CHAPTER 1

AN INTRODUCTION TO ENZYMES

1.1 Enzyme and its biological activity.

Enzymes are macromolecular catalysts playing a crucial role in biochemical process. Like any catalysts, the enzymes increase the rate of the reaction without any change in their chemical composition. In most cases, a catalyst acts by reducing the energy of activation. As a consequence, the reaction equilibrium is not disturbed. In general, enzymes are globular proteins made of folded polypeptide chains throughout the amino acid sequence of the enzyme. The folding topology of the enzyme has its own characteristic patterns, which are responsible for a specific function. The catalytic site or active site, another common name, usually located in the groove on the surface of the enzyme. With this site, substrate can access to bind with the enzyme to form enzyme-substrate complex. Then there will be the chemical change of the substrate and this will turn out to be enzyme-product complex. The interaction between the enzyme and the product molecules will be broken down releasing the product and free enzyme. This product will be of further used in the next step along the biochemical pathway. The free enzyme will be ready to act and initiate the reaction again.

All enzymes are proteins. Many enzymes cannot work without any cofactor. The active component, enzyme with cofactor, is termed *holoenzyme* [44]. The inactive component, enzyme without cofactor, is termed *apoenzyme*. There are two generous kinds of cofactors so called *coenzymes* and *metal ions*. Coenzymes are organic molecules that can change the structure themselves during the reaction and returned back after termination while metal ions that help in binding of substrate and catalyzes the reaction is unchanged. Table 1.1 shows some examples of enzymes incorporating with metal ions or coenzyme. A cofactor that bound tightly to enzyme is called *prosthetic group*. The enzyme function can be damaged if its cofactor is removed.

Table 1.1. Examples of enzymes containing metal ions or coenzymes [44].

Enzyme	Metal ion	Coenzyme
Cytochrome oxidase	Fe, Cu	Porphyrin
Catalase	Fe	Porphyrin
Succinic dehydrogenase	Fe	FAD
Tyrosinase	Cu	
Ascorbic acid oxidase	Cu	
Carbonic anhydrase	Zn	
Carboxypeptidase	Zn	
Pyruvate carboxylase	Zn, Mn	Biotin
Glucose oxidase		FAD
Glyceraldehyde 3-phosphate dehydrogenase		NAD^+
Glucose 6-phosphate dehydrogenase		$NADP^+$
Dihydrofolate reductase		$NADP^+$
Glutamic oxaloacetic transaminase		Pyridoxal phosphate

1.2. Specificity of enzyme to substrate

Enzyme has its own characteristic feature that is its specific in action. [44; 49] Enzyme has two prominent points that are high efficiency and specificity to substrate. Comparing to general catalysts, the reaction catalyzed by enzyme undergoes faster than that of the one without any catalysts. For examples, consider the inter-conversion of A and B. Suppose that in the absence of enzyme the forward rate constant (k_F) is 10^{-4} s^{-1} and the reverse rate constant (k_R) is 10^{-6} s^{-1}. The equilibrium constant K is given by the ratio of these rate constants:

$$A \underset{10^{-6}\ s^{-1}}{\overset{10^{-4}\ s^{-1}}{\rightleftharpoons}} B$$

$$K = \frac{[B]}{[A]} = \frac{k_F}{k_R} = \frac{10^{-4}}{10^{-6}} = 100 \qquad (1.1)$$

The equilibrium concentration of B is 100 times that of A, whether or not enzyme is present. However, it would take more than an hour to approach this equilibrium without enzyme, whereas equilibrium would be attained within a second in the presence of a suitable enzyme.

The interaction between the substrate and enzyme can have either binding or catalyzing function. That is binding sites link to specific groups in the substrate, ensuring that the enzyme and substrate molecules are held in a fixed orientation with respect to each other, with the reacting group or groups in the vicinity of catalytic sites. Substrate can occupy at the active site not only because of its geometrical fitness but also chemical suitability. The intermolecular interactions typically found in the enzyme-substrate binding are hydrogen bonds and van der Waals attraction.

1.2.1 The Fischer "Lock-and-Key" hypothesis

The enzyme specificity implied the presence of complementary structural features between enzyme and substrate [44]. That is a substrate might fit into its complementary site on the enzyme as a key fits into a lock. According to the lock-and-key model as shown in figure 1.1, all structures remain fixed throughout the binding process.

