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Protein Purification

Nison Sattayasai
Khon Kaen University
Thailand

1. Introduction

There are many thousand kinds of proteins with different properties and functions in a cell. To study a protein, one has to obtain the highly purified intact form of the protein. A major protein is not so difficult to be purified while a minor one may need many purification steps and high skills on the techniques. This chapter describes the principles underlying techniques for separation and purification of proteins extracted from cells and tissues. The strategy involves extraction and purification.

2. Extraction of protein

Generally both extraction and purification processes should be done under a cold condition, mostly at 0-4oC, except for some proteins. An ice box or another cooling system is normally needed for sample cooling. The extraction procedure varies according to the type of sample and physicochemical properties of the protein. The first step is to disrupt cells or tissues. The more gentle procedure is used the more intact protein is obtained. Most of animal cells and tissues are soft and easy to break, so gentle methods can be applied. For bacteria, fungi and most plant cells which have tough cell envelope, more vigorous procedures are required. Extraction buffer or solvent is also important. Buffer with suitable concentration, ionic strength, and pH is used for extraction of water-soluble protein. In some case, mild detergent or other appropriate dissociating substance is added to the extraction buffer. Protease inhibitors are sometimes necessary to prevent destruction of the extracted proteins by proteases. From the fact that the protein extract obtained from subcellular component has contaminants less than that obtained from whole cell and will be easier to be purified. So separation of subcellular components may be done before the protein extraction. Disruption of cells should be adjusted to get intact sub-cellular components. The required cell component is obtained by centrifugation under appropriate condition.

2.1 Extraction of water-soluble protein from animal cells and tissues

The proteins which are components of fragile unicellular tissues such as animal blood cells can be extracted by using hypotonic buffer solution. If the sample contains different types of cells, separation of the cell types before the extraction will make the purification easier. This osmosis-based method is also used for animal cells grown in culture media. In

some case a mild surfactant is included in the extraction buffer. Sonication or freeze/thaw cycle or a mild mechanical agitation may be used to help disruption of the cells and dissociation of the cellular components. For multicellular soft tissue, a hand homogenizer (Fig. 1) or an electrical one with optimal size is a good choice. The tiny pestle using with a micro-centrifuge tube is also commercially available for the sample with a small volume. Equipments with stronger breaking power such as a blade blender are needed for extraction of proteins from tougher tissue such as animal muscle. Cell disruption by nitrogen decompression is another method for animal cells and some plant cells. In this method, large quantities of nitrogen are dissolved in the cells under high pressure in a vessel. When the pressure is suddenly released, the dissolved nitrogen becomes bubbles. The expanding bubbles cause rupture of the cell. Intact organelles are also obtained by this method.
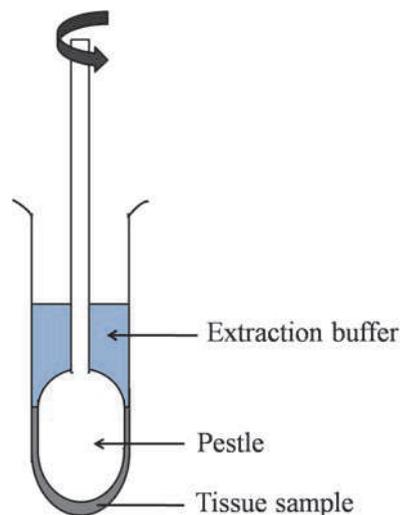

Fig. 1. Homogenization of tissue by a hand homogenizer.

2.2 Extraction of water-soluble protein from unicellular organisms

This group of organisms includes bacteria, yeast, fungi and some algae. Their cell envelopes are tougher than those of animal cells. The stronger methods are needed for disruption of the cells. A simple method is to shake the suspension of cells, as well as spores, with small glass beads or the other kind of beads. If the collision with the beads is done in a blender, the method is called a bead mill. Sonication is another way to break the cells. The solution used to suspend the cells can be only an appropriate buffer, or with the addition of some enzyme and/or mild detergent. The examples of the enzymes are lysozyme, cellulase and chitinase. Nonionic and zwitterionic detergents, especially Triton X-100 and CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate), are commonly used since they are mild and have little effects on protein denaturation. To disrupt the unicellular organisms with very strong envelope, a high-pressure press apparatus may be needed such as French press, Microfluidizer processors. In these systems, the cell suspension in a suitable chamber

is pressurized (up to 30,000 psi) by using a hydraulic pump. Shearing force is generated when the pressurized suspension is squeezed pass a very narrow outlet into the atmospheric pressure (Walker, 2005).

2.3 Extraction of water-soluble protein from plant tissue

Grinding with or without sands, in the presence of an appropriate buffer, by using a pestle and mortar actually works well with plant sample. If the plant tissue is too strong to be ground, rapid freeze with liquid nitrogen will make the plant more fragile. The frozen plant tissue is ground before shaking in the buffer (Fido et al., 2004). Some plant cells can be disrupted by the mean of nitrogen decompression.

