CHAPTER II
GENERAL PRINCIPLES AND LITERATURE REVIEWS

2.1 General and nature of myoglobin

Myoglobin was a small, water-soluble globular protein which served as an intercellular oxygen storage site (Torrens, Castellano, Campos, & Abad, 2009). It is present in vertebrates in red skeletal and cardiac muscle and has long been thought to contribute to oxygenation of tissues (Nichols & Weber, 1989). The first three-dimensional myoglobin structure was solved, initially by Kendrew and coworkers in 1958 (Kendrew et al., 1958). Myoglobin has a molecular weight of about 18 kDa and consists of a single polypeptide chain of about 153 amino acids residues non-covalently enfolded around a heme group. Heme group is protoporphyrin with an iron atom at its centre, is situated in a pocket of original structure, which is estimated to line mainly by non-polar amino acid residues (Marengo, 2006). The molecular interactions between the heme and its globins are very complex. The central iron atom is covalently linked to proximal histidine. On the other side of the heme, the distal histidine residue is one of the important residues in the control of ligand entry. Although, the heme group is firmly buried in the protein matrix, it can be detached under denaturating condition (Katta & Chait, 1991).

Primary structures (amino acid sequences) of myoglobin from various species have also been reported, which are useful for structural comparison. According to molecular structure of myoglobin, it exhibited spectral characteristics such as the Soret absorption peak at 408 nm (Chauvet & Archer, 1972; Jiang, lting, Stayton & Sligar, 1996).

The heme iron in myoglobin reversibly binds small ligands such as dioxygen (O_2) and carbon monoxide (CO) (Nienhaus et al., 2002). Myoglobin could be existed in different states depending on the oxidation state of the heme (Hsu & Woody, 1969; Vojtechovský, Chu, Berendzen, Sweet, & Schlichting, 1999). The ligand-binding process in myoglobin has also been studied by various techniques, indicating that ligand binding was not a simple process consists of a series of sequential reactions as

the ligand moves through the protein to the binding site at the heme iron (Elber & Karplus, 1987; Antonini & Brunorl, 1991; Vojtechovsky et al., 1999). Furthermore, changes in the ligand binding to the heme group produced substantial changed in its absorption spectrum (Zhao et al., 1995). The oxidative state of myoglobin dictated the expressed color of muscle (Hunt et al., 2004).

The states of myoglobin are oxymyoglobin, deoxymyoglobin, and metmyoglobin (Mancini & Hunt, 2005). Different forms of myoglobin produce different colors, resulting in purple (deoxymyoglobin), bright red (oxymyoglobin), cherry red (carboxymyoglobin) and brown (metmyoglobin) (Hunt et al., 2004; Mancini & Hunt., 2005; Matheu, Merkx, & Marc, 2005). The previous works were of general interest for more understanding of relationship between myoglobin and meat color (Hunt et al., 2004; Mancini & Hunt, 2005).

Studies on the structural and functional aspects of myoglobin from various animal species have been reported. Robert Olek et al. reported that myoglobin was found in cardiac and red skeletal muscles and particularly abundant in diving mammals. These studies were also done with myoglobins from human (Jeffreys et al., 1984), bovine (Chen, Huffman, Egbert, & Smith, 1992), whale (Zhao et al., 1995), water buffalo (Dosi et al., 2006), elephant (Bartnicki, Mizukami, & Romero-Herrera, 1983) and etc. Myoglobin from fish muscle was also interesting to study, because its genetic evolution was different from that of mammalian muscle (Zare, 2004). Many reports confirmed that the fish myoglobin was different from mammalian myoglobin (Madden et al., 2004). For instance, amino acid compositions of fish myoglobin contained a cystein residue which is not present in mammalian species (Brown, Martinez, Johnstone, & Olcott, 1962). In addition, Nichols et al. also reported that fish myoglobins were more readily oxidized than mammalian myoglobins. Other studies also showed that the differences among those myoglobins observed were with thermal denaturation profile and autooxidation rate (Chanthai, Neida, Ogawa, Tamiya, & Tsuchiya, 1996; Chow, 1999).

