CHAPTER 1

INTRODUCTION

1.1 Statement and significance of the problem

Public awareness of the importance of dietary lipids and lipoproteins has increased due to their association with coronary heart disease (CHD). Many studies have demonstrated that, especially in affluent countries with high fat consumption, there is an association between blood lipid levels and the development of atherosclerosis. Decades of basic research have contributed to our knowledge concerning lipid and protein contents of lipoproteins, as well as their roles in atherogenesis. Low-density lipoprotein (LDL) is the major lipoprotein responsible for the delivery of cholesterol to cells. Elevated LDL levels may promote atherosclerosis. In addition, because LDL is a small lipoprotein, it can infiltrate into the intima layer of arteries. This oxidized LDL is taken up by macrophages through scavenger receptors. Macrophages that take up too much oxidized LDL subsequently become foam cells, which are major components of fatty streaks, an early precursor of atherosclerotic plaques [(1)]. There is considerable heterogeneity in LDL in density and size. Two main LDL phenotypes have been identified: pattern A, characterized by the predominance of large, buoyant LDL (bdLDL) particles and pattern B, characterized by an excess of small, dense LDL (sdLDL) particles [(2)]. The sdLDL have been shown to be more proatherogenic than the larger forms due to their lower binding affinity for the LDL receptor, prolonged residence time in plasma, increased penetration into the arterial wall, higher binding to extracellular matrix components of the arterial wall [(3)]

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

and greater susceptibility to oxidative stress [4]. Evidence suggests that the sdLDL

and greater susceptibility to oxidative stress [4]. Evidence suggests that the sdLDL

phenotype is closely associated with CHD [5-7]. However, it is widely recognized that most current methods used for measurement of sdLDL have several limitations, including being technically demanding, time-consuming, costly and unsuitable for routine clinical laboratories. To overcome these problems, a novel method should be developed.

Previous proteomic study has suggested that sdLDL of patients with the metabolic syndrome and type 2 diabetes were rich in apo C-III and depleted of apo C-I, apo A-I and apo E compared with matched healthy controls with the A phenotype. Moreover, there was an association between high apo C-III in sdLDLs and their affinity for arterial proteoglycans (PGs). Thus, these high apo C-III, coupled with the augmented affinity with arterial PGs and low apo A-I (which has been expected to protect LDL from modification [8]) of sdLDL might contribute to the increased risk of cardiovascular disease [9]. However, there is still limited evidence concerning LDL subfractions and their protein composition. Thus, detailed information about protein profiles of LDL subfractions may reflect molecular mechanisms that lead to CHD.

Proteomics is a new tool used to identify and characterize proteins in organisms or cells. Advances in proteomic technologies have been increasingly used for the purpose of better understanding of molecular mechanisms underlying diseases and discovering novel protein targets for therapeutic innovations. The application of proteomics profiling of serum/plasma and other clinical samples has increased over the last several years. Many different technologies have been applied, including two-dimensional gel electrophoresis and mass spectrometry (MS). Up to now, liquid

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

chromatography (LC) and electrospray ionization (ESI) in combination with tandem mass spectrometry has been shown to be an effective tool for protein analysis, and has been widely applied in many fields of interest for a greater understanding of human diseases. Elevated plasma concentrations of LDL increase the risk of CHD, but most current methods used to characterize lipoproteins suffer from several limitations. Moreover, the association between these factors is unclear. Thus, protein profiles of LDL subfractions may help identify the molecular mechanisms underlying coronary disease, and may thus be applied to the development of novel techniques for identification of sdLDL and bdLDL.

1.2 Literature reviews

1.2.1 Lipoproteins and apolipoproteins

Approximately 40% of the average person's caloric intake consists of lipids, about 35% from saturated animal lipids, and 5% polyunsaturated vegetable lipids. Triglycerides are the major component animal lipids (98% to 99%) along with smaller amounts of cholesterol and other lipids.

The digestive phase occurs inside the lumen of the intestines. Lipid is first solubilized through the emulsification process. Bile is released from the gall bladder into the small intestine and breaks up lipids into smaller units called micelles. Micelles contain unesterified cholesterol, monoglycerides, fatty acids, phospholipids, and conjugated bile acids [10] which promotes the action of digestive enzymes. Cholesterol esterase cleaves the ester linkages to release free fatty acids and free cholesterol. Lipase hydrolyzes triglycerides to release free fatty acids forming monoglycerides, diglycerides and glycerol while phospholipase A acts on the

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

phospholipids. Once lipids enter the intestinal mucosal cells, they are packaged into chylomicrons which contain triglycerides, cholesterol, phospholipids and proteins called apolipoproteins. Enterocytes synthesize apo B-48 (the major apolipoprotein of chylomicrons), apo A-I, apo A-II and apo A-IV which form the surface layer of chylomicrons [11]. Chylomicrons are secreted into the lymphatic system and eventually enter the circulatory system.

Lipoproteins Metabolism

Lipoprotein lipase (LPL) hydrolyzes triglycerides on chylomicrons, which subsequently become chylomicron remnants and are rapidly taken up by the liver. The liver secretes very low-density lipoprotein (VLDL) into the circulatory system. It undergoes lipolysis by the action of LPL and is converted to intermediate-density lipoprotein (IDL), which can be further transformed by lipolysis into LDL that is responsible for the delivery of cholesterol to cells. These chylomicrons, VLDLs, IDLs and LDLs are called lipoproteins

Lipoproteins are spherical particles and range in size from 10 to 1200 nm. As the name implies, lipoproteins consist of both lipids and proteins called apolipoproteins (Figure 3). Lipids are insoluble in water and thus unable to be transported to the tissues to accomplish their metabolic functions. The existence of proteins enables the lipids to remain partially soluble in plasma and facilitates their transportation. The hydrophobic triglycerides and CE are found in the core region of lipoproteins while the phospholipids and cholesterol are found on the surface. They also contain apolipoproteins which are located on their surfaces.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

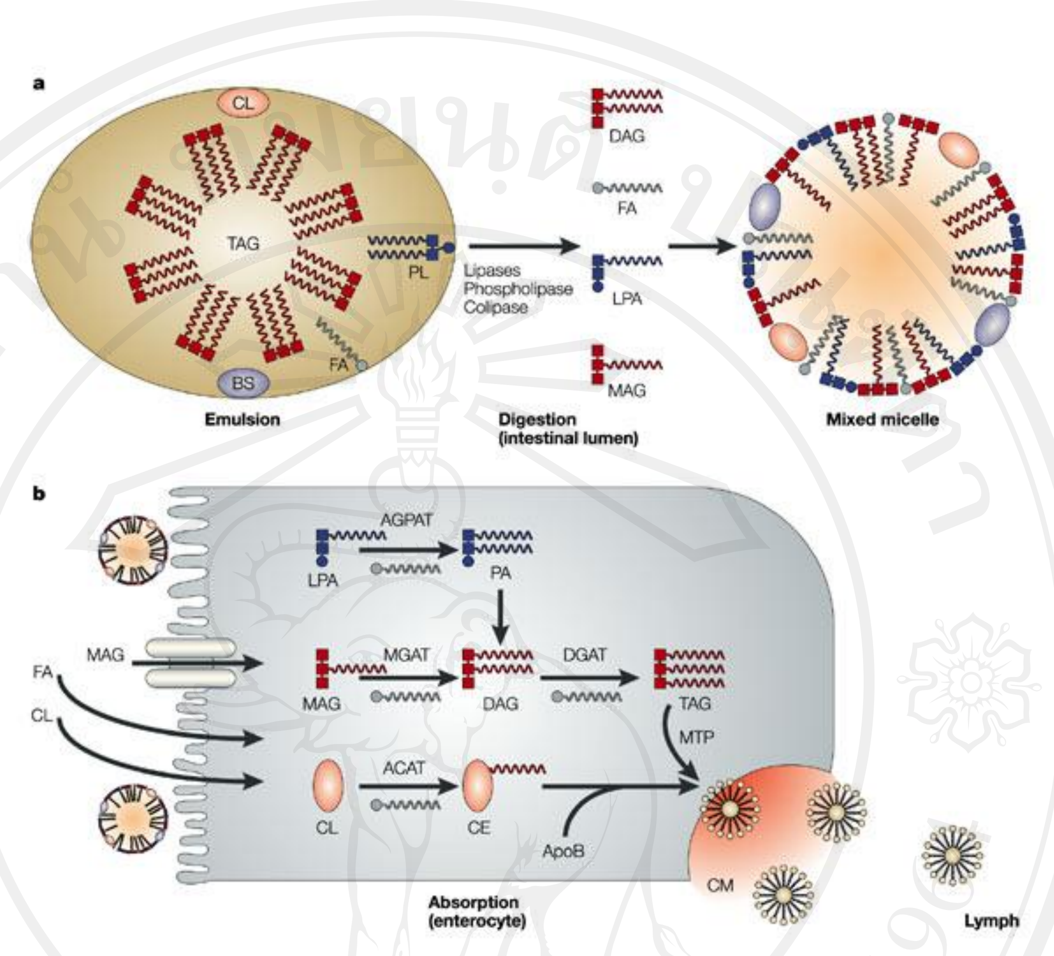

Figure 1 Lipid digestion and absorption a) Lipids are mixed with bile salts (BS) and digestive enzymes to form monoacylglycerol (MAG), diacylglycerol (DAG), free fatty acid (FA), cholesterol (CL), lysophosphatidic acid (LPA) which subsequently absorb at the brush border membranes of the enterocytes. b) FA and MAG are reesterified by MAG acytransferase (MGAT) and DAG acytransferase (DGAT) to form triacylglycerol (TAG). Cholesterol is acylated by acyl-CoA cholesterol acyltransferase (ACAT) to form cholesterol ester (CE). LPA is acylated by 1-acyl-glycerol-3-phosphate (AGPAT) to form phosphatidic acid (PA) which is converted into TAG. TAG is packaged, along with CE and apolipoproteins into chylomicrons (http://www.nature.com/nrd/journal/v3/n8/fig_tab/nrd1469_F2.html)