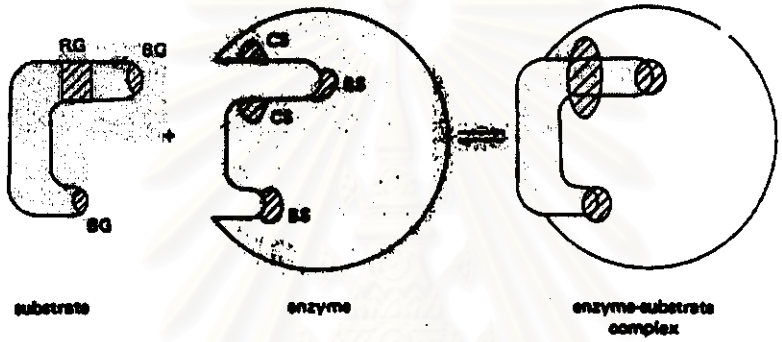

Figure 1.1 Diagrammatic representation of the interaction between an enzyme and its substrate, according to the lock-and-key model. (BS = a binding site on the enzyme, CS = a catalytic site, BG = a binding group on the substrate and RG = reacting group, i.e. a group undergoing enzyme-catalyzed reaction) [44].

1.2.2 The Kohland "Induced-Fit" hypothesis

The data from several spectroscopy techniques such as X-ray diffraction and nuclear magnetic resonance (NMR) have revealed that there are some differences in structure between free and substrate-bound enzyme [44]. Such previous hypothesis, lock-and-key model, cannot explain it. There is a conformational change in three-dimensional structure due to the binding of the substrate to an enzyme. The reasons that can be used to explain this is that the interactions between a substrate and its binding sites may have replaced previously linkages existing between each binding sites and neighboring groups on enzyme. And the water molecules presented with substrate at the active sites may increase the non-polarity of that region.

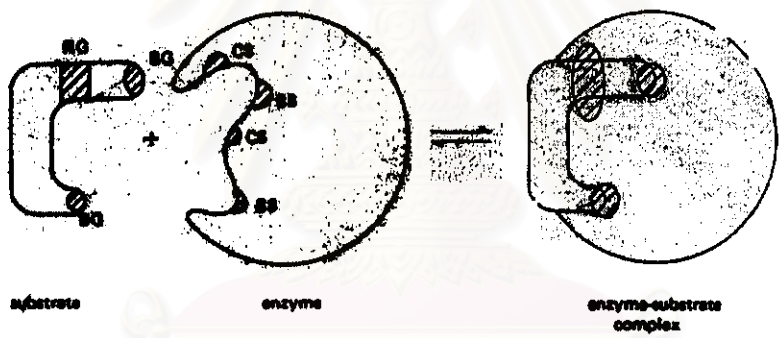

Figure 1.2 Diagrammatic representation of the reaction between an enzyme and its substrate, according to the induced-fit model [44].

1.2.3 High efficiency in catalysis of enzyme

This is due to several reasons. *The proximity of substrate and enzyme* will lead to the suitable in position and direction of binding [44]. Thus the reaction can occur. Some enzymes undergo the reaction by *covalent catalysis*. There will be the covalent bond between substrate and enzyme, which is easy to vanish and yield the final product. *Acid-base catalysis* occurs in organic substance and some enzymes. That is some amino acid residues in active site region can act as proton donor or acceptor. The carbonium ion, as the intermediate, will form the ionic bond with the substrate. This helps to stabilize the enzyme-substrate complex. The active site often includes both polar and non-polar amino acid residues, creating an arrangement of hydrophilic and hydrophobic microenvironment not found elsewhere on an enzyme molecule. *The environment at the active site of the enzyme* such as non-polarity helps in stabilizing some interaction i.e. ionic interaction. Furthermore this can increase the ability of many groups to react with the substrate. Some enzyme make some *strains* occurred by distort the substrate molecule. These result in the stretching, and thus weakening, of a bond that is ready to be cleaved. In some enzyme, when substrate bind to enzyme it will make some change in enzyme conformation. This will induce, so called *induce fit*, the groups in active site region to move to the suitable position of the catalysis. Such new conformation tends to turn back to the previous state by disintegrating and gives the product.