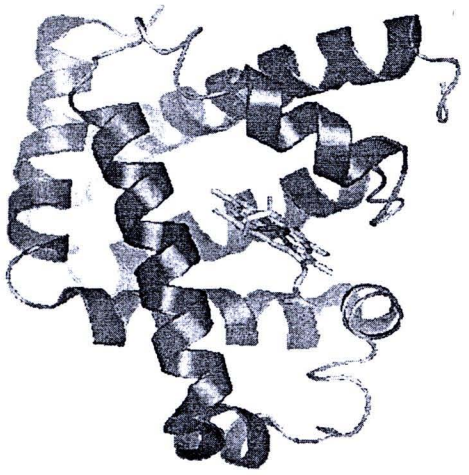

Figure 2.1 Three dimensional structure of sperm whale myoglobin consists of single polypeptide chain and heme prosthetic group

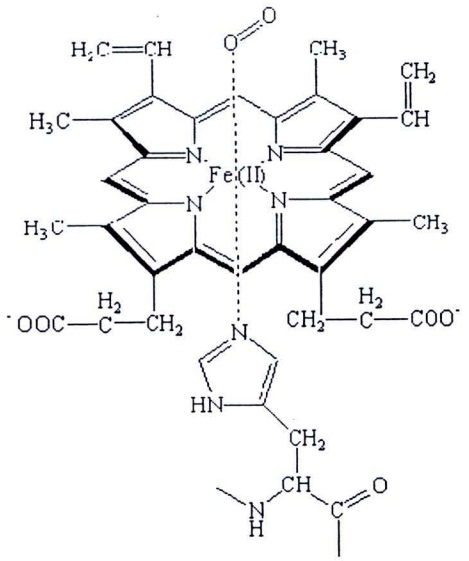

Figure 2.2 The heme prosthetic group of myoglobin consists of propylinling and iron(II) atom connecting to oxygen molecule and histidine.

2.2 Isolation and purification of myoglobin

Myoglobin from various sources showed different structural properties. The isolation and purification of myoglobin obtained by different procedures have been previously described. The purified horse heart myoglobin has succeeded in preparing by Theorell's procedure (Bowen, 1948). Several works have been reported many processes of isolating and purifying myoglobin from different animals by modification of there all's procedure (Fridovich, 1962).

Commonly, these procedures were generally derived from extraction either with water or buffer and salting out with ammonium sulfate or organic solvent (Fridovich, 1962; Jeffreys et al., 1984; Bizzarri & Cannistraro, 1993). Based on the complexity of the extracted solution containing crude myoglobin, further purification processes were also needed such as gel filtration column chromatography (Ueki, Chaujen, & Ochiai, 2005; Chaijan, Benjakul, Visessanguan, & Faustman, 2007), ion-exchange column chromatography (Brown, 1996) and affinity column chromatography (Torrens et al., 2009).

Depending on different matrix factors in muscle, Chaijan et al. (2007) reported that purification of yellowfin tuna myoglobin was performed as follows; myoglobin extracted with cold Tris-HCl buffer (pH 8.0) and applied to a Sephacryl S-200HR column (100×2.5 cm, i.d.), eluting with the same buffer. As previously reported, other species of fish myoglobins were extensively purified by Sephadex G-75 (Chaijan et al., 2005), Sephadex G-100 (O'Brien et al., 1992) gel filtration chromatography and also by ion exchange chromatography with DEAE cellulose (Chaijan et al., 2005) and carboxymethyl cellulose column (Brown, 1961).

Likewise, myoglobin in muscles from various mammals such as bovine (Chen et al., 1992), elephant (Bartnicki et al., 1983) and penguin (Ponganis, Starke, Horning, & Kooyman, 1999) was investigated. For instance, Emperor penguin myoglobin could be isolated and purified by precipitating with ammonium sulfate precipitation. The myoglobin solution was accomplished by Sephadex G-200 column and finally purified by ion-exchange chromatography on DEAE column (Tamburrini et al., 1999). Also, the Sephacryl S-200 gel filtration column has been reported to purify myoglobin from bovine muscle (Chen et al., 1992).