2.4 Extraction of lipid-soluble protein

Most of lipid-soluble proteins are membrane proteins. These proteins may be called proteolipids which are extracted from the samples by using organic solvents such as a mixture of chloroform and methanol (Velours et al., 1984). An aqueous solution containing mild detergents such as Triton X-100 and CHAPS is an alternative way to dissolve the proteins from cells or organelles. Strong detergents can be used but they may cause permanent denaturation of the proteins.

2.5 Extraction of aggregated protein

High expression of recombinant proteins in bacterial host cell often form insoluble aggregates which is known as inclusion bodies. This form of proteins is very difficult to be solubilized. To extract any protein from bacterial inclusion bodies, strong denaturants such as 6M guanidine-HCl or 6 to 8M urea are included in the solubilizing solution. This solution efficiently extracts the aggregated protein. However, the extracted protein is also denatured and sometimes can not be renatured (Rudolph & Lilie, 1996). The other procedure is to use a mixture of mild detergents such as a combination of Triton X-100, CHAPS and sarkosyl. This method is less efficiency but more native forms of the protein can be recovered (Tao et al., 2010).
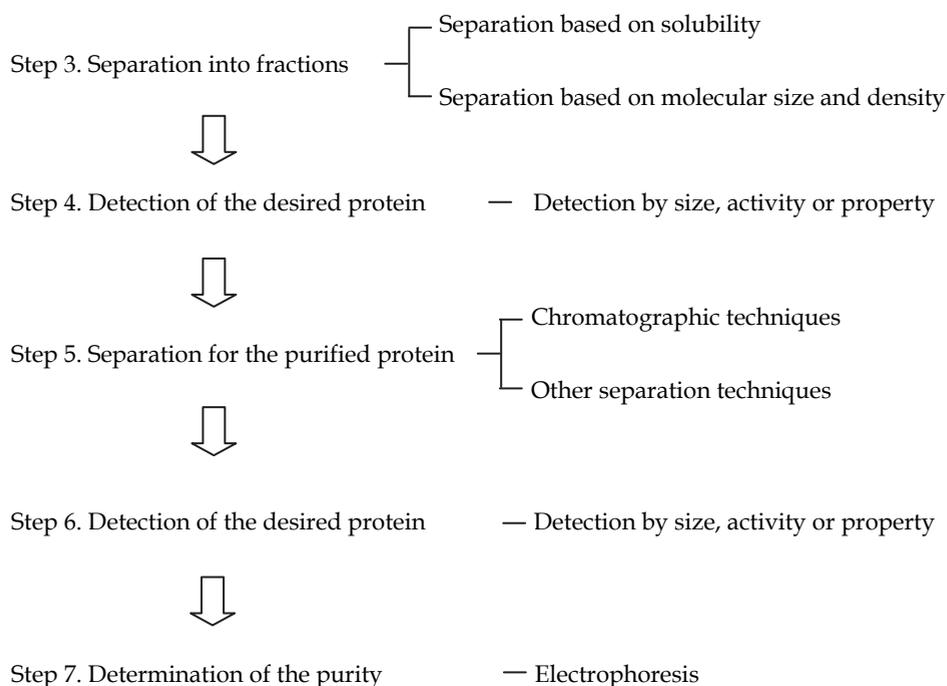
3. Purification of protein

To purify any protein, various separation techniques are used depending on physical and chemical properties of the protein. The purification process can be concluded as follows.

Step 1. Crude extract of protein

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Step 2. Detection of the desired protein   ⸺ Detection by size, activity or property

⇩

Step 3. Separation into fractions ┬── Separation based on solubility

                                   └── Separation based on molecular size and density

Step 4. Detection of the desired protein ── Detection by size, activity or property

Step 5. Separation for the purified protein ┬── Chromatographic techniques

                                             └── Other separation techniques

Step 6. Detection of the desired protein ── Detection by size, activity or property

Step 7. Determination of the purity ── Electrophoresis

It is not necessary to follow all steps or all techniques in the process for every protein. Some step or some technique is not necessary for some protein. For example, myoglobin is easily observed by its red color or detected by using UV-visible spectrometric method, no need to use any sophisticated procedure. Principles of separation techniques are as followings.

3.1 Separation based on solubility

Solubility of hydrophilic protein depends on its charge and hydrogen bonding with water molecule. Net charge of a protein is zero at its isoelectric pH (pI); so the protein molecule is easy to aggregate and then precipitate. The method is called isoelectric precipitation. If the pI is not known, high concentration of some salt can precipitate protein by destroying hydrogen bond between protein and water molecule. The method is called salting out or salt precipitation. Ammonium sulphate is normally used since it has high ionic strength. Proteins in crude extract can be separated into a number of fractions by gradually addition of the salt. The precipitated proteins are redissolved and used for further steps. Combination of the two methods is used for precipitation of some protein.

