Denaturation of Proteins

Introduction:

Denaturation of proteins involves the disruption and possible destruction of both the secondary and tertiary structures. Since denaturation reactions are not strong enough to break the peptide bonds, the primary structure (sequence of amino acids) remains the same after a denaturation process. Denaturation disrupts the normal alpha-helix and beta sheets in a protein and uncoils it into a random shape.

Denaturation occurs because the bonding interactions responsible for the secondary structure (hydrogen bonds to amides) and tertiary structure are disrupted. In tertiary structure there are four types of bonding interactions between "side chains" including: hydrogen bonding, salt bridges, disulfide bonds, and non-polar hydrophobic interactions. which may be disrupted. Therefore, a variety of reagents and conditions can cause denaturation. The most common observation in the denaturation process is the precipitation or coagulation of the protein.

Heat:

Heat can be used to disrupt hydrogen bonds and non-polar hydrophobic interactions. This occurs because heat increases the kinetic energy and causes the molecules to vibrate so rapidly and violently that the bonds are disrupted. The proteins in eggs denature and coagulate during cooking. Other foods are cooked to denature the proteins to make it easier for enzymes to digest them. Medical supplies and instruments are sterilized by heating to denature proteins in bacteria and thus destroy the bacteria.

Alcohol Disrupts Hydrogen Bonding:

Hydrogen bonding occurs between amide groups in the secondary protein structure. Hydrogen bonding between "side chains" occurs in tertiary protein structure in a variety of amino acid combinations. All of these are disrupted by the addition of another alcohol.

A 70% alcohol solution is used as a disinfectant on the skin. This concentration of alcohol is able to penetrate the bacterial cell wall and denature the proteins and enzymes inside of the cell. A 95% alcohol solution merely coagulates the protein on the outside of the cell wall and prevents any alcohol from entering the cell. Alcohol denatures proteins by disrupting the side chain intramolecular hydrogen bonding. New hydrogen bonds are formed instead between the new alcohol molecule and the protein side chains.

In the prion protein, tyr 128 is hydrogen bonded to asp 178, which cause one part of the chain to be bonding with a part some distance away. After denaturation, the graphic show substantial structural changes.

Acids and Bases Disrupt Salt Bridges:

Salt bridges result from the neutralization of an acid and amine on side chains. The final interaction is ionic between the positive ammonium group and the negative acid group. Any combination of the various acidic or amine amino acid side chains will have this effect.

As might be expected, acids and bases disrupt salt bridges held together by ionic charges. A type of double replacement reaction occurs where the positive and negative ions in the salt change partners with the positive and negative ions in the new acid or base added. This reaction occurs in the digestive system, when the acidic gastric juices cause the curdling (coagulating) of milk.

The example on the left is from the prion protein with the salt bridge of glutamic acid 200 and lysine 204. In this case a very small loop is made because there are only three other amino acids are between them. The salt bridge has the effect of straightening an alpha helix.

The **denaturation** reaction on the salt bridge by the addition of an acid results in a further straightening effect on the protein chain as shown in the graphic on the left.

Heavy Metal Salts:

Heavy metal salts act to denature proteins in much the same manner as acids and bases. **Heavy metal salts** usually contain Hg^{+2}, Pb^{+2}, Ag^{+1} Tl^{+1}, Cd^{+2} and other metals with high atomic weights. Since salts are ionic they disrupt salt bridges in proteins. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt.

This reaction is used for its disinfectant properties in external applications. For example $AgNO_3$ is used to prevent gonorrhea infections in the eyes of new born infants. Silver nitrate is also used in the treatment of nose and throat infections, as well as to cauterize wounds.

Mercury salts administered as Mercurochrome or Merthiolate have similar properties in preventing infections in wounds.

This same reaction is used in reverse in cases of acute heavy metal poisoning. In such a situation, a person may have swallowed a significant quantity of a heavy metal salt. As an antidote, a protein such as milk or egg whites may be administered to precipitate the poisonous salt. Then an emetic is given to induce vomiting so that the precipitated metal protein is discharged from the body.

Heavy Metal Salts Disrupt Disulfide Bonds:

Heavy metals may also disrupt disulfide bonds because of their high affinity and attraction for sulfur and will also lead to the denaturation of proteins.

Reducing Agents Disrupt Disulfide Bonds:

Disulfide bonds are formed by oxidation of the sulfhydryl groups on cysteine. Different protein chains or loops within a single chain are held together by the strong covalent disulfide bonds. Both of these examples are exhibited by the insulin in the graphic on the left.

If oxidizing agents cause the formation of a disulfide bond, then reducing agents, of course, act on any disulfide bonds to split it apart. Reducing agents add hydrogen atoms to make the thiol group, -SH.