Ultrafiltration was one of a technique used to prepare myoglobin from any muscles (Frost & Zydney, 2004). Sannier et al. reported that inorganic ultrafiltration membranes with molecular weight cutoff 30 and 150 kDa could be used to separate myoglobin from yellowfin tuna red muscles (Sannier, Lecoeur, Zhao, Garreau, & Piot, 1996). In 1992, myoglobin from bird species was separated at alkaline pH by ammonium sulfate extraction followed by ultrafiltration (O'Brien et al., 1992). The myoglobin was extracted from human psoas muscle or myochardium and then purified by preparative flat bed isoelectric focusing in a granulated gel (Samaan, Caudie, & Quincy, 2005).

In addition, the use of centrifugal partition chromatography was proposed to purify myoglobin by using aqueous two phase system as eluent (Sutherland et al., 2008). This technique was successfully used to purify myoglobin in high concentration without precipitation. For the determination of myoglobin purity, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was generally used for preliminary testing (Chaijan, Benjakul, Visessanguan, & Faustman, 2005).

2.3 Identification and characterization of myoglobin

Previous reports on some properties of myoglobin from any animals mostly involved their molecular weight, isoelectric point, spectral properties, autoxidation rate, thermal stability and amino acid compositions. In 2001, Wen-Lee Chen and co-workers studied on the physico-chemical properties of milkfish myoglobin in terms of pigment content, autoxidation rate constant and free energy of unfolding myoglobin (Wen-Lee & Chau-Jen, 2001). Characterization techniques of myoglobin such as polyacrylamide gel electrophoresis (Chen et al., 1992; Zhao et al., 1995), capillary electrophoresis (CE) (Ebbing, Jeremy, & Charles 2004), UV-Visible spectrophotometry, spectrofluorophotometry (Zhou et al., 2007), isoelectric focusing (Wu et al., 1989) and MS/MS (Ponce-Alquicira &Taylor, 2000) were widely used to study myoglobin properties. Wu et al. reported the characterization of myoglobin from sheep heart by PAGE, isoelectric focusing and by use of a 4-30% SDS-polyacrylamide gradient gel (Wu et al, 1989). MS/MS methodology was developed and applied both to define the molecular weight (Stewart et al., 2004) and amino acid

sequences (O'Brien et al., 1992). In 2000, myoglobins from different meat species analyzed by electrospray mass spectrometry (ESI-MS) have been reported (Ponce-Alquicira & Taylor, 2000). Nobuhiko et al. reported that the matrix assisted laser desorption ionization spectrometry (MALDI) could be used to analyze the molecular mass and primary structure of bullet tuna myoglobin (Ueki, Chow, & Ochiai, 2005). Liquid chromatography coupled with high resolution and accuracy techniques such as LC-SF-ICPMS (Harrington, Elahi, Merson, & Ponnampalavanar 2004) and LC-MS/MS (Stewart et al., 2004) was also used to identify myoglobins from various muscles. In addition, flow injection technique was developed for identification of surface activity of myoglobin (Bramanti et al., 2006).

2.4 Asian swamp eel (*Monopterus albus*)

Asian swamp eels (order Synbranchiformes) are not true eels (order Anguilliformes). This fish species are native to Asia, from Northern India and Burma to China. They were probably introduced to North America by aquarium release, stocking as a food source, or escaped from fish farms during flooding events (Siang et al., 2007). Swamp eels are often found standing vertically on the bottom part of its elongated body while stretching toward the surface of the water with its head. This is because like some other fishes of the purely tropical or subtropical fishes they can breathe atmospheric oxygen. The swamp eel fish supplementary respiratory organ is a gill sack that can be seen clearly as it stretches to breathe.

Swamp eels are highly adaptable fish which are preferred environmentally includes a wide variety of freshwater habitats: shallow wetlands, stagnant waters, marshes, streams, rivers, ditches, canals, lakes, reservoirs, ponds, mud, underneath a dried up pond. Most of fish species can breathe air, allowing them to survive in deoxygenated water for long periods (Siang et al., 2007). Moreover, swamp eels appeared to tolerate cold temperatures well (successfully established in areas where temperatures fall below freezing) and were able to tolerate wide range of water oxygen levels.