3.2 Separation based on size and density

The techniques include centrifugation, dialysis and molecular filtration. A high speed centrifugation (10,000-20,000g) is normally used to remove cell debris and large organelles from crude protein extract if the desired protein is a water-soluble molecule. The supernatant is then used for further purification steps. To know the location of the desired

protein in cell, the cell disruption should be a soft procedure to get intact organelles and the other subcellular structures. The subcellular structures including organelles are then fractionated by using differential centrifugation and/or density gradient centrifugation. In differential centrifugation, the subcellular particles are separated according to their sizes into fractions by the stepwise increase of the centrifugal force. Density gradient centrifugation is a procedure to separate particles including protein aggregates by their densities. A liquid density gradient may be pre-formed before use for the separation. Various materials can be used to make the gradients such as sucrose, Ficoll. In addition the liquid density gradient can be formed during the separation. By this way, the sample is layer on top of a suitable concentration of cesium chloride. The strong centrifugation force of ultracentrifuge causes migration of cesium chloride into the bottom of the tube. The liquid density gradient is then formed by the gradient concentration of cesium chloride (Ohlendieck, 2005). The density gradient is also spontaneously generated during centrifugation by using Percoll (polymer-coated silica particle).

Dialysis is a widely used method. The method is simple, but time consuming because the separation depends on diffusion. The sample is placed inside a dialysis bag prepared from a tube made of semipermeable membrane. Rely on commercially available dialysis tube, only small molecules whose sizes less than 10 kDa is removed from the sample to the surrounding medium. So this technique is normally used to remove salts from the solution of protein. Dialysis is also used to concentrate protein solution. Water molecules are removed from the inside of dialysis bag by using a hydrophilic polymer such as polyethylene glycol.

Molecular filtration (or ultrafiltration) is similar to dialysis. A membrane with specific pore size is used to fractionate proteins. By using pressure or centrifugation force, only the molecules smaller than the pore pass through the membrane (Fig 2). There are various sizes of the membrane pore; the largest one can cut off the large molecule whose mass is almost 100 kDa. So proteins in the sample can be separated into various ranges of molecular masses by using the membranes with different pore sizes. The protein solution is also concentrated by this technique.
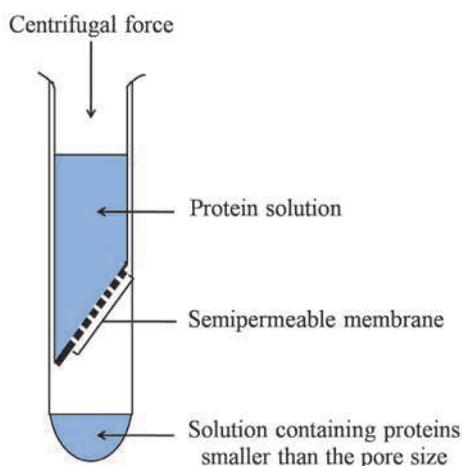

Fig. 2. Separation of proteins by using molecular filtration.

3.3 Chromatographic technique

Chromatography is the powerful method for detection and purification of biological substances. The principle of chromatographic separation is distribution or partition of separated molecules between two immiscible phases called mobile phase and stationary phase. Chromatographic methods are classified by different criteria including physical shape of stationary phase, nature of mobile phase and/or stationary phase, mechanism of separation or the other properties of the chromatographic systems. So the methods are called depending on their popularity. For examples, paper chromatography is called according to the material used as the stationary phase; thin layer chromatography (TLC) and column chromatography are named by the physical shapes of the stationary phases. Gas-liquid chromatography has gas mobile phase and liquid stationary phase. By using mechanisms of separation, chromatography is classified as adsorption chromatography, partition chromatography, size-exclusion chromatography, ion-exchange chromatography and affinity chromatography. Column chromatography is the most popular method for purification of proteins.

The conventional column chromatography is performed under low pressure. The mobile phase flows through the stationary phase in the column by the gravity or by low-pressure pump(s). So it can be called low pressure liquid chromatography (LPLC). High performance liquid chromatography (HPLC) is an advanced version of column chromatography. High pressure pumps, high-quality materials used as stationary phase and detectors with high sensitivity are used to improve the separation and to reduce the operating time. HPLC is now very popular for detection and purification of biological molecules. The other chromatographic methods are fast protein liquid chromatography (FPLC), capillary chromatography, reversed-phase liquid chromatography etc. FPLC is similar to HPLC but its operating pressure is lower than that of HPLC. LPLC, HPLC and FPLC are widely used for purification of proteins. In these methods, proteins are normally separated according to their size, charge or binding affinity. The separated proteins are eluted from the column as peaks which can be seen by various means such as absorption spectrometry, spectrofluorimetry (Wilson, 2005).

3.3.1 Size-exclusion chromatography

The other names of this method are gel filtration chromatography and gel permeation chromatography. This chromatographic technique also separates proteins by molecular sizes. Size-exclusion chromatography is the best to conserve native structure and function of the purified protein since wide varieties of buffers can be selected to obtain the suitable condition for the protein. The stationary phase of size-exclusion chromatography normally contains porous hydrophilic gel beads (Fig. 3). The gel beads used for LPLC are made of agarose, dextran, polyacrylamide, and chemical derivatives of these substances. The beads used for HPLC and FPLC are made of stronger materials such as cross-linked dextran and polystyrene. The cross-linked polystyrene can be used for separation of hydrophobic substances in the presence of organic solvents. The principle of the technique is the diffusion of molecules into the porous cavities of the beads. The molecules larger than the pores can not enter inside the beads where as the smaller ones can do. Since the pores have many sizes, the molecules including proteins are separated according to their molecular masses. The larger molecules pass the column faster than the smaller molecules (Fig. 4). Size-