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

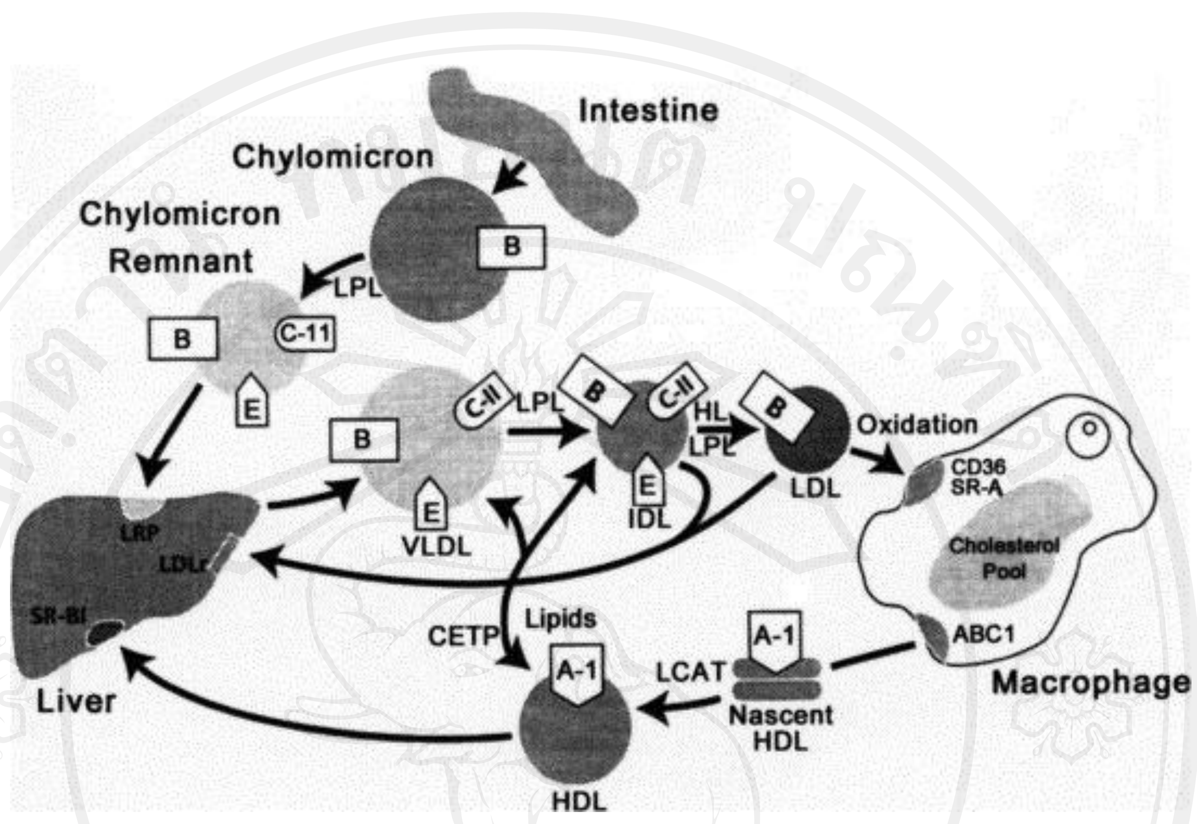

Figure 2 Schematic overview of lipoprotein metabolism [12]

The linkages of the core lipids with the phospholipid and apolipoproteins are the noncovalent interactions: hydrogen bonds and van der Waals forces. A weak binding between lipids and apolipoproteins allows them to be exchanged between plasma lipoproteins. The sizes of the lipoprotein particles differ due to their different proportions of lipids and proteins. The larger lipoprotein particles contain more lipids relative to protein, which results in their lighter density. They have larger core regions that are high in triglycerides and CE, and also contain fewer proteins [13]. The various lipoprotein particles can be separated into different density fractions (Table 1) [14].

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

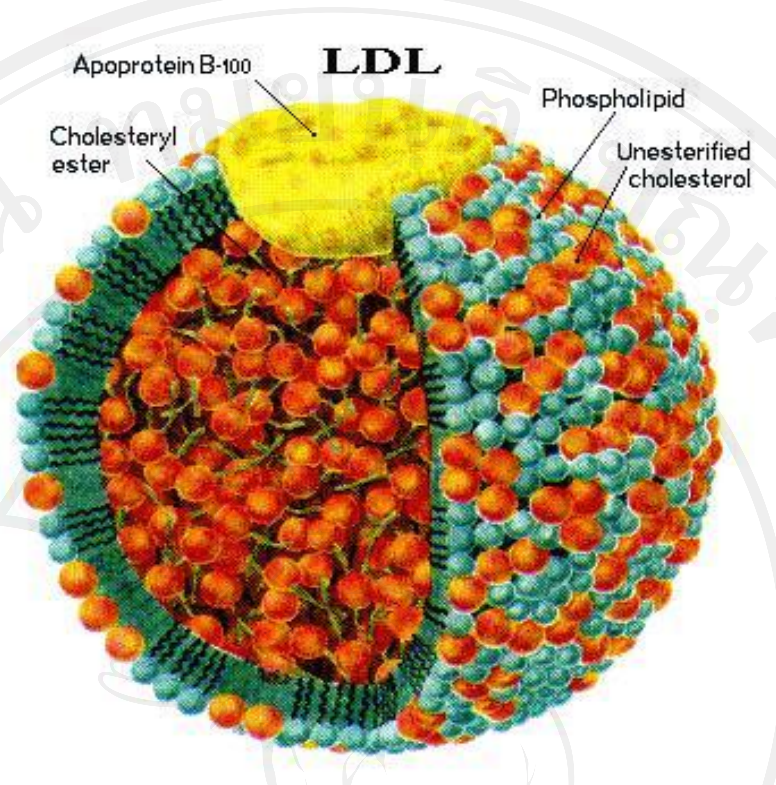

Figure 3 Schematic model of lipoproteins structure

(http://www.intramed.net/UserFiles/vinetas/22038.jpg)

Table 1 Density and electrophoretic mobilities of lipoproteins of human plasma

Lipoprotein class	Density (g/ml)	Electrophoretic mobilities in agarose gel
High density (HDL)	1.063-1.21	α
Low density (LDL)	1.019-1.063	β
Intermediate density (IDL)	1.006-1.019	β or pre-β
Very low density (VLDL)	0.95-1.006	pre-β
Chylomicrons	< 0.95	origin

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Chylomicrons represent the largest and most buoyant lipoprotein particles. Because of their high triglyceride and low protein content, they also have the lowest density. This low density permits high concentrations of chylomicrons to float on top of the serum or plasma. The major protein component is apo B-48, but they also contain apo A-I, apo A-II, apo A-IV[15], apo C-I, apo C-II, apo C-III and apo E. Chylomicrons are produced in the intestine, then enter the lymphatic system and reach the circulation via the thoracic duct and jugular vein. They collect apolipoprotein C-II and apo E through interactions with HDL. Apo C-II activates lipoprotein lipase (LPL), which hydrolyzes triglycerides to release fatty acids. Along with hydrolysis process, the cores of the chylomicrons are reduced in size and subsequently become the chylomicron remnants. Apo C-II and apo C-III are then transferred back to HDL. These free fatty acids can be taken up by cells and used as a source of energy or re-esterified and stored in adipose tissue. Chylomicron remnants are then released into the circulatory system. They consist of CE, apo B-48 and apo E. These remnants are rapidly taken up by the liver through interaction of apo E with specific receptors on the surface of liver cells [1]. The delivery of CE to the liver is used to synthesize bile acids, or they may be packaged into VLDL particles; whereas apo B-48 undergoes a degradation process. This pathway is called the “exogenous pathway”.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