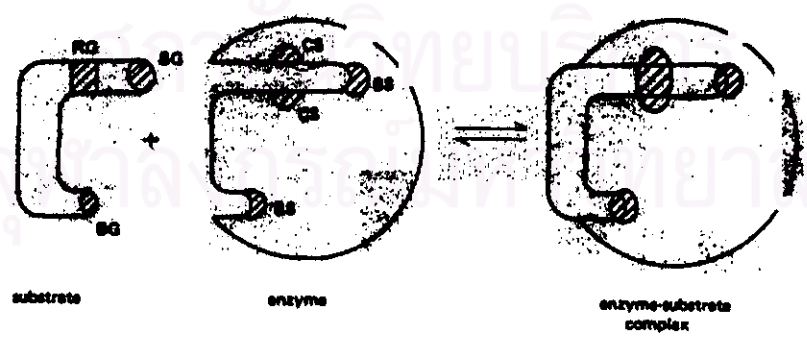

Figure 1.3 Diagrammatic representation of the interaction between an enzyme and its substrate, incorporating a 'strain' effect [44].

1.3 Protein Structure

Proteins are macromolecules found abundance in living organisms [47; 49]. Proteins are made up of amino acids linked together by peptide bonds thus called polypeptide. Peptide bond is geometrically planar as shown in figure 1.4.

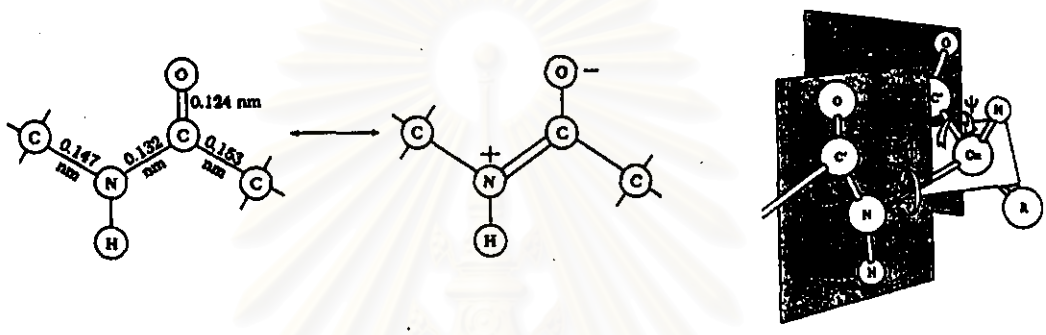

Figure 1.4 The basic characteristics of peptide bond [47].

The carbonyl oxygen and the amide hydrogen about the peptide bond are in the trans position, as is that of the α-carbons. This places the side-chain R groups alternately on either side of the polypeptide backbone as shown in figure 1.5.

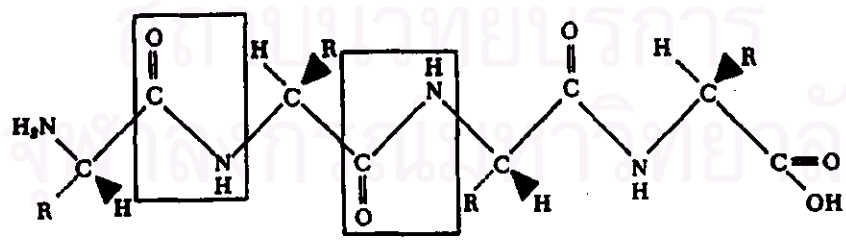

Figure 1.5. The structure of polypeptide, R is the sidechain of the amino acid [47].

Amino acid sequences, which are determined by the genetic code of the cells, are specific and give unique properties to each protein. 20 naturally occurring amino acids, classified according to their polarity, are shown in figure 1.6.