exclusion chromatography can also be used for estimation of molecular mass of protein. A set of suitable proteins with known molecular masses are separated by the same condition as the purified protein. The fraction numbers or elution volumes are then plotted against log molecular masses of the standard proteins. The standard curve is used to estimate molecular mass of the purified protein. To obtain the good result, one have to choose the right beads since different types of beads are suitable for different molecular masses of the proteins.
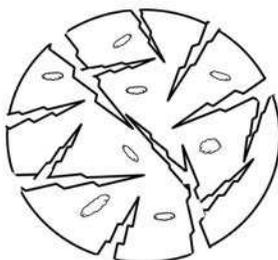
Fig. 3. A model demonstrates the pores in the hydrophilic gel bead used in size-exclusion chromatography.
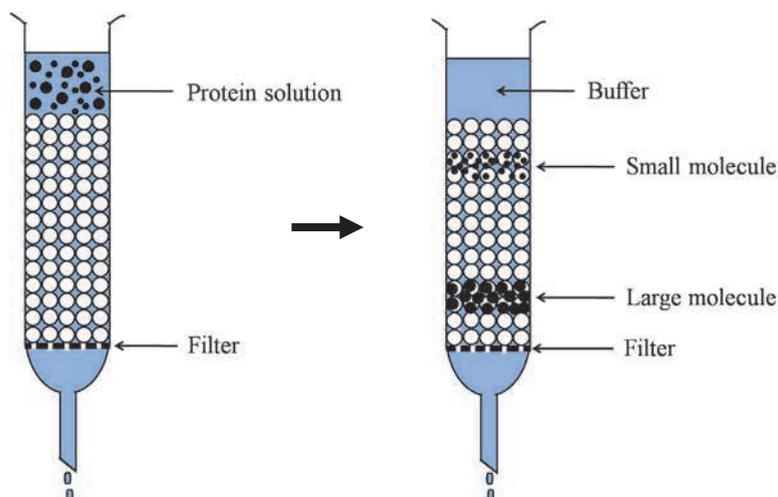
Fig. 4. Separation of two proteins by using size-exclusion chromatography. The protein mixture is loaded on the top of the gel. Then the large molecules pass the column faster than the small molecules.

3.3.2 Ion-exchange chromatography

This chromatographic technique conducts the separation according to magnitude of net electric charge of the proteins. There are two types: anion-exchange chromatography and cation-exchange chromatography. The material packed in the column is called ion exchanger which also have two types, anion and cation exchangers. Anion exchangers possess positively charged groups while cation exchangers have negatively charged groups.

Anion-exchange chromatography consists of anion exchanger and used for separation of proteins containing net negative charges. In contrast cation-exchange chromatography consists of cation exchanger and used for separation of proteins containing net positive charges. There are various varieties of ion exchangers depending on the matrix materials and ionic groups. Cellulose, agarose, dextran and polystyrene are used for LPLC while polystyrene, polyethers and silica are strong enough for HPLC. The matrix is chemically modified to contain ionic groups which are weakly acidic, strong acidic, weakly basic, or strong basic. The matrix with weakly acidic group (cation exchanger) as well as that with weakly basic group (anion exchanger) is suitable for separation and purification of most proteins. Choice of the exchanger depends on the pH range in which the protein is stable and the pI value of the exchanger. Generally the pH of the system should make opposite charges between the stationary phase and the protein (Fig. 5). Elution of proteins from the column can be isocratic or gradient system. The pH and ionic strength of elution buffer is important for getting well separation of proteins.
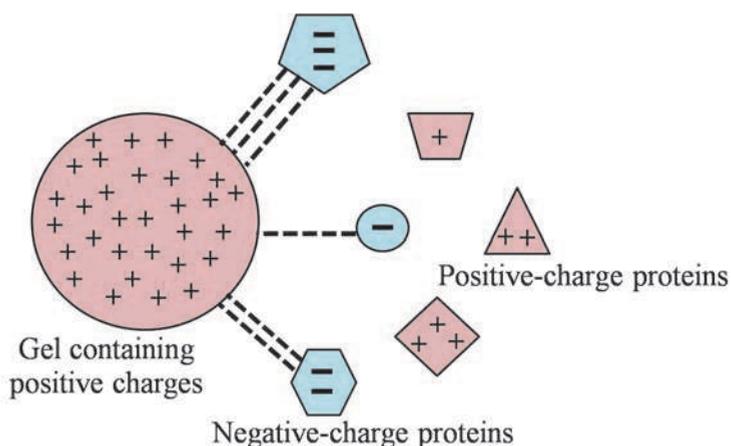

Fig. 5. Principle of ion-exchange chromatography. The anion-exchanger binds to negative-charge proteins but positive-charge proteins. The binding strength varies according to the charges of the proteins.