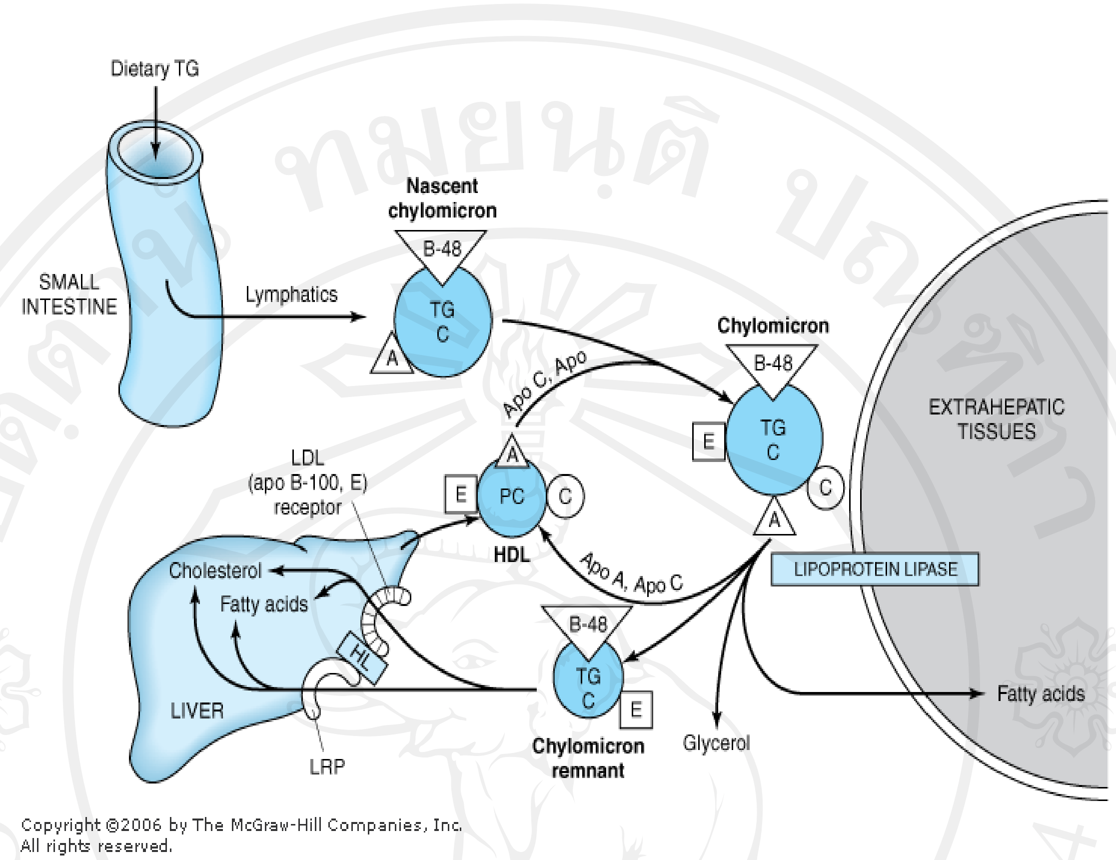

Figure 4 Schematic representation of the exogenous pathway (http://2.bp.blogspot.com/eDsdmzF2JEw/TcKYgh8muII/AAAAAAAAAtw/wpm8nhT2y9g/s1600/Chylomicron.jpg)

In the endogenous pathway, the liver secretes triglycerides-rich lipoproteins called VLDL. These triglycerides are produced in the liver *de novo* or by reesterification of free fatty acids and then packaged into VLDL. Hepatocytes synthesize triglycerides from carbohydrate and fatty acids. These triglycerides are secreted with phospholipids and apo B-100 as nascent VLDL [14]. They contain apo B-100, apo E and apo C on their surface. Additional apo C is transferred through interaction with HDL. Free cholesterols are released and taken up by HDL. These cholesterols are esterified by lecithin cholesterol acyltransferase (LCAT) and then

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

transferred back by the action of cholesterol ester transfer protein (CETP) for the exchange of triglycerides. Consistent with chylomicrons, VLDL particles secreted into the circulation undergo the lipolysis process. Apo C-II activates LPL, which hydrolyzes triglycerides in VLDL to release free fatty acids. Apo C is transferred back to HDL. VDLD particles are then converted to VLDL remnants. Some VLDL remnants are taken up by the liver, while the remainder are converted to smaller and denser particles called IDL. Some IDL particles are removed from circulation through the interaction between apoE and hepatic receptor or undergo hydrolysis by LPL to form LDL. These LDL collect CE from HDL and then delivered to cells. In humans, hepatocytes remove about 50% of IDL[13].

LDL is the major lipoprotein responsible for the delivery of cholesterol to cells through LDL receptors. LDL receptors are present on both the hepatocytes and peripheral tissues. They recognize both apo B-100 and apo E on LDL particles. Once LDL binds to the receptors, they are internalized by endocytosis. After LDL dissociates from receptors, the receptors subsequently return to the cell surface for reuse [13]. For LDL, several enzymes degrade apo B-100 into amino acids and convert CE to free cholesterols, which are utilized for the synthesis of cell membranes, steroid hormones and bile acids. The result of abnormalities in the LDL receptor is elevated LDL in the circulation, which can lead to atherosclerosis. Patients with familial hypercholesterolemia disease have only half the normal amount of LDL receptors, which results in decreased hepatic uptake of LDL by the liver. The LDL that accumulates in the plasma often leads to the development of coronary heart disease.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

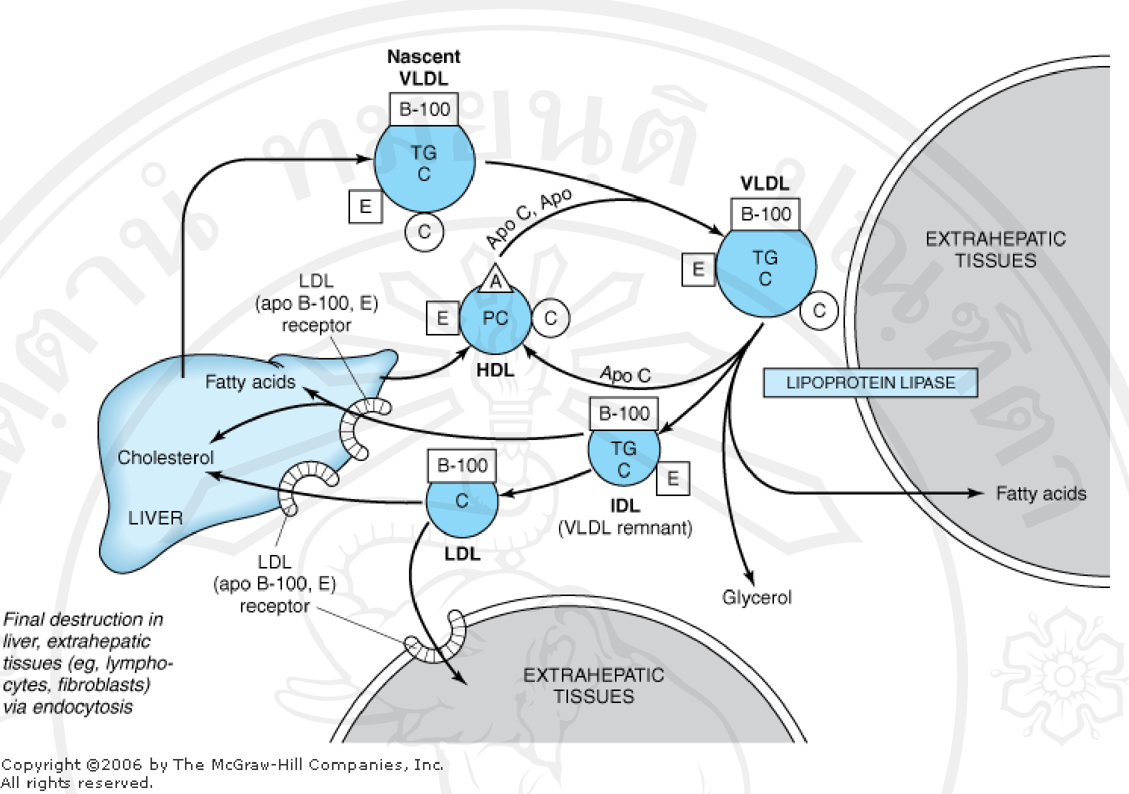

Figure 5 Schematic representation of endogenous pathway

(http://2.bp.blogspot.com/Ugl0efBdxII/TcKYkYRdDHI/AAAAAAAAAt4/LU0bjR3P3Ac/s640/VLDL.jpg)

One of the major roles of HDL is to maintain the equilibrium of cholesterol in peripheral cells by the reverse cholesterol transport pathway. This pathway is composed of several processes, including the cholesterol efflux from cells to HDL, cholesterol esterification, transfer of esterified cholesterol from HDL to lipoproteins or direct uptake to the liver. HDL is secreted from the liver or intestine as discoidal-shaped nascent particles. They consist of phospholipids, apolipoproteins and small amounts of unesterified cholesterol. The major apolipoproteins associated with HDL are apo A-I, apo A-II and apo C (C-I, C-II and C-III). The discoidal HDL particles

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

acquire more unesterified cholesterol from cell membranes and from other lipoproteins. LCAT converts free cholesterol into CE, in the presence of its cofactor, apo A-I. This increased volume core-HDL becomes the functional spherical form. CE of HDL may be transferred back to the liver by the action of HDL as donors to LDL or VLDL through the action of cholesterol ester transfer protein (CETP). An exchange of TG from VLDL or LDL for CE in HDL occurs via the CETP. The transferred CE are then transported to the liver in these lipoproteins and internalized by receptor-mediated endocytosis [15]. CE are also directly delivered to the liver by HDL receptors such as scavenger receptor class B type I (SR-BI) [1].