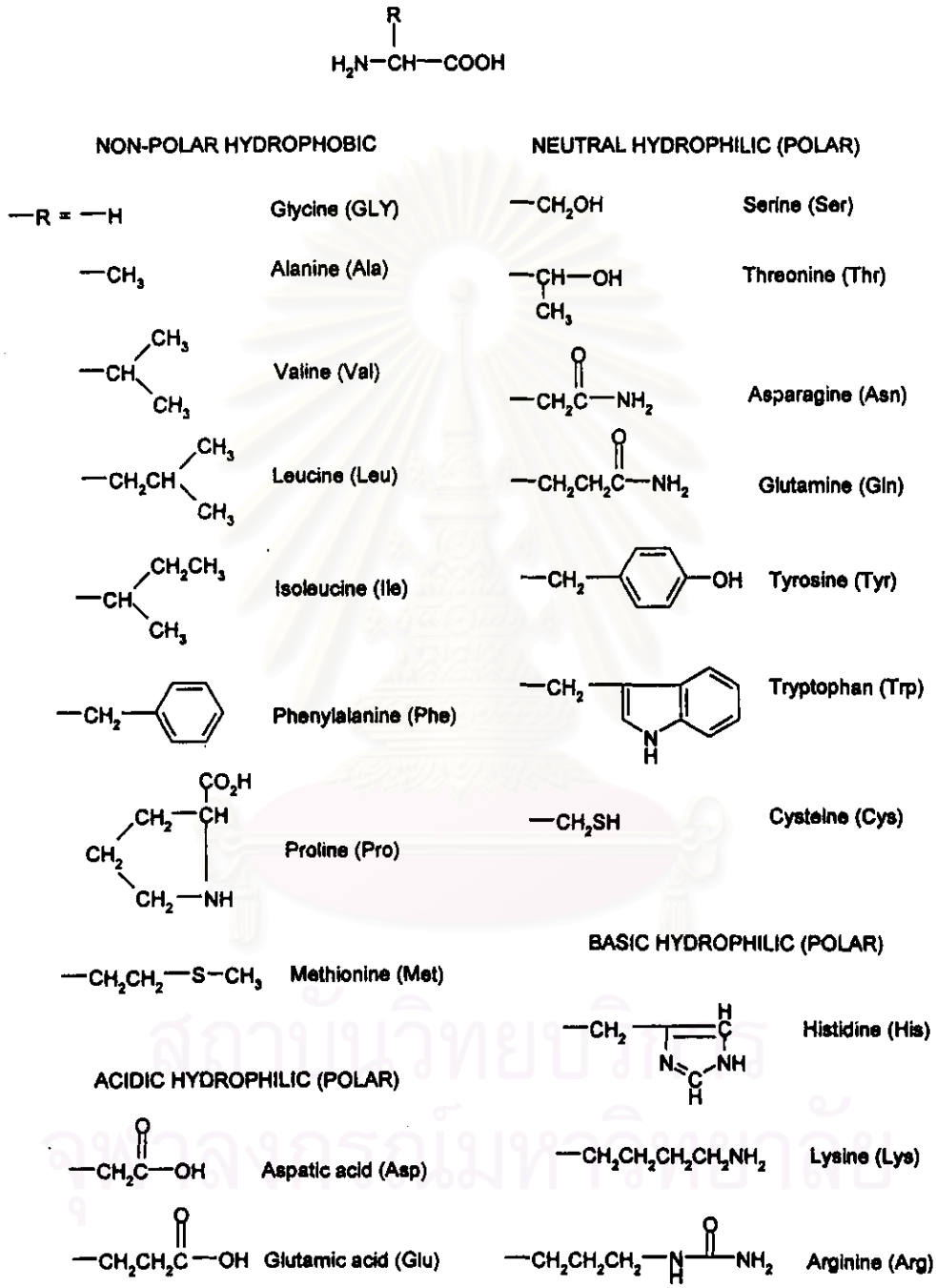

Figure 1.6 A classification of side chains (R-groups) for the 20 naturally occurring L-α-amino acids [44].

Protein structure can be categorized into four levels, i.e. primary, secondary, tertiary and quarternary structures [47]. The *primary structure* that determines is the native conformations are the sequence of amino acids in a given protein. *The secondary structure* is defined by the local conformation of the protein backbone that has the repeating pattern of polypeptide chain and stabilized by hydrogen bonding. In other words, the secondary structure means steric relationship between the neighboring amino acids in primary structure and various conformations of each part of peptide chain which fold, coil or pleat in that peptide. The most important secondary structures are *α-helix* and *β-pleated sheet*. The α-helix is a compact rod like structure with 3.6 amino acids per turn and the distance between the helix is 5.4 Å. α-helix is stabilized by the hydrogen bond between CO of residue *i* and NH of residue *i+4*.