3.3.3 Affinity chromatography

This is an efficient technique but the material used as stationary phase is costly. The separation of molecules is based on binding affinity between macromolecules and macromolecules, or between macromolecules and low molecular mass ligands. The binding is specific for a certain molecule or a group of molecules. For examples, monoclonal antibody binds to its antigen only while avidin can bind to any biotin-coupled proteins. In this technique, one of the partner molecules is immobilized on a matrix by using a chemical reaction (Fig. 6). The matrix can be polyacrylamide, polystyrene, cross-linked dextrans and agarose etc. There are a number of chemical substances which react to different functional groups of the immobilized molecule. The chemical reaction must not destroy the binding activity. So it is necessary to use the right reaction for the immobilization. However, there are a lot of commercially available affinity matrices for different purposes such as protein A

Sepharose for purification of immunoglobulins, mannan-agarose for purification of mannose-binding lectins. (Fig. 7). After washing off the impurities, the matrix-bound protein is eluted by change of the pH of elution buffer, or by a competing substance. Affinity chromatography is always operated in a simple way by packing the matrix in a small column. Sophisticated system is not necessary for this technique.
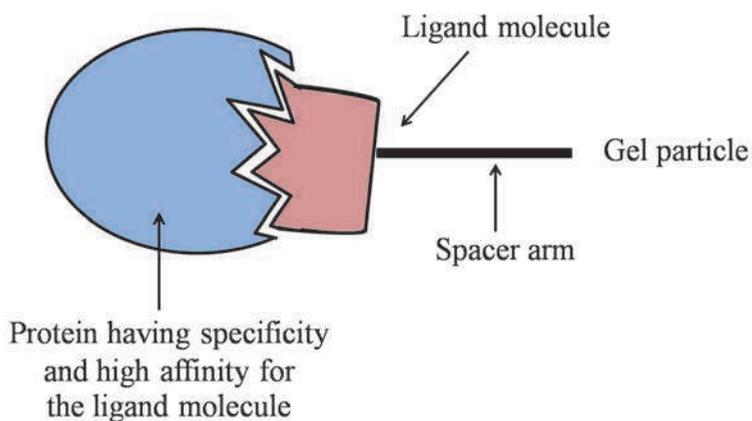

Fig. 6. Principle of affinity chromatography. The ligand is immobilized on a matrix and used for purification of its partner molecule.
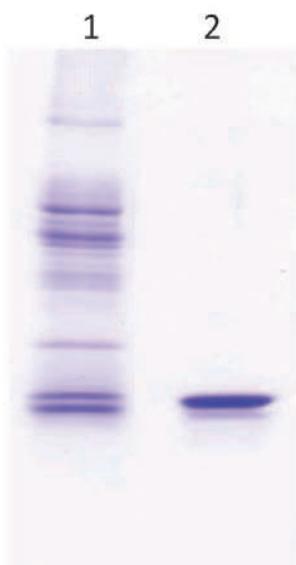
Fig. 7. One-step purification of mannose-binding lectin from *Dendrobium chrysotosum*. The affinity chromatography was performed by using mannan-agarose column. The crude extract (lane 1) and the purified lectin (lane 2) were separated on SDS-PAGE and then stained by Coomassie Brilliant Blue R250.

3.3.4 Hydrophobic interaction chromatography

This chromatographic method separates proteins on the basis of hydrophobicity, the same as reversed-phase liquid chromatography (RPLC). The stationary phases of both methods are hydrophobic ligands attached to a matrix. However, hydrophobicity and number of the ligands in hydrophobic interaction chromatography (HIC) is less than those in RPLC (Fig. 8). So HIC may be referred to as a milder form of RPLC. RPLC is mainly used for separation of peptides and low molecular mass proteins that are stable in aqueous-organic solvents. HIC is suitable for purification of proteins since it uses less extreme condition for elution of the adsorbed proteins. The hydrophobic ligands of HIC are mainly alkyl (ethyl to octyl) or phenyl or polyamide groups; the matrices are cross-linked agarose or silica. For the general procedure of HIC, the sample is applied onto the column equilibrated with a mobile phase of relatively high salt concentration. The adsorbed proteins are then eluted by a solvent of decreasing salt concentration. HIC is suitable for separation of proteins after salt precipitation and/or ion-exchange chromatography since the proteins already dissolves in the solution of high salt. Although HIC gives only moderate resolution, it opens new possibilities for purifying a number of biomolecules such as receptor proteins, membrane proteins (Wilson, 2005).