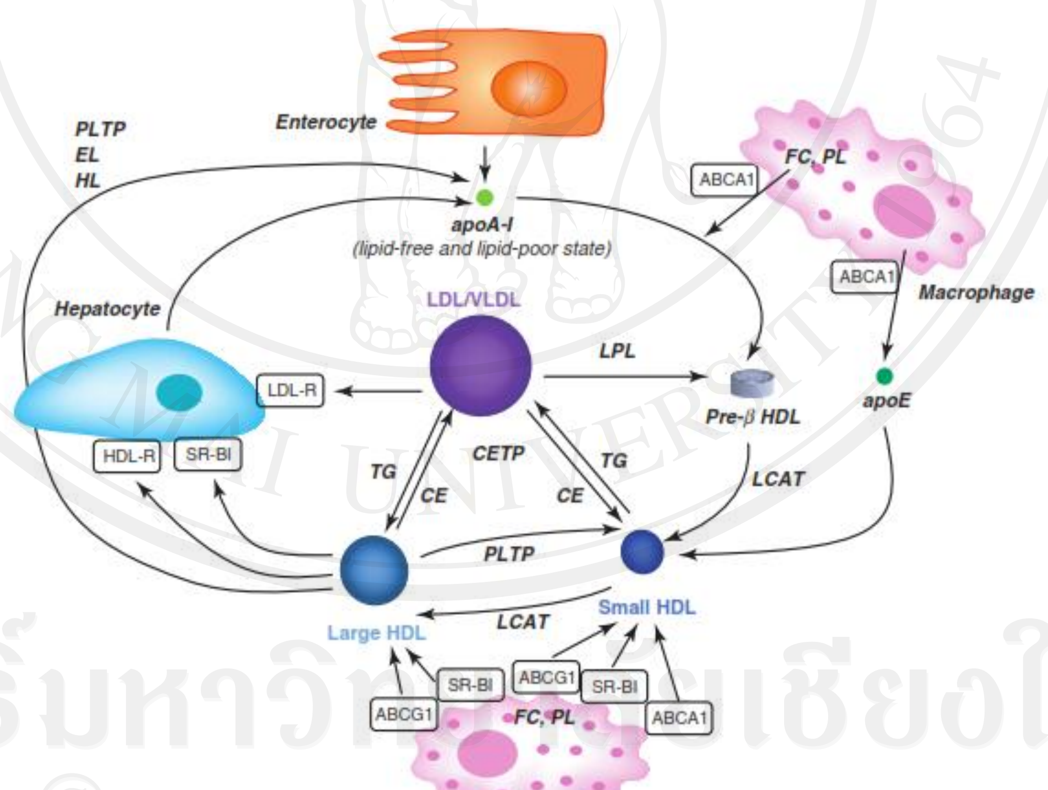

Figure 6 The reverse cholesterol transport pathway [16]

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

As indicated earlier, all lipoproteins contain several different kinds of apolipoprotein. These apolipoproteins provide the structural element of lipoprotein particles and maintain stability. They also act as ligands for specific receptors and activators or inhibitors of enzymes involved in lipoprotein metabolism. The characteristics and functions of the major human plasma apolipoproteins are summarized in Table 2 [13].

Table 2 Classification and properties of major human plasma apolipoproteins

Apolipoprotein	**Molecular weight (Daltons)**	**Chromosomal location**	**function**	**Lipoprotein carrier (s)**
apo A-I	29,016	11	Activates LCAT	Chylomicrons, HDL, LDL, VLDL
apo A-II	17,414	1	Modulates the binding of HDL by SR-BI [17]	Chylomicrons, HDL, LDL, VLDL
apo A-IV	44,465	11	Activates LCAT	Chylomicrons, HDL, LDL, VLDL
apo B-100	512,723	2	Binds to LDL receptors	VLDL, IDL, LDL

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 2 (Continued) Classification and properties of major human plasma apolipoproteins

Apolipoprotein	**Molecular weight (Daltons)**	**Chromosomal location**	**function**	**Lipoprotein carrier (s)**
apo B-48	240,800	2	Essential for the assembly, secretion of chylomicrons (18, 19)	Chylomicrons
apo C-I	6,630	19	Activates LCAT	Chylomicrons, VLDL, HDL, LDL
apo C-II	8,900	19	Activates LPL	Chylomicrons, VLDL, HDL, LDL
apo C-III	8,800	11	Inhibits LPL	Chylomicrons, VLDL, HDL, LDL
apo E	34,145	19	Binds to LDL and hepatic receptors	Chylomicrons, VLDL, HDL, LDL

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Apolipoprotein A-I (apo A-I) is the major protein of HDL, constituting about 70-80% of HDL protein. It is synthesized in the liver and small intestine. Apo A-I is largely helical in structure. Studies have suggested that the expression of apo A-I inhibited the development of atherosclerosis. These findings demonstrated the anti-atherogenic properties of apo A-I, which may be involved in supporting the cholesterol efflux. Apo A-I is associated to the reverse cholesterol transport pathway. This pathway consists of three steps, including collection of unesterified cholesterol from peripheral cells to HDL, esterification by LCAT, and then transportation of CE to the liver. The presence of apo A-I has been suggested to be involved in the cholesterol efflux and LCAT activation in the reverse transport cholesterol pathway [(20)]. The gene for apo A-I is part of a gene cluster on the long arm of chromosome 11 that includes the genes for apo C-III and apo A-IV [(15)].

Apolipoprotein A-II (apo A-II) comprises about 20% of HDL protein. The roles of apo A-II have not been fully elucidated. Studies suggest that apoA-II acts as pro-atherogenic in contrast to anti-atherogenic apoA-I. It may play a role in the remodeling of HDL. Previous study has shown that apo A-II modulates the binding of HDL by Scavenger Receptor BI (SR-BI) [(17)]. This receptor plays an important role in direct uptake of HDL to the liver and promotes the efflux of free cholesterol from cells to HDL. Studies have demonstrated larger atherosclerotic lesions in apoA-I/apoA-II transgenic mice than the apo A-I transgenic mice. This effect was probably due to apoA-I containing both Lys and Arg as positively charged amino acids, while apoA-II contains only Lys. Some studies suggest that that Arg residues of apoA-I are important for LCAT activation[(21)].

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Apolipoprotein A-IV (apo A-IV) is synthesized in the small intestine. The present view is that apo A-IV is involved in LCAT activation, although not as effective as apo A-I. It has also been suggested that apo A-IV may be necessary for the maximal activation of LPL by apo C-II [15].

Apolipoprotein B-100 (apo B-100) is the full-length translation product of the apo B gene. It is synthesized in the liver and found in LDL, and to a lesser extent in VLDL and IDL [11]. Apo B-100 contains LDL receptor binding domain (amino acids 3100 to 3400) which allows the specific binding of LDL to cells through the LDL receptor [15].

Apolipoprotein B-48 (apo B-48) is found only in chylomicrons. It is produced from the apo B gene and results from the posttranscriptional modification of apo B-100 mRNA. A single base substitution produces a stop codon at residue 2153 [22]. Apo B-48 is not recognized by the LDL receptor because it does not contain the LDL receptor binding domain.

Apolipoprotein C (apo C) is synthesized in the liver. It transfers between the TG-rich lipoprotein and HDL. Three major forms have been identified: apo C-I, apo C-II and apo C-III, which have 57, 78 and 79 amino acids, respectively. Apo C-I activates LCAT, while apo C-II act as an activator of LPL, which results in hydrolyzation of triglycerides to release fatty acids to the cells for metabolism and storage. The role of apo C-III is prevention of the action of LPL.

Apolipoprotein E (apo E) is involved in the remove of chylomicrons and VLDL remnants from the plasma. It is synthesized in the liver and incorporated into HDL. This apo E is then transferred from HDL to VLDL or chylomicrons. There are three major isoforms of apo E including apo E2, apo E-3 and apo E-4. Apo E-2 differs

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

from apo E-3 by only a single amino acid, Cys being substituted for Arg at residue 158. Apo E4 also differs from apo E-3 by only a single amino acid, Arg being substituted for Cys at residue 112 [15]. Apo E3 and E4 are recognized by hepatic receptors. However, apo E-2 does not bind to these hepatic receptors. Thus, homozygous apo E-2 are unable to clear the chylomicron remnants, resulting in increased risk of coronary heart disease.

1.2.2 Biochemical basis of LDL heterogeneity

LDL particles are a heterogeneous group of particles that differ in size, density and composition. They can be separated into subfractions by density ultracentrifugation or gradient gel electrophoresis. The largest and most buoyant subfraction called buoyant LDL (bdLDL) has a size higher than 26.5 nm and a density range of approximately 1.019 to 1.039 g/mL. The remainder is progressively smaller and denser and known as small, dense LDL (sdLDL). Two main LDL phenotypes have been identified: pattern A, characterized by the predominance of large, buoyant LDL particles and pattern B, characterized by an excess of small, dense LDL particles.