2.5 Fundamental methods for isolation and purification of myoglobin

Myoglobin from various sources showed different structures and properties. Therefore, the isolation and purification of myoglobin by the different procedures have been previously described. Firstly, red muscle was extracted with cool water or buffer and then followed by isolation of myoglobin, and remove of interfering species and other protein.

2.5.1 Protein precipitation

Precipitation is widely used for product recovery of biomolecule especially proteins. The method is usually induced by addition of salt or organic solvent or by changing the pH to alter the nature of the solution. The most common type of precipitation for proteins is salt induced precipitation (salting out). Protein molecules contain hydrophobic amino acids and hydrophilic amino acids. After protein folding in aqueous solution, hydrophobic amino acids usually form protected hydrophobic areas while hydrophilic amino acids interacted with the molecules of solvation and allow proteins to form hydrogen bonds with the surrounding water molecules. Hydrophilic protein surface are enough, the protein can be dissolved in water. When the salt concentration is increased, some of the water molecules are attracted by the salt ions, which decreases the number of water molecules available to interact with the charged part of the protein. As a result of the increased demand for solvent molecules, the protein-protein interactions are stronger than the solvent-solute interactions; the protein molecules coagulate by forming hydrophobic interactions with each other. This process is known as salting out. Ammonium sulfate was the most common salt used for this purpose because of high solubility and strong ionic strength and solubility changed little with temperature. It is cheap and the density of even a concentrated solution is less than that of protein could be centrifuged down from the concentrated solution. Therefore, differential ammonium sulfate precipitation provides a pre-purification step for proteins and is one of the classification method used for protein isolation.

2.5.2 Protein desalting

After protein precipitation step with salt, the protein must be presented in a high salt environment. The salt could be removed from protein extract by dialysis bag (dialysis tubing cellulose membrane). Dialysis is a common laboratory technique, and operates on the same principle as medical dialysis. Typically a solution of several types of molecules is placed into a semipermeable dialysis bag, such as a cellulose membrane with pores, and the bag is sealed. The sealed dialysis bag is placed in a container of a different solution, or pure water. Suitable size molecules (smaller than pore size of dialysis membrane) can passed through the tubing (often water, salts and other small molecules) tend to move into or out of the dialysis bag, in the direction of decreasing concentration. Larger molecules (often proteins, DNA, or polysaccharides) that have dimensions significantly greater than the pore diameter are retained inside the dialysis bag. One common reason for using this technique would be to remove the salt from a protein solution.

2.5.3 Column chromatography

Column chromatography is one of the most common methods of protein purification. Like many of the techniques on this site, it is as much an art form as a science. Proteins vary hugely in their properties, and the different types of column chromatography allow you to exploit those differences. Most of these methods do not require the denaturing of proteins. To be very general, a protein is passed through a column that is designed to trap or slow up the passing of proteins based on particular properties (such as size, charge, or composition).

2.5.3.1 Gel filtration (size exclusion) chromatography

Gel filtration or size-exclusion chromatography (SEC) is a popular method to separate biomolecules based on their size. The column is packed with a matrix of fine porous beads. The beads have very small holes. As the protein solution passed to the column, small molecules enter the pores beads. Larger molecules are excluded from the holes, and pass quickly between the beads. These larger molecules are eluted first. The smaller molecules have a longer path to travel, as they get stuck over and over again in the maze of pores running from bead to bead. Therefore, these

smaller molecules take longer to make their way through the column and are eluted last.

The process for separation protein on gel filtration chromatography, primarily, it is applied to the separation of biopolymers such as proteins and nucleic acids, i.e. water-soluble polymers. This system is also called gel filtration, typically with beads of dextran or agarose serving as gel matrix. Smaller molecules pass significantly slower through the column than larger molecules. SEC does not require electric current and the sieving effect will not separate small molecules first.