Which chromatographic procedure should be used for purification of a particular protein? Ion-exchange chromatography is usually the first chromatographic technique to be done for removal of unwanted proteins since the matrix is relatively cheaper and has higher binding capacity. Size-exclusion chromatography is usually operated after that. However the operation sequence can be reversed. One-step purification of protein from crude extract can be successful by using affinity chromatography (Fig. 7). Gel filtration chromatography or ion exchange chromatography is sometimes operated in accompany with affinity chromatography. Hydrophobic interaction chromatography (HIC) is another chromatographic techniques used for purification of some proteins.
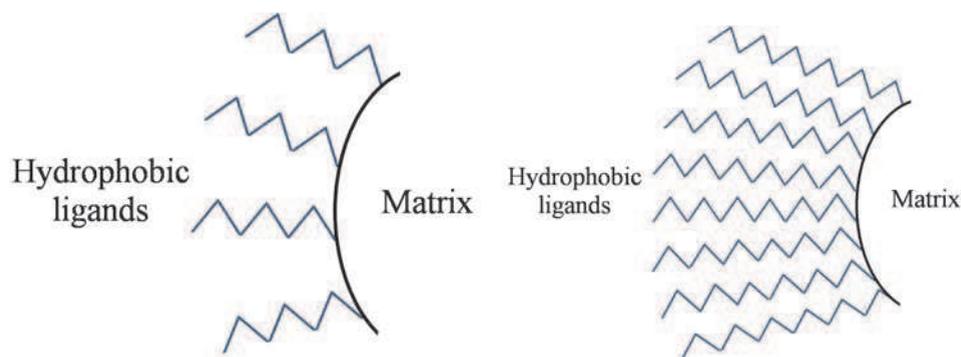

Fig. 8. Comparing the structures of materials used as stationary phases in hydrophobic interaction chromatography (A) and reversed-phase liquid chromatography (B).

3.4 Other separation techniques

There are some minor methods used for purification of protein such as electroelution of the protein separated by SDS-PAGE. The stained protein band is cut out of the gel. The gel slices

with a suitable buffer are packed in a dialysis bag. The bag is placed in the buffer between two electrodes. The protein is then electrically eluted from the gel slices. One can make a simple electroelution cell or can take any commercially available apparatus. SDS is removed from the protein by dialysis in the presence of a nonionic detergent. Only a small amount of protein is obtained by electroelution and it is possibly denatured.

Most proteins have been normally purified by the above techniques. To obtained intact hydrophilic protein, the purification process mainly includes salting out and chromatographic technique(s). However some proteins can be purified by simply specific procedures. For an example, molecular filtration in combination with sodium chloride solution was successfully used for purification of glutamine synthetase (Fig. 9) (Arunchaipong et al., 2009). So the simply specific procedure may be made for purification of some protein if the protein has the specific property.
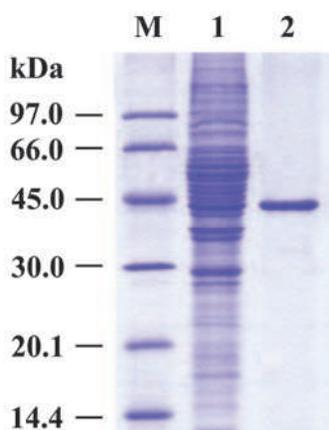

Fig. 9. Purification of glutamine synthetase from chick retina by using molecular filtration in the presence of 0.7M sodium chloride. The crude extract and the purified protein were separated on SDS-PAGE and then dye-stained. Lane M, molecular mass marker; lane 1, crude protein extract; lane2, purified enzyme.

4. Detection of the desired protein

Actually, purification process includes many steps and is laborious. Therefore it is necessary to be certain that the desired protein is present in the crude protein extract and all purification steps. For the protein with known molecular mass or size, the most versatile method is gel electrophoresis, especially SDS-polyacrylamide gel electrophoreis. Determination of enzymatic activities or other properties such as absorption spectra are also widely used. In some case the sophisticated technique, LC-MS/MS, is performed for identification of the protein after separation by electrophoresis.

4.1 Electrophoresis

It is the most widely used technique for separation, detection and determination of the purity of protein. It is sometimes used for protein purification. The technique is based on

migration of charged proteins in an electric field. Electrophoresis of proteins is generally conducted in a gel medium, mostly polyacrylamide. The widely used polyacrylamide gel electrophoreses (PAGE) are sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF).

4.1.1 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

SDS-PAGE is normally employed for estimation of purity and molecular mass of protein. This versatile method is reproducible and low-cost. For the principle, sodium dodecyl sulfate (SDS) in the system binds to most proteins in amount approximately proportional to the molecular mass of the proteins. So each protein has similar charge-to-mass ratio and migrates proportional to its molecular mass. The widely used SDS-PAGE is the Laemmli protocol, as well as its modified protocols, which consists of the discontinuous buffer system. In this buffer system, there are two parts of polyacrylamide gel, stacking gel and separating gel. There are differences in composition, concentration and pH among electrode buffer, stacking buffer and separating buffer (Fig. 10). The usefulness of the buffer stem is stacking effect which occurs in the stacking gel. The effect increases concentration of proteins in the sample which helps well
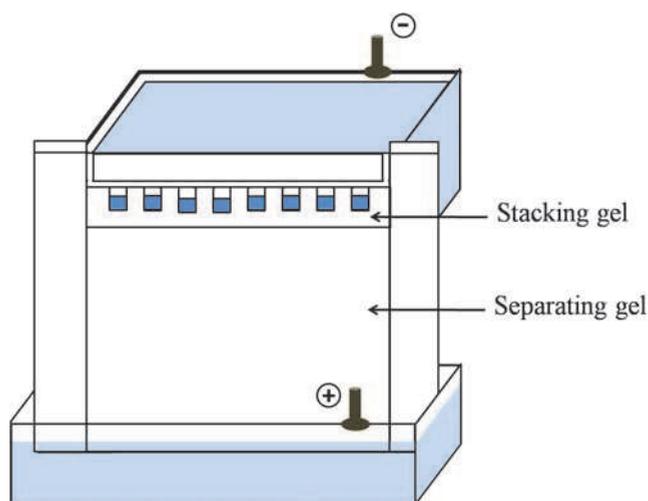