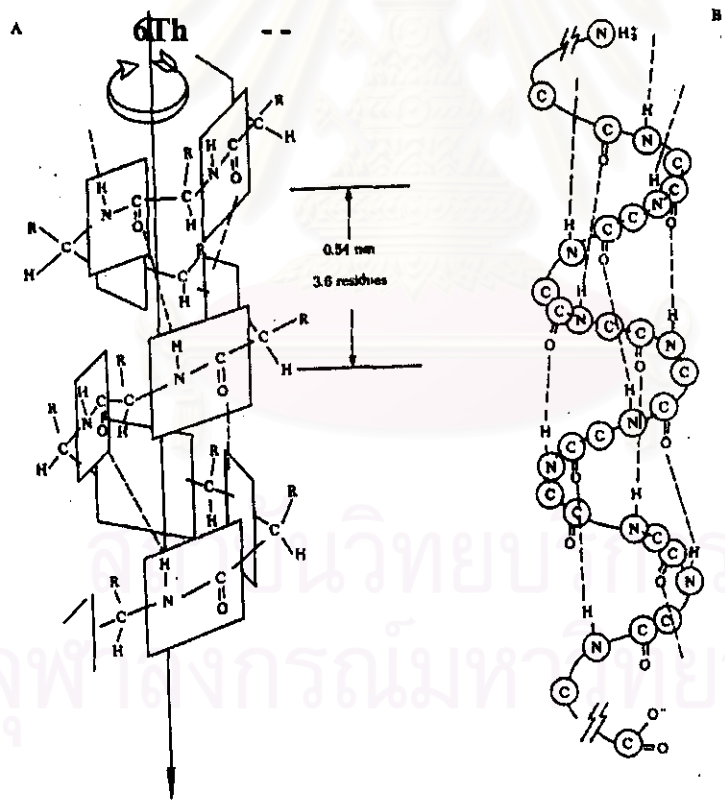

Figure 1.7. Representation of the α-helical structure of proteins [47].

A. Scheme illustrating the relative arrangement of planar peptide units.

B. Intrahelical hydrogen bonds formed between amide hydrogens and carbonyl oxygens.

The β-pleated sheets are the peptide chains run together. There are hydrogen bonds between CO of one chain and NH of another chain. If two peptide chains run in the same direction, these will be called *parallel β-pleated sheet*. On the other hands, they will be called *anti-parallel β-pleated sheet* if two peptide chains run in opposite direction.

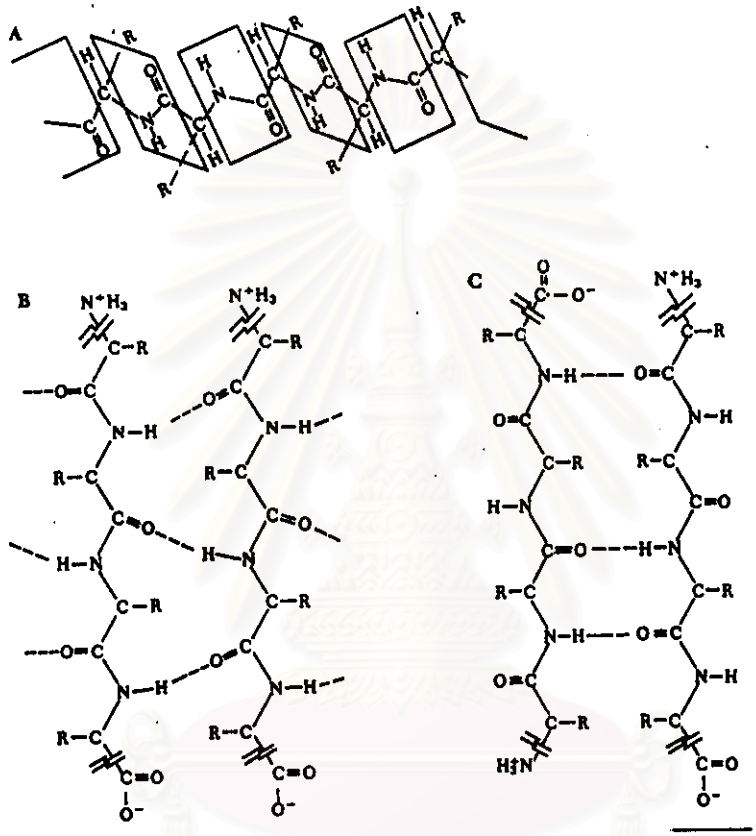

Figure 1.8 Some aspects of the β-pleated sheet or the β-conformation [47].