Small, dense LDL is often accompanied by increased triglycerides (TG) and low high-density lipoprotein (HDL). High plasma free fatty acids levels caused by insulin resistance or defects in the acylation stimulatory protein (ASP) pathway can result in increased hepatic uptake of free fatty acids, which leads to greater triglycerides formation. This increased triglyceride production leads to the secretion of larger TG-rich VLDL particles which are called $VLDL_1$. They undergo the process of lipolysis by lipoprotein lipase to form LDL. The TG in the core of VLDL is

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

exchanged for CE in LDL by the action of cholesterol ester transfer protein (CETP), resulting in CE-depleted TG-enriched LDL that are subsequently converted into smaller, and denser particles by hepatic lipase. The TG-enriched LDL is hydrolyzed by HL and then becomes small, dense LDL. The TG-enriched HDL is similarly modified by hepatic lipase and becomes smaller HDL that is catabolized by the kidney. This process causes decreased levels of HDL-C which may interfere with the reverse cholesterol transport pathway [2, 23-25]

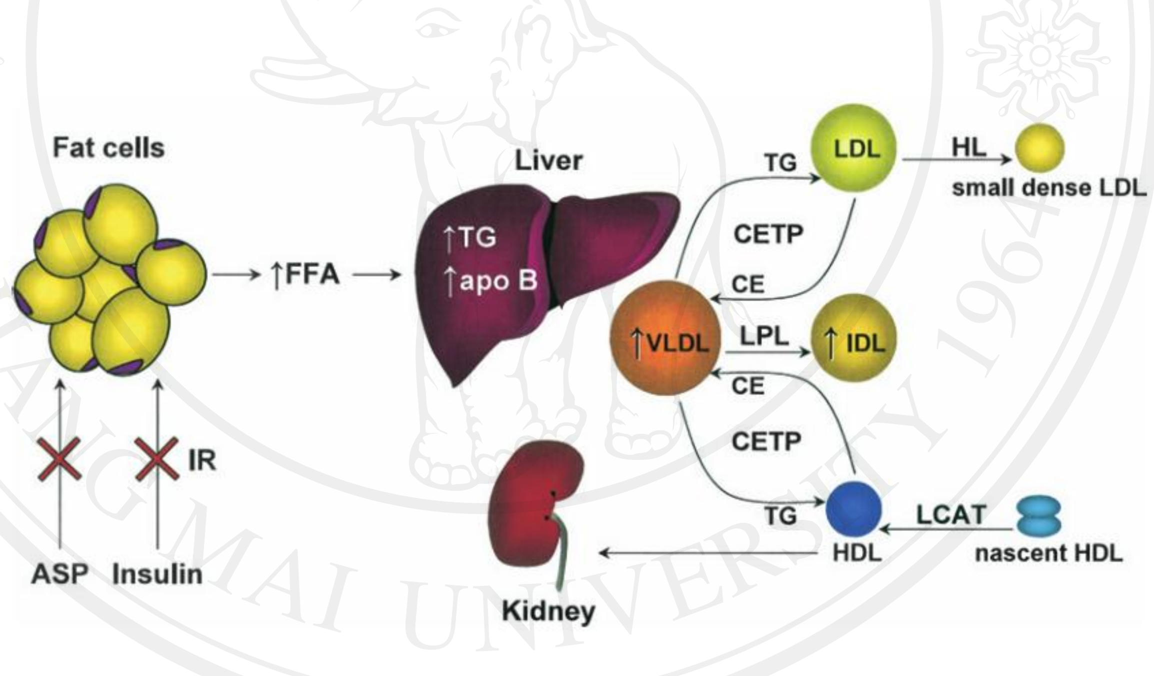

Figure 7 Mechanism of the small, dense LDL production [25]

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

1.2.3. Health effects of LDL abnormalities

Elevated low-density lipoprotein cholesterol (LDL-C) is a risk factor for coronary heart disease (CHD) which is the leading cause of death in the world. It caused by deposition of lipids and waste products in the narrowed and hardened arteries, and is called atherosclerosis.

Studies have demonstrated that lowering of LDL-C decreases the incidence of CHD. The atherogenic risk of elevated plasma LDL-C is due to its small size, as it can infiltrate into the extracellular space of the vessel wall, where it can undergo oxidation and be internalized by macrophages through scavenger receptors. Macrophages that take up high amounts of lipid are transformed into foam cells, which are the predominant cell type of fatty streaks, an early precursor of atherosclerotic plaques.

Atherosclerosis, a major cause of CHD, is a process that causes plaque formation in the arteries which supply blood to cardiac muscle. It involves the deposition of lipid in the arterial wall, which causes the narrowing of the lumen of the artery. The process of atherosclerosis proceeds slowly with the involvement of macrophages, monocytes, lymphocytes and smooth muscle cells.

High levels of low-density lipoprotein (LDL) cholesterol is one of the risk factors for atherosclerosis. Elevation of plasma LDL levels results in penetration of LDL into the arterial wall. When LDL particles become trapped in an artery, they can be oxidized, resulting in the release of LDL oxidation products such as malondialdehyde, 4-hydroxynonenol and lipid peroxides. These products may return to react with apo B-100 of LDL particles. After alteration of antigen expression, the apo B-100 is recognized and taken up by macrophages through the scavenger receptors. This internalization leads to the accumulation of CE in macrophages,

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

leading to their transformation into foam cells. Also, modified LDL is chemotactic for monocytes and can up-regulate the expression of genes for macrophage colony stimulating factor and monocyte chemotactic protein derived from endothelial cells. These new monocytes then enter the lesions.

The earliest type of lesions is called fatty streaks, comprising foam cells, monocytes, and T lymphocytes along with smooth muscle cells. In advanced lesions, the fibrous cap which conceals the lesion covers a mixture of leukocytes, lipid, and debris, which can result to the formation of a necrotic core. Rupture of the fibrous cap that often occurs at the thin site can result in thrombosis. The continuing influx and activation of macrophages results in thinning of the fibrous caps. These macrophages secrete matrix metalloproteinases and proteolytic enzymes which can cause degradation of the matrix, leading to thrombosis and plaque rupture [26, 27].

Interestingly, the presence of small, dense LDL has been associated with an approximately three-fold increased risk for coronary artery disease [28]. Studies have suggested a predictive role for LDL size. It is believed that small, dense LDL particles are more atherogenic than the larger ones due to their lower binding affinity for the LDL receptor, prolonged residence time in plasma, increased penetration into the arterial wall, higher binding to extracellular matrix components of the arterial wall and greater susceptibility to oxidative stress.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

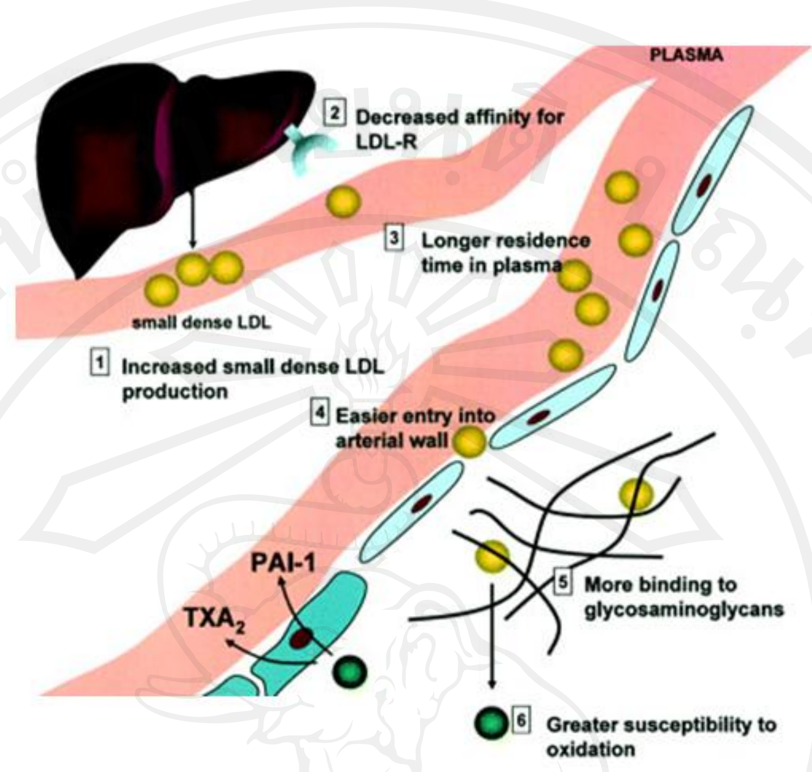

Figure 8 Atherogenicity of small, dense LDL

(http://content.onlinejacc.org/content/vol50/issue18/images/medium/coverfig.gif)

1.2.4. Lipoprotein separation techniques

Several methods have been used for separation of serum lipoproteins. Lipoprotein separations take advantage of physical properties such as density, size and charge. The varied ranges in density of each lipoprotein class enable them to be separated by ultracentrifugation. Electrophoresis separations take advantage of differences in charge and size.

Electrophoresis methods are used to separate the major lipoprotein classes. Agarose gel electrophoresis is the classic technique that is most widely used for separation of the various lipoproteins by differences in their net charges. The fraction must undergo staining with a lipid stain such as Oil Red O. The lipoprotein bands result from the differences in the migration rate of each lipoprotein. High-density

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

lipoprotein (HDL) is an alpha lipoprotein, low-density lipoprotein (LDL) is a beta lipoprotein, very low-density lipoprotein (VLDL) is a pre-beta lipoprotein and intermediate-density lipoprotein (IDL) is located between beta and pre-beta. Chylomicrons are found at or near the point of application [11] (Table 1).

For many years, nondenaturing polyacrylamide gradient gel electrophoresis (GGE) has been used to characterize lipoprotein particle size. Such characterizations have been performed commonly on HDL and LDL. Moreover, there is a composite gradient gel which enables characterization of both LDL and HDL in the same gel by 3-31% PAGE. The preparation of gradient gels is more cumbersome and time-consuming.

