microcystin, Nodularin.
8. Biological reductant – Glutathione.
9. Artificial sweetener – Aspartame.
10. Muscle peptides – Creatine, Carnosine, Anserine.

Table 2.1 lists the common peptides and their biological significance.

Q. 7. What are domains, folds, motifs and supersecondary structures?

Ans. **Domain**

Proteins range in molecular weight from a thousand to more than a million. Proteins composed of about 250 amino acids or less often have a simple, compact globular shape. However, larger globular proteins are usually made up of two or more recognizable and distinct structures or modules.

Domains are individually or independently folding compact regions or units connected by short segments in the overall tertiary structure of proteins. Proteins can have just one or