Fig. 10. Polyacrylamide gel electrophoresis. The electrophoresis set composes of the upper and lower chambers which are filled with an electrode buffer. The gel is polymerized in the space between two glass plates and then connected between the two chambers. Protein samples are load in wells at the top of the gel. Protein molecules will migrate into the gel under an electric field. For SDS-PAGE, the proteins migrate from cathode to anode.

separation of the proteins in the separating gel. SDS-PAGE can be used to estimate molecular mass of the protein by using a calibration curve between relative mobility and log molecular mass of standard protein. There are the other protocols of SDS-PAGE including urea-SDS-PAGE, Tricin-SDS-PAGE etc. The other types of polyacrylamide gel electrophoresis are also available such as acid-urea-PAGE, native-PAGE. Although SDS-PAGE is commonly used, some proteins may need specific PAGE for the detection. The

proteins separated on polyacrylamide gel can be visualized by many types of staining. The staining with dye especially Coomassie Brilliant Blue R250 is most widely used since it is easy, low cost and effective. Staining with some other dyes and silver staining are also available for specific purposes. Examples are Sudan Black B for staining of proteolipids, Thymol for staining of glycoproteins (Hames, 1981; Holtzhauer, 2006).

4.1.2 Isoelectric focusing

IEF is used for determination of isoelectric pH (pI) of protein. The technique is also useful for separation, detection and determination of the purity of protein. Proteins are separated according to their pI in a medium containing pH gradient. For analytical slap gel, the medium commonly used is polyacrylamide gel or agarose gel. The pH gradient is established by a mixture of low molecular mass organic acids and bases which is called ampholytes (Berg et al., 2002). The proteins in the mixture migrate in an electrical field depending on their charges and then standstill in the gel at the pH equal to their pI (Fig. 11). The separated proteins can be visualized by an activity staining or a dye staining. The activity stain depends on the protein property. There is a problem concerning with the dye staining of IEF gel. The ampholytes can be stained with some dyes especially Coomassie Brilliant Blue R250. So it must be removed by appropriate solutions before staining.
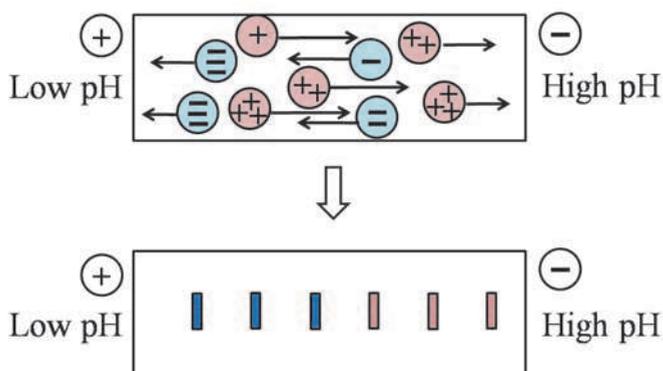

Fig. 11. Principle of isoelectric focusing. A pH gradient is established between two electrodes by using an electric field, a mixture of ampholytes and suitable electrode buffers. Each protein migrates in the gel and then stop moving at the pH equal to its pI.

For some work, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is performed to obtain a very high resolution. The technique is a sequential separation of proteins. The protein mixture is separated by using IEF in the first dimension and then SDS-PAGE in the second dimension. High skill is needed to perform the conventional procedure. Fortunately, the commercially available apparatus are designed to make it much easier and more efficient. Since the cost of the technique is rather high, it is done only when necessary.

After dye staining or silver staining, the protein separated by SDS-PAGE or 2D-PAGE may be identified by using Liquid Chromatography-Mass spectrometry (LC-MS). The protein band is cut from the gel and then in-gel digestion with some protease which is mostly

trypsin. The peptide fragments are separated by HPLC and injected to a series of Mass Spectrometers. This mean of mass spectrometric analysis is called tandem mass spectrometry. The MS spectra of the peptides are used to search in the data bases through the internet. The amino acid sequences of some peptides are obtained as well as the protein(s) which has them as parts of molecule (Berg et al., 2002).

4.2 Enzyme activity or the other property of protein

Apart from electrophoresis, there are some other methods for detection of the desired protein during purification process. Various specific techniques are used depending on the property of the protein. Many proteins can be stained in gel by using their enzyme activity. Since the native conformation is important for the activity, IEF and native-PAGE are compatible with activity stains. SDS-PAGE is not suitable for activity staining of protein, excepted that the proteins can be re-natured by soaking the gel with non-ionic detergent. The enzyme activity is also determined in solution. Its activity is measured by absorption spectrometry and the activity unit can be determined. Specific activity calculated from enzyme activity and the amount of protein in milligram is normally used for determination of the purity fold during the purification process. Some enzyme activity may be detected by other techniques such as paper chromatography (Arunchaipong et al., 2009).