Ultracentrifugation methods are used in research laboratories, but are uncommon in the clinical laboratory. The method involves separation of lipoprotein classes from plasma after adjustment of the density with potassium bromide. However, ultracentrifugation suffers from several limitations. It requires huge quantities of plasma or serum. Moreover, it is a time-consuming procedure which may take several days to complete. The long exposure of sample to very high g-forces and salt concentrations may lead to alteration of the lipoprotein or loss of apolipoproteins. Other problems arising are oxidation of the lipoprotein and bacterial contamination. In spite of being expensive and technically demanding, ultracentrifigation remains a major method for separation of lipoproteins. The most widely used nomenclature defines major five classes of lipoproteins based on their density. Chylomicrons are particles that have a density less than 0.94 kg/L. VLDL has a density between 0.94 and 1.006 kg/L. IDL has a density greater than 1.006 kg/L but less than 1.019 kg/L. LDL has a density between 1.019 and 1.063 kg/L. HDL has

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

a density of 1.063-1.21kg/L. According to Guerin's method, there are 12 subfractions of lipoprotein including VLDL (d < 1.017 kg/L), IDL (d= 1.018-1.019 kg/L), LDL-I (d = 1.019-1.023 kg/L), LDL-II (d= 1.023-1.029 kg/L), LDL-III (d = 1.029-1.039 kg/L), LDL-IV (d = 1.040-1.050 kg/L), LDL-V (d = 1.050-1.063 kg/L), HDL_{2b} (d = 1.063-1.091 kg/L), HDL_{2a} (d = 1.091-1.110 kg/L) HDL_{3a} (d= 1.110-1.133 kg/L), HDL_{3b} (d= 1.133-1.156 kg/L) and HDL_{3c} (d = 1.156-1.179 kg/L) by density gradient ultracentrifugation [(29)].

Due to several limitations of current methods, the Quantimetrix lipoprint LDL system was developed. Recently, there has been increased interest in the Quantimetrix lipoprint LDL system because it requires only 25 µL of plasma or serum and has a total analysis time less than 3 hours. Moreover, it is the only method that has been approved by the Food and Drug Administration [(30)]. However, one problem associated with this technique is the expensive cost.

LDL has also been separated by nuclear magnetic resonance (NMR) spectroscopy. It determines lipoprotein subclasses based on the spectral characteristics of methyl groups of the four types of lipids in the particles: phospholipid, cholesterol, CE and triglyceride. The principle is based on the magnetic property specific to lipoproteins that causes the lipids in larger particles to broadcast signals that are characteristically different in frequency and shape from the lipid signals emitted by smaller particles[(31)]. The LDL by NMR includes 3 LDL subclasses. Nuclear magnetic resonance is only performed by LipoScience Inc.

Recently, Hirano *et al.* developed a simple assay for the quantification of sdLDL (d = 1.044-1.063 g/ml). It comprises two steps including precipitation of lipoprotein with density <1.044 g/ml by using heparin-magnesium and subsequent

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

measurement of cholesterol in the supernatant. The lipoproteins with d <1.044 g/ml that are precipitated by heparin and magnesium include the VLDL, IDL, and large, buoyant LDL particles. After precipitation, the supernatant consists of lipoproteins with d >1.044 g/ml including sdLDL and HDL. The sdLDL-C can be quantified by measuring the LDL-cholesterol in the supernatant [(32)].

Previous study provided a novel homogeneous enzymatic assay for determination of sdLDL-C. The reagents were used to eliminate others lipoproteins except sdLDL. Cholesterol was then measured [(33)].

1.2.5. Proteomics

The Human Genome Project (HGP) began in 1990 with a major goal to identify the approximately 20,000-25,000 genes in human DNA [(34)]. This knowledge provides clues to understanding diseases and how to diagnose and treat them. However, alternative splicing and post translational modification (glycosylation, phosphorylation, etc) results in different forms of proteins. Hence, these 20,000-25,000 genes identified in human genome might produce millions of different proteins.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

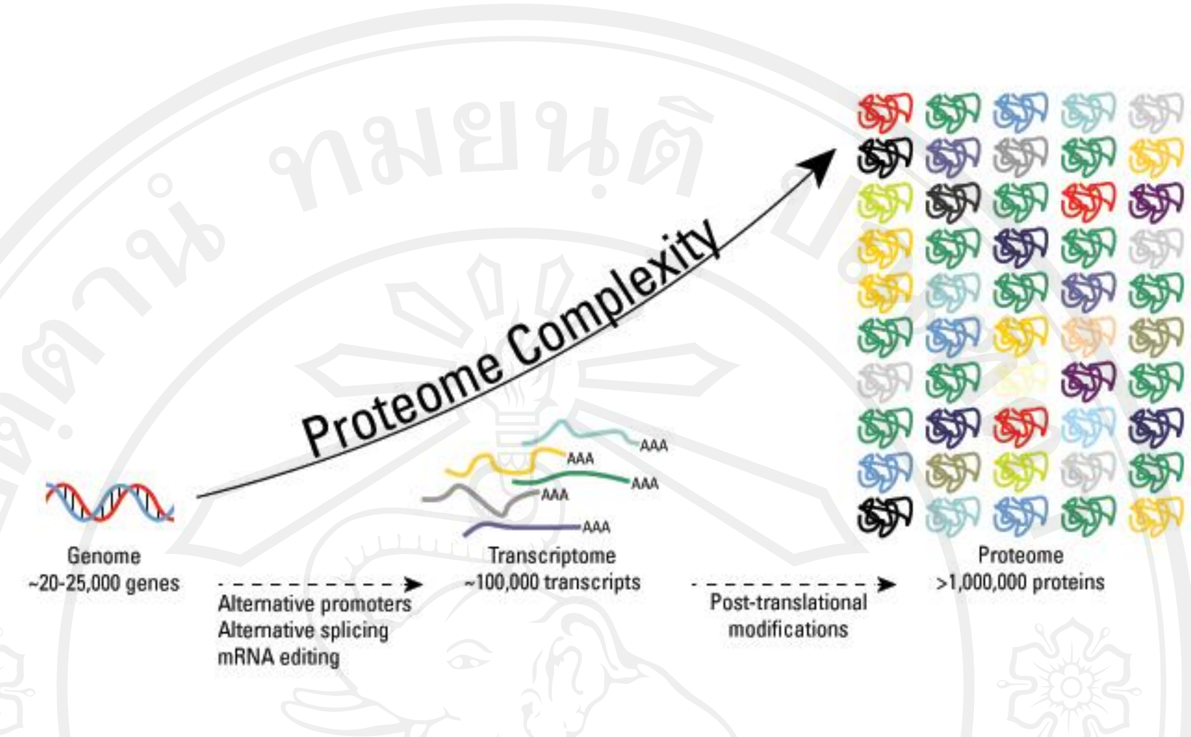

Figure 9 Schematic overview of proteomics diversity [35]

The term "proteome" refers to the set of proteins expressed by the genome. It is more complex than the genome due to alternative splicing and post translational modification (PTM). Several different proteins can be produced from each gene through RNA splicing and PTM. In eukaryotes, a pre-messenger RNA (pre-mRNA) must undergo several processes before translation. Most eukaryotes genes consist of introns and exons. After transcription, introns of pre-mRNA are removed by a splicing process, which leaves the resulting exons that are aligned in mRNA [36]. Different mRNAs translate into different protein isoforms. Therefore, a single gene may code for various proteins.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

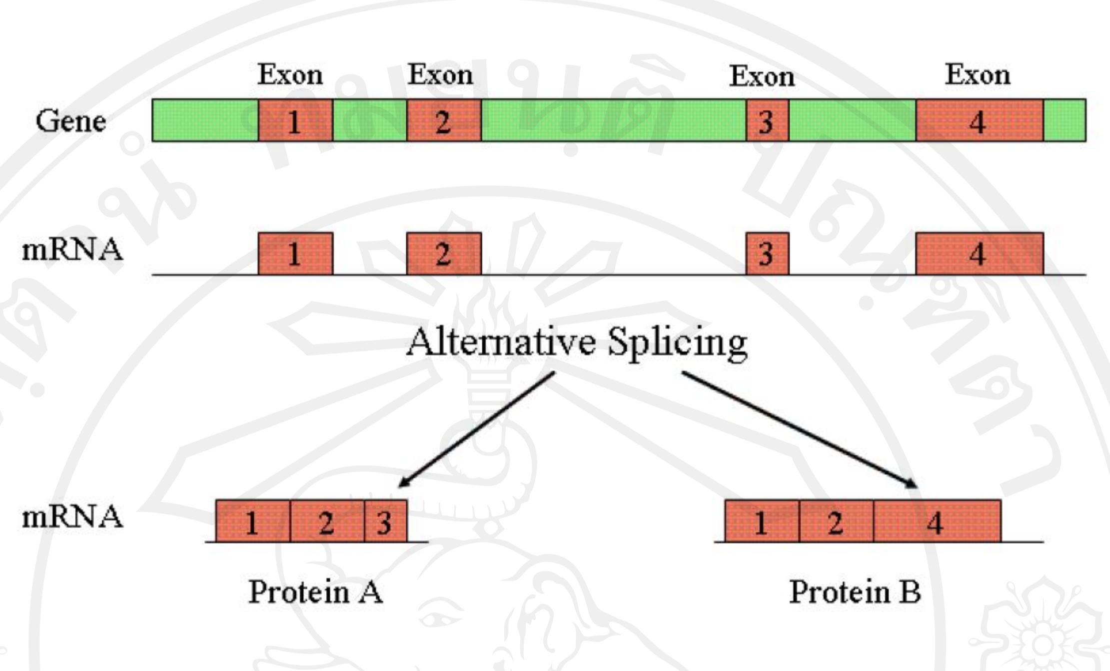

Figure 10 Schematic representation of alternative splicing [37].