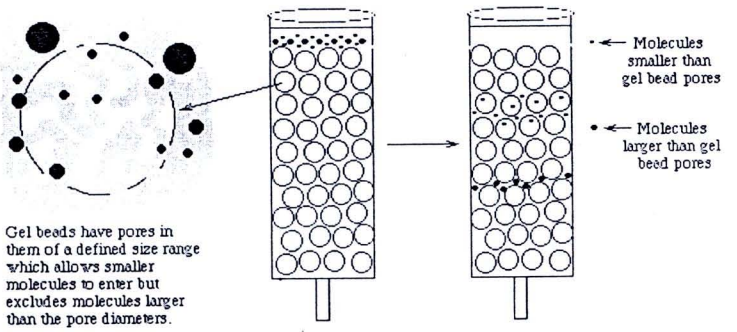

Figure 2.3 The separation process of protein mixture on gel filtration chromatography.

2.5.3.2 Ion exchange chromatography

Ion exchange chromatography relies on charge-charge interactions between the proteins in your sample and the charges immobilized on the resin (stationary phase) of your choice. Ion exchange chromatography can be subdivided into cation exchange chromatography, in which positively charged ions bind to a negatively charged resin; and anion exchange chromatography, in which the binding ions are negative, and the immobilized functional group is positive. Once the solutes are bound, the column is washed to equilibrate it in your starting buffer, which should be of low ionic strength, and then the bound molecules are eluted off using a gradient of a second buffer which steadily increases the ionic strength of the eluent solution. Alternatively, the pH of the eluent buffer can be modified as to give your protein or the matrix a charge at which they will not be interacted and interesting molecule elutes from the resin.

2.5.5 Ultrafiltration

Ultrafiltration is a variety of membrane filtration in which hydrostatic pressure forces a liquid against a semipermeable membrane. Ultrafiltration is used for separating dissolved molecules in solution on the basis of size which means that molecules larger than the membrane pore size rating will be retained at the surface of the membrane. This separation process is used in industry and research for purifying and concentrating macromolecular solutions, especially protein solutions.

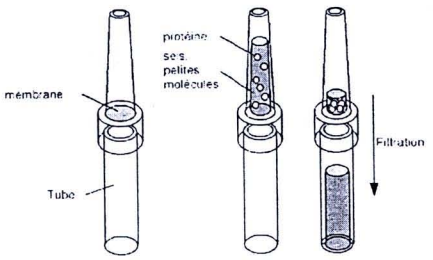

Figure 2.4 The separation and concentration of protein on ultrafiltration unit

2.6 Fundamental techniques for identification and characterization of myoglobin

2.6.1 Gel electrophoresis of protein

Gel electrophoresis can provide information about the molecular weight of proteins, the subunit structures of proteins, and the purity of a particular protein preparation. It is relatively simple to use and highly reproducible. The most common use of gel electrophoresis is the qualitative analysis of complex mixture of proteins.

In gel electrophoresis, proteins are separated on the basis of charge, and the charge of a protein can be either negative (-) or positive (+) charge, depending upon the pH of the buffer. In normal operation, a plat of gel is partitioned into two sections, known as the separating or running gel and the stacking gel. Electrodes are attached to the ends of the gel and an electric current passed through the partitioned gels. If the electrodes are arranged in such a way that the upper bath is cathode (-), while the lower bath is anode (+), anions are allowed to flow toward the anode, the system is known as an anionic system. Flow in the opposite direction, with cation flowing to the cathode is a cationic system.

The support medium for electrophoresis can be formed into a gel within a tube or it can be layered into flat sheets. The tubes are used for easy one dimensional separation, while the sheets have a larger surface area and are better for two-dimensional separations.

In sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), proteins are treated with sodium dodecyl sulfate (SDS) before electrophoresis so that the charge density of all proteins is made roughly equal. When these samples are electrophoresed, proteins are separated according to mass. SDS-PAGE allows estimation of the molecular weight and composition of protein. SDS-PAGE is also used for routine separation and analysis of proteins because of it is rapid, simplicity and resolving capability.

Non denatured polyacrylamide gel electrophoresis (native-PAGE) is probably the most highly resolving electrophoretic method yet developed for separating proteins. In electrophoresis, proteins are separated by mass and charge-density. In gel electrophoresis, the support media (acrylamide gel) also contributes to the separation power of the method. Using the organic monomer, acrylamide, to make the gel by free radical polymerization results in very uniform pore sizes which can be reproduced each time a gel is poured without a lot of variation. This method of gel electrophoresis allows one to separate native proteins according to difference in their mass and charge density. The buffer in the gel is suitable for maintaining the protein in its native state. Thus, their enzymes activity can be assayed after the electrophoretic separation.