A. Alternating relationship between planar peptide units.

B. Parallel arrangement of polypeptide chains stabilized by hydrogen bonding.

C. Antiparallel arrangement of polypeptide chains stabilized by hydrogen bonding.

The tertiary structure is given by the steric relationship between the amino acid of peptide chains that are in long distance. And it also refers to the conformation of all chain of peptide that composes of various parts of secondary structure in it. From the X-ray diffraction studies, one can classified tertiary structure into 5 types: i) all α-protein that is the protein with only α-helix; ii) all β-protein that is the protein with only β pleated sheet; iii) α+β -Protein that is the protein with both α-helix and β pleated sheet which are in the long distance; iv) α/β-Protein that is the protein with alternated contains both α-helix and β pleated sheet; v) coil protein that is a small protein with a small amount of α-helix and β pleated sheet but a lot of disulfide bonds or metal ions to stabilize the structure. As mentioned earlier, the tertiary structure of most enzymes are in globular form.

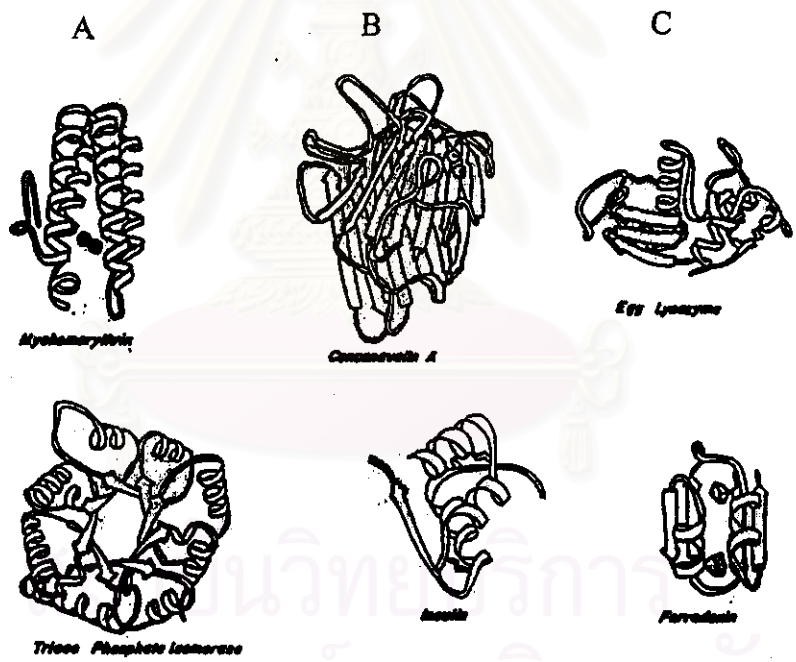

Figure 1.9 The tertiary structure of protein [47].

A all α-protein B all β-protein C α+β protein

D α/β protein E coiled protein with disulfide

F coiled protein with metal

The *quarternary structure* is the complete three-dimensional structure, including the interaction between the component polypeptide chains.

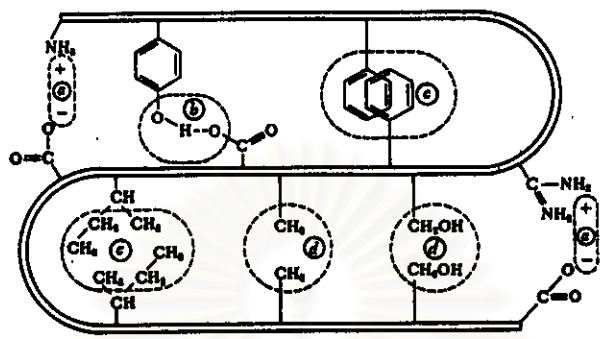

Figure 1.10 Some noncovalent bonds that stabilize proteins. a, Electrostatic interaction; b, hydrogen bonding between tyrosine residues and carboxylate groups; c, hydrophobic interaction; d, Van derWaals interactions.