PTM is a covalent addition of functional groups and which occurs at the side chains of proteins; it includes phosphorylation, methylation, acetylation, glycosylation, etc. It is also a process of covalent cleavage of peptide backbones by the action of proteases [38]. Hence, a gene product can be translated into several different modified forms of protein.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

1. Covalent modification

2. Cleavage of protein backbone

Figure 11 Schematic categories of PTM 1) covalent modification of amino acid side chain 2) cleavage of the backbone at a specific peptide bond [38]

Proteomics is the study of proteins and their interactions. Protein separation techniques had developed such as two- dimensional gel electrophoresis (2-DE) and liquid chromatography (LC), combined with the analysis of proteins using mass spectrometry (MS).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Proteomics technologies

Two-dimensional gel electrophoresis (2-DE)

Background

Gel electrophoresis is a method used to separate molecules by charge and size. The most widely used methods are sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). It separates different proteins according to size as they migrate through gel matrix. Isoelectric focusing (IEF) is also a common way to separate proteins according to their isoelectric point.

Since SDS-PAGE alone can be insufficient to separate numerous proteins, there was great interest in coupling two independent separation techniques. 2-DE is a technique for separation of proteins based on charge and mass. Proteins are separated by charge in the first dimension and by mass in the second dimension. There are 5 steps in the process including sample preparation, first separation using isoelectric focusing, equilibration with SDS buffer in the presence of DTT and iodoacetamide (IAA), second separation using SDS-PAGE and protein visualization. However, the reverse dimensions are used as well. Most studies have used SDS-PAGE in the first dimension [39, 40]. The reason that SDS-PAGE is commonly used in the second dimension are easier staining, and better performance of further analysis techniques. Blotting and mass spectrometry are very sensitive to the ampholytes that are used during isoelectric focusing process [41].

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

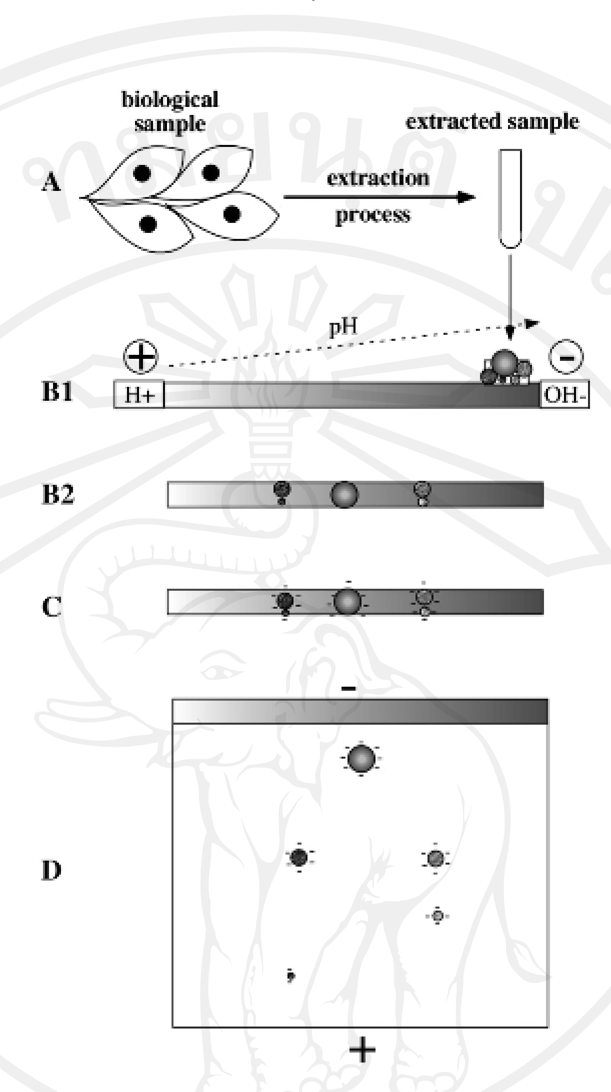

Figure 12 Principle of two dimensional gel electrophoresis [41]**.** (A) Proteins are extracted from a biological sample. (B1). Extracted sample is loaded onto pH gradient. (B2) Proteins will move through the gel matrix until they reach the pH that is equal to their pI. (C) Proteins carry negative charge by equilibrating in SDS-buffer. (D) Proteins are separated according to their mass.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

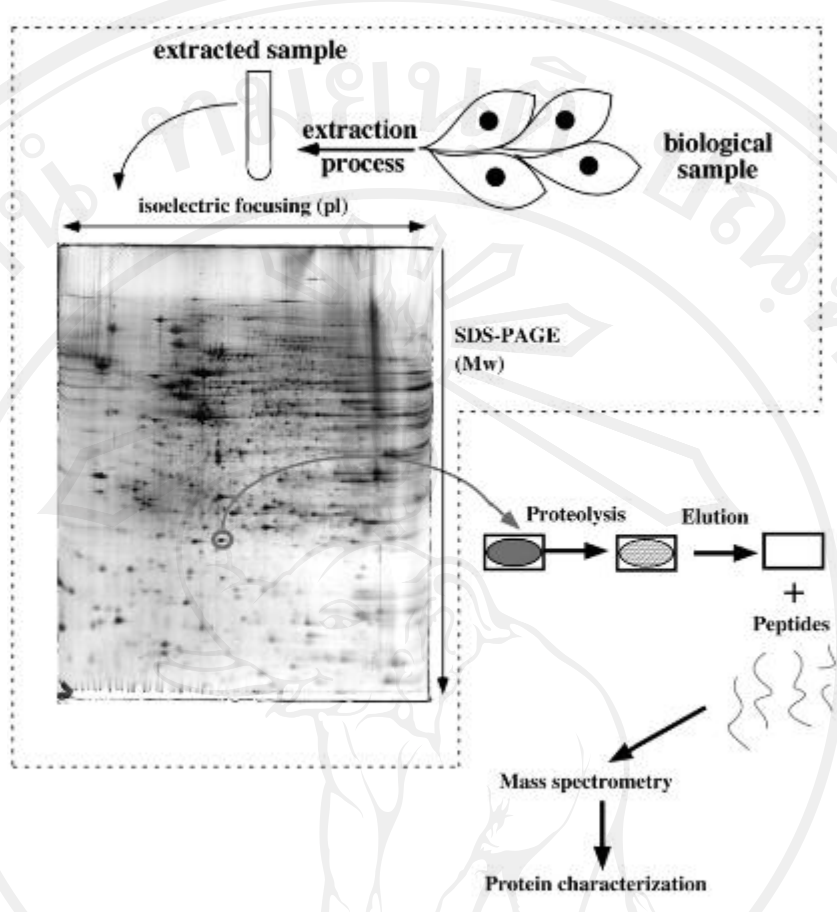

Figure 13 Overview of 2-DE method [41]

Sample preparation

The sample should be completely soluble in lysis buffer. The protein charge must not be altered; this may be accomplished by avoiding charged detergents such as SDS. Even though SDS is a potential solubilizing detergent for hydrophobic proteins, its anionic charge provides limitation for a nondenaturing IEF electrophoresis. The zwitterionic chaotropic agents such as urea and thiourea [42] are often used to denature proteins. They disrupt hydrogen bond structures in proteins. However, these agents cannot dissolve cellular lipids efficiently. Another neutral detergent is always added

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

such as CHAPS (3-[(3-cholamidopropyl) dimethyl ammonio]-1-propanesulfonate). Since SDS cannot be used in IEF buffer, hydrophobic and membrane proteins become a limitation due to lack of detergents for solubilization [43]. One of the major causes for 2-DE streak pattern is the poor protein solubilization. Hence, the choice of detergent in the sample solution should deserve special attention. The presence of salt in the sample disturbs isoelectric focusing, which result in streak patterns in 2-DE gels. Special attention also has to be paid to protein modifications. Several different proteins can be produced from post translational modification such as phosphorylation and glycosylation. However, there are modifications during the sample preparation such as oxidation.

Table 3 Common modifications [44]

Residue (3 & 1 letter symbols)	*Residue structure (R = side chain)*	*Average mass*	*Monoisotopic Mass*	*Common modifications (and nominal molecular weight additions)*
Glycine (Gly & G)	OC—CH—NH, with H	57.05	57.02146	
Alanine (Ala & A)	OC—CH—NH, with CH_3	71.08	71.03711	
Serine (Ser & S)	OC—CH—NH, with CH_2OH	87.08	87.03203	Phosphorylation Glysosylation
Proline (Pro & P)	OC—CH—N—, ring	97.12	97.05276	
Valine (Val & V)	OC—CH—NH, with $CH_2CH_2CH_3$	99.13	99.06841	
Threonine (Thr & T)	OC—CH—NH, with $CH(OH)CH_3$	101.11	101.04768	Phosphorylation Glysosylation (*O*-linked sugars)

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3 (Continued) Common modification

Residue (3 & 1 letter symbols)	*Residue structure (R = side chain)*	*Average mass*	*Monoisotopic Mass*	*Common modifications (and nominal molecular weight additions)*
Cysteine (Cys & C)	OC—CH—NH, R = CH_2SH	103.15	103.00919	Carbamidomethyl *S*-propionamide *S*-pyridylethyl
Leucine (Leu & L)	OC—CH—NH, R = $CH_2CH(CH_3)_2$	113.16	113.08406	
Isoleucine (Ile & L)	OC—CH—NH, R = $CH(CH_3)CH_2CH_3$	113.16	113.08406	
Asparagine (Asn & N)	OC—CH—NH, R = $CH_2C(=O)NH_2$	114.10	114.04293	N-linked glycosylation (Sequon Asn-X-Ser/Thr)
Aspartic acid (Asp & D)	OC—CH—NH, R = $CH_2C(=O)OH$	115.09	115.02694	Esterification, methyl ester
Glutamine (Gln & Q)	OC—CH—NH, R = $CH_2CH_2C(=O)NH$	128.13	128.05858	
Lysine (Lys & K)	OC—CH—NH, R = $(CH_2)_4NH_2$	128.17	128.09496	Acetylation Homo-arginine
Glutamic acid (Glu & E)	OC—CH—NH, R = $CH_2CH_2C(=O)OH$	129.16	129.04259	Esterification, methyl ester
Methionine (Met & M)	OC—CH—NH, R = $CH_2CH_2SCH_3$	131.20	131.04049	Oxidation