Many proteins are not enzymes but they may have other activities or properties. Some activities or properties are used for detection of the proteins. For examples, Anti-microbial activities can be detected by microbiological methods. Glycoproteins are pursued during purification process by using lectins. In this case, the binding activity has to be done on a membrane surface since the protein molecules can not freely move in the gel matrix and the specific binding will be interfered. The membrane method is called Western blot analysis. In this method the proteins are separated using SDS-PAGE. The separated proteins are then electrically transferred onto a membrane sheet made of nitrocellulose or polyvinylidene fluoride (PVDF) (Fig. 12). The protein bands are adsorbed on the membrane surface. The membrane is then used for binding with a lectin-linked enzyme. Only glycoprotein band(s) binds to the lectin-linked enzyme. The band(s) can be vistualized by addition of a suitable substrate. Western blot analysis is also suitable for detection of protein by specific antibody.

⊕   Anode
    Filter paper
    Nitrocellulose membrane
    Polyacrylamide gel
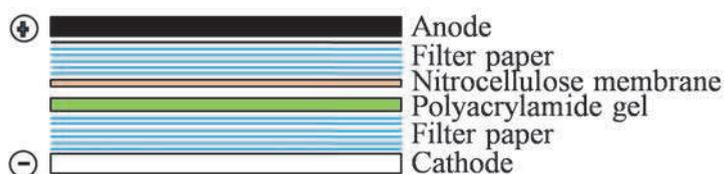   Filter paper
⊖   Cathode

Fig. 12. Diagrammatic representation of electrical transfer of proteins. The transfer of protein from polyacrylamide gel onto a membrane is also called western transfer. The western transfer shown in the picture is the semi-dry procedure. After separation of proteins by using polyacrylamide gel electrophoresis, the gel and the membrane sheet are sandwiched between filter papers and electrodes. The membrane (such as nitrocellulose) and the papers are wet with a transfer buffer. The proteins in the gel are transferred to the membrane by application of an electric field.

5. Determination of protein concentration

Determination of protein concentration is normally required during the purification process. The protein concentration is useful for determination of specific activity. There are a number of protocols to determine protein concentration such as ultraviolet absorption, Bradford method and Lowry method. Ultraviolet absorption (at 280 nm) of protein solution is the simplest method. This method is relatively sensitive and does not destroy protein; so it is used for detection of proteins eluted from chromatographic column. However, the absorption at 280 nm can be interfered by other substances such as nucleic acids. Bradford method relies on the binding between protein and the dye Coomassie Brilliant Blue G. The protein-bound dye has maximal absorption at 595 nm. In Lowry method, the protein solution is mixed with a copper sulphate solution and the Folin reagent (a mixture of sodium tungstate, molybdate and phosphate) to produces blue purple color. The color solution is quantified by the absorbance at 660 nm (Walker, 2005). However all methods used do not give true concentration of protein since it has to used some protein to make a standard calibration curve. Bovine serum albumin (BSA) is mostly used because it is high purity and relatively low cost.

6. Conclusion

Protein purification involves extraction and purification. In extraction process, there are different procedures to disrupt cells or tissues as well as different extraction solvents, depending on the nature of the cells or tissues. Animal cells and tissues can be disrupted by gentle methods while unicellular organisms and plant tissues require the more vigorous procedures to break the cell envelopes. Normally the extraction solvents for most hydrophilic proteins are suitable buffers except that some detergents are included in some cases. Lipid-soluble proteins are extracted by some organic solvents, or buffers containing detergents. A number of procedures are involved in the purification process such as separation based on solubility, separation based on size and density, chromatography. Chromatographic techniques are widely used and can be operated as LPLC, HPLC or FPLC. Many steps are normally required for purification of most proteins but some proteins can be purified by only simple procedures. Detection of the desired protein should be done during the purification process and electrophoreses are widely operated. Detections by activities or other properties are also used for some proteins.

7. Acknowledgment

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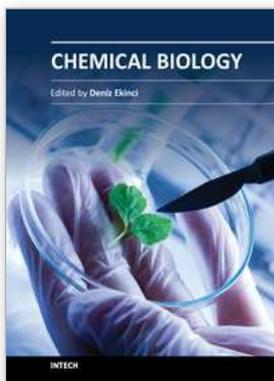
Chemical Biology

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Chemical biology utilizes chemical principles to modulate systems to either investigate the underlying biology or create new function. Over recent years, chemical biology has received particular attention of many scientists in the life sciences from botany to medicine. This book contains an overview focusing on the research area of protein purification, enzymology, vitamins, antioxidants, biotransformation, gene delivery, signaling, regulation and organization. Particular emphasis is devoted to both theoretical and experimental aspects. The textbook is written by international scientists with expertise in synthetic chemistry, protein biochemistry, enzymology, molecular biology, drug discovery and genetics many of which are active chemical, biochemical and biomedical research. The textbook is expected to enhance the knowledge of scientists in the complexities of chemical and biological approaches and stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of chemical biology.

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