2.6.2 Isoelectric focusing

The protein is amphoteric molecules called zwitterions that contain both positive and negative charges depending on the functional groups present in the molecule. The net charge on the molecule is affected by pH of their surrounding environment and can become more positively or negatively charged due to the loss or gain of protons (H^+).

Isoelectric focusing is an electrophoretic method in which proteins are separated on the basis of their isoelectric point (pI). It makes use of the property of proteins that their net charges are determined by the pH of their local environments.

Proteins carry positive, negative, or zero net electrical charge, depending on the pH of their environmental surrounding. Protein are positively charged in solution at pH values below their pI and negatively charged above their isoelectric points. Thus, at pH values below the pI of a particular protein, it will migrate toward the cathode during electrophoresis. At pH above its pI, a protein will move toward the anode. A protein at its isoelectric point will not move in an electric field.

When a protein is placed in medium with a linear pH gradient and subjected to an electric field, it will initially move toward the electrode with the opposite charge. During migration through the pH gradient, the protein will either pick up or lose protons. As it does, its net charge and mobility will decrease and the protein will slow down. Eventually, the protein will arrive at the point in the pH gradient equaling its pI. There, being uncharged, it will stop migrating. If a protein at its pI should happen to diffuse to a region of lower pH, it will become protonated and be forced toward the cathode by the electric field. If, on the other hand, it diffuses into a pH higher than its pI, the proteins condense, or focus, into sharp bands in the pH gradient at their individual, characteristic pI values.

2.6.3 Matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS)

Matrix assisted laser desorption/ionization-time of flight mass spectrometry is a soft ionization technique (MALDI-TOF-MS) used in mass spectrometry, allowing the analysis of biomolecules and macromolecules such as protein, polymer and large organic molecules. MALDI-TOF-MS is used for determine molecular weight and peptide mass fingerprint of proteomics. In this mass spectrometric method, a solid matrix is used for absorb light of the laser beam. The sample is mixed with a matrix solution and allowed to co-crystallize on a target plate. When the target is fired with the laser, the matrix absorbed the laser light energy which vaporizes of matrix (matrix desorbs from the surface) and this carry some of the sample with it. At the time that the laser is pulsed a voltage is applied to the target plate to accelerate the ionized sample towards a time-of-flight mass analyzer for analysis of protein molecular weight and peptide mass fingerprint.

2.6.4 UV-Visible spectrophotometry

The light absorption in the visible (about 400 to 800 nm) and ultraviolet (about 180 to 400 nm) regions of the spectrum results from transition between electronic states by simultaneous change in vibrational and rotational states. Absorption of radiation in this region by organic molecule are either participated directly in bond formation or localized about such atoms as oxygen, sulfur, nitrogen and the halogens. Electrons involved in double and triple bond of organic molecule such as aromatic molecules lager the region of the molecules over which π-electron conjunction can occurs, the longer than wave length of the absorption and the more intense that resulting absorption band. Thus, species with unsaturated bonds generally exhibit useful absorption peaks. The unsaturated organic functional groups that can absorb in the ultraviolet or visible regions are known as chromophore (Skoog et al., 2004).

In proteins, three classes of chromophores are found, the peptide bond, amino acid side chains, and prosthetic groups. First, the peptide bond, the smallest of the three chromophores has the absorption maximum at 190 nm. Second, side chains of the residues are given the electronic absorption. The absorption is caused by delocalized electrons.

And finally, a typical prosthetic transition group represent as protoheme IX. The electronic transition of this molecule comes from the porphyrins, the iron atom and the axial ligands. Three types of transition are important: (i) porphyrin π-π^* transitions are a very intense band near 400 nm called the Soret band and two band near 550 nm, (ii) ligand field transitions, these transitions occur between molecular orbitals that are mainly localized on the central iron atom, and (iii) charge transfer transitions, these are transitions between orbitals mainly localized on the iron and orbitals localized on the ligands (heme). Ligand to metal and metal to ligand transitions are distinguished depending on whether the excitation lifts an electron out of a heme orbital into orbital ort vice versa (Makinen, 1979)