1.4 Structural studies of protein

The biological activity of proteins depends on the three-dimensional structure of globular protein and is due to the arrangement of its crucial side-chains, prosthetic groups or cofactors in a way to make the protein function [49]. Most proteins work at ambient temperature. Thus creates internal mobility for protein, which is possibly important for the protein function. Generally, some parts of protein are directly relevant for protein function so called *active site*, which is usually located in the cavity of the protein. Many proteins undergo significant structural changes when performing their function. Thus, the mobility may be necessary for proteins so that it is adaptable to target molecules.

There are several experimental methods for studying the structure of protein. These techniques that can provide information about internal mobility of proteins include fluorescence spectroscopy, hydrogen exchange measurements by infrared or tracer techniques, EPR spin label studies, etc. The most important methods nowadays are X-ray diffraction and Nuclear Magnetic Resonance. Both methods have their strengths and

weaknesses, so they will undoubtedly coexist as complementary methods in the foreseeable future.

1.4.1 X-ray diffraction

The basic elements of the dynamics of proteins are atomic fluctuations. The most detailed data are provided by temperature factors obtained in crystallographic refinements of X-ray structure [46]. *The single-crystal x-ray crystallography* is the method to obtain a detailed picture of a large molecule such as protein by interpreting the diffraction of x-rays from many identical molecules in an ordered array like a crystal. This method determines the structure of a protein by x-ray crystallography entails growing high-quality crystals of the purified protein. Measuring the directions and intensities of x-ray beams diffracted from the crystal, and using the computer to simulate the effects of an objective lens and thus produce an image of the crystal's contents. Finally, interpreting the image by entails displaying using computer graphics and then building the molecular model that is consistent with the image.

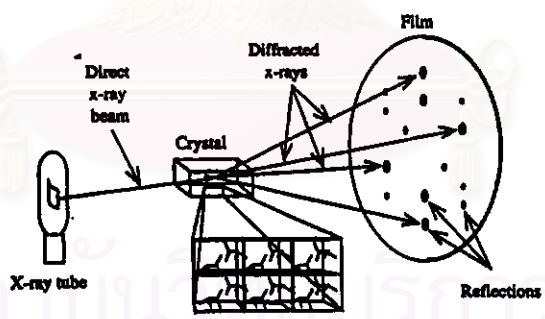

Figure 1.11 Crystallographic data collection. The crystal diffracts the source beam into many distance beams, each of which produces a distinct spot (reflection) on the film. The positions and intensities of these reflections contain the information needed to determine molecular structures [46].

1.4.2 Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) is particularly a useful method for studying of protein mobility [49]. NMR provides a model of the protein in solution, rather than in the crystalline state. The method in brief is that determining the structure of proteins by using various NMR techniques, refining the structure using NOE intensities and chemical shifts then computing the distance geometry and restrained molecular dynamics. This method is unique as the only method that determines the three-dimensional structures of proteins in solutions. It is fully complementary to X-ray crystallography, but also provides direct and site-specific information on protein dynamics and provides a direct link to chemical properties that relates to function.

1.4.3 Computer-Aided Molecular Modeling

Studying the conformational fluctuation using the experiment has many disadvantages [49]. For examples, the X-ray crystallography provides structural information for the system. From crystal structure, an image of biomolecules with fix atomic position is obtained, we can neither emphasize the parts of a molecule nor we can see evidence of motion or disorder in a crystal structure. However, this detail description of motional phenomena can be obtained from the computer simulations of protein. Still there are limitations in experimental approaches to biomolecular dynamics as to the information obtained from them, for instance, if we concern with the time scale of motions, the frequency spectrum covered by NMR is incomplete. So we may rationalize the motional models with an inaccurate data.

Computer simulations have been brought to fulfil that incomplete point. The most important thing is to change the static view of the biomolecular structure to a dynamic picture. Molecular dynamics (MD) is one of the most useful computer simulation techniques today. MD allows studying the dynamics of large macromolecules, including biological systems such as proteins, nucleic acids (DNA, RNA), membranes. Dynamical events may play a key role in controlling processes, which affect functional properties of

the biomolecule. The basic principle of the computer simulation will be presented again in Chapter 3.