2.6.5 Fluorescence spectroscopy

Fluorescence spectroscopy is one of electromagnetic spectroscopy. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light of a lower energy. Fluorescence is the phenomenon in which adsorption of light of given molecule is followed by the emission of light at longer wavelengths. The distribution of wavelength-dependent intensities that cause fluorescence is known as the fluorescence excitation spectrum and the distribution of wavelength-dependent intensities of emitted energy is known as the fluorescence emission spectrum. For intrinsic fluorescence of protein and peptides, proteins contain aromatic amino acid residues (tryptophan, tyrosine and phenylalanine) which may contribute to their intrinsic fluorescence. Cofactors such as porphyrin also exhibit fluorescence. The fluorescence of folded protein is a mixture of the fluorescence from individual aromatic residues. Protein fluorescence is generally excited at 280 nm or at longer wavelengths, usually at 295 nm. Most of the emissions are due to excitation of tryptophan residues, with a few emissions due to tyrosine and phenylalanine. Chang in intrinsic fluorescence can be used to monitor structural change in protein.

2.6.6 Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

Liquid chromatography-mass spectrometry (LC-MS, or alternatively HPLC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. LC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. Generally its application is oriented towards the specific detection and potential identification of chemicals in the presence of other chemicals (in a complex mixture).

Liquid chromatography-mass spectrometry (LC-MS) is a powerful technique used for many applications in proteomics studied. In this technique, a liquid chromatography is used to separate different compounds. The stream of separated compound is fed on-line into the electrospray ion source, a voltage of metallic filament is applied. This filament emits electrons which ionize the compounds. The

ions can then further fragment, yielding predictable patterns. The fragment ions pass into the mass spectrometer analyzers and are eventually detected. To derive sequence of individual peptides, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was used. This powerful technique can be used for sequencing a large number of peptide and protein identification with protease digestion.

2.6.7 Extinction coefficient

Absorption spectroscopy is a common method for finding the concentration of proteins or protein complexes in a solution. Proteins absorb light at specific wavelengths can be defined by the equation $A = \log (I_0/I)$. This equation states that an absorbance at a specific wavelength (A) is equal to the log of the ratio of incident light intensity (I_0) to transmitted light intensity (I). This can be better understood when looking at equation below. This absorbance (A) is directly proportional to the concentration of the protein in solution. To identify this concentration an equation known as the Beer-Lambert Law is used.

$$A = \varepsilon bc$$

In this equation "A" is the absorbance for of the sample measured. The molar extinction coefficient (ε) is a constant used to define the absorbance of the protein. If the extinction coefficient of protein is known, it can be used to determine the concentration of a protein in solution. The path length (b) is the thickness of the sample and is usually standardized at 1 cm. The concentration of the protein in the sample is denoted as "c" (Williamson, 2003).

2.6.8 Autoxidation rate of myoglobin

Myoglobin (Mb) is present in vertebrates in red skeletal and cardiac muscle and has long been thought to contribute to oxygenation of tissues. This its faction acting as the O_2 buffer to counteract the fluctuating needs of O_2 in muscle. Autoxidation rate of myoglobin was studied on stability as a function of oxygen supply. Among these, stability properties are of particular importance in vivo. Like

all known dioxygen carriers synthesized so far with transition metals, the oxygenated forms of oxymyoglobin (MbO_2) is oxidized easily to their ferric met forms (metmyoglobin or metMb), which cannot be oxygenated and are therefore physiologically inactive. The autoxidation reaction is mainly dependent on pH, temperature and salt concentration (Chanthai et al., 1998). The rate-determining step of the autoxidation reaction in aqueous solution is first order. This process was followed by a plot of experimental data as $\ln([MbO_2]_t/[MbO_2]_0)$ vs. time t, where the ratio of MbO_2 concentration after time t to that at initial time t=0) can be monitored by the absorbance change, for instance, at α-peak of the protein (581nm in the case of bovine heart MbO_2). From the slope of each straight line, the observed first-order rate constant, k_{obs} in h^{-1}, was determined (Shikama, 1998). The lower k_{obs} value was refer to myoglobin higher oxygen affinity than higher k_{obs} value.