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 3 (Continued) Common modifications

Residue (3 & 1 letter symbols)	*Residue structure (R = side chain)*	*Average mass*	*Monoisotopic Mass*	*Common modifications (and nominal molecular weight additions)*
Histidine (His & H)	OC— CH — NH, CH_2, imidazole ring (H, N, N)	137.14	137.05891	
Phenylalanine (Phe & F)	OC— CH — NH, CH_2, phenyl ring	147.18	147.06841	
Arginine (Arg & R)	OC — CH — NH, HN — $(CH_2)_3$, H_2N—C=NH	156.19	156.10111	
Tyrosine (Tyr & Y)	OC — CH — NH, CH_2, HO-phenyl ring	163.18	163.06333	Phosphorylation
Tryptophan (Trp & W)	OC— CH—NH, CH_2, indole ring (H, N)	186.21	186.07931	Oxidation

Isoelectric focusing

In isoelectric focusing, proteins are separated based on their charge (isoelectric point, pI) in a pH gradient generated by carrier ampholytes. The net charge of the protein is the sum of positive and negative charges of the amino acid side chains of proteins. When protein mixtures are applied in a pH gradient, different proteins have different net charge. Proteins migrate in a pH gradient until they reach a position where their net charges are zero. Proteins with positive charge move toward the cathode, while, proteins with negative charge move toward the anode to a pH where proteins have no net charge [44]. First attempts aimed to create a polyacrylamide gel with a pH gradient in a test tube [45]. Recently, commercial IPG (immobilized pH

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

gradient) strips have been widely used. They are acrylamide gels that contain a built-in pH gradient. These dried gel strips must be rehydrated prior to use.

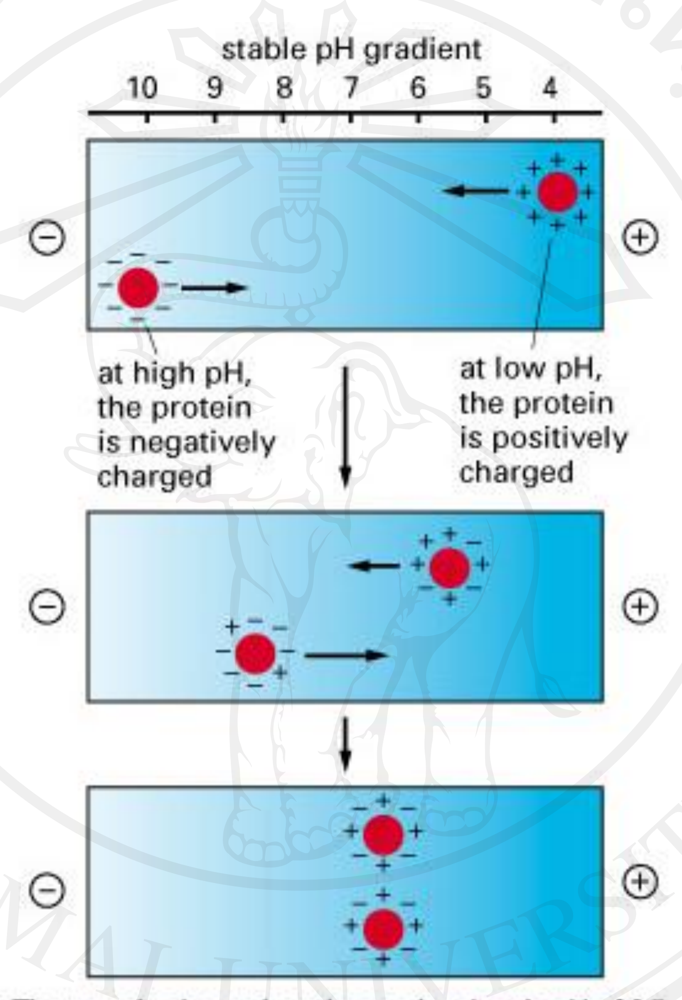

Figure 14 Schematic representation of protein separation by isoelectric focusing

(http://www.channelwolf.com/lvv/sem6/index_files/image1749.jpg)

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Equilibration

Prior to the second dimension run, the IPG strips are equilibrated with a solution containing DTT, IAA and SDS. Equilibration is the process of preparing proteins for the second dimension. Proteins should be completely unfolded and carry negative charges. There are two steps during the process. Dithiothreitol (DTT) is added in order to cleave disulfide bonds between cysteine residues and complete unfolding of proteins. It reduces disulfide linkages to free sulfhydryl groups in proteins. To prevent the reforming of disulfide bonds, iodocetamide (IAA) is used as an alkylating agent. It binds covalently with thiol groups of cysteine, converting them to carbamidomethyl cysteine, thus preventing formation of disulfide bonds. Alkylation with iodoacetamide results in the covalent addition of a carbamidomethyl group and prevents disulfide bond formation. Proteins that were reduced, alkylated and saturated with SDS are ready for transferring to the second dimension.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

After equilibration, IPG strips are applied onto the edge of the SDS gel. Hot agarose is added in order to accomplish the gel continuity, avoid having the strip floating in buffer solution and to prevent air bubbles [44]. Proteins are separated according to their molecular weight. SDS-proteins complexes are formed and move toward the anode.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

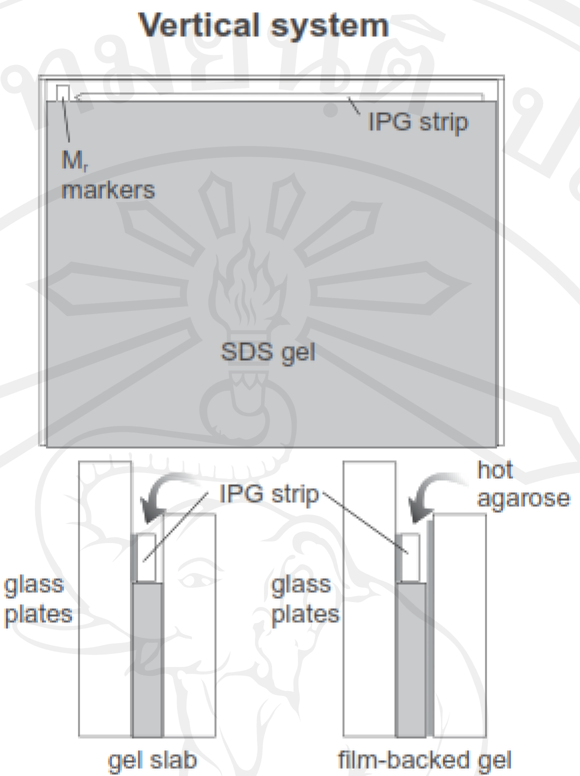

Figure 15 Transfer of the IPG strip ontp the SDS gel [44]

Visualization method

Coomassie Brilliant Blue (CBB)

Proteins can be visualized by numerous staining methods. The classical technique that is most widely used for 2-DE visualization is Coomassie Blue staining. It is a chemical form of disulfonated triphenylmethane compound. Coomassie G has a greenish tint, while Coomassie R has a reddish tint of the blue dye. Coomassie G differs from Coomassie R by the addition of two methyl groups. The mechanism of gel staining is based on the formation of protein/dye complexes with van der Waals forces and electrostatic interactions, which stabilizes the negatively charged anionic form of the dye, producing the blue color. It is necessary for background destaining

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

of classical Coomassie-staining gels. Modern protocol with G colloidal form was introduced for the purpose of avoiding the destaining problem, while original CBB-gels suffered from highly colored background staining.

Silver staining

Silver staining is one of the protocols used for protein detection by electrophoresis separation. The general procedure includes acidic and alkaline silver stain. The mechanism depends on an oxidation step followed by a reduction step that converts silver ions into metallic silver. For the alkaline method, formation of silver diamine complexes are developed in an alkaline environment (ammonium hydroxide), followed by reduction of free silver ions to metallic silver in an acidic developer with formaldehyde. On the other hand, the acidic method uses silver nitrate in water (weakly acidic pH), followed by reduction of free silver ions at alkaline pH in solution with formaldyhyde (carbonate, thiosulfate). Thiosulfate is added in order to reduce the background [46, 47]. There are many steps during the process including (i) fixation, to precipitate proteins and remove interference compounds in gels (Tris, glycine, SDS (ii) sensitization, to increase image formation. The compounds used in this step bind to proteins and silver ion, or reduce silver ion to metallic silver [48, 49] (iii) silver impregnation with acidic or alkaline silver and (iv) image development with formaldehyde in carbonate solution (acidic stain) or citric acid (alkaline stain) [47, 50]. Silver staining is more sensitive than Coomassie staining. However, some silver stain methods are not compatible for protein identification with mass spectrometry (MS). Some silver stain procedures require glutaraldehyde, which alkylate α-amino groups of proteins [46]. Acidic silver staining is compatible with MS via omitting

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

glutaraldehyde from the sensitizing solution and formaldehyde from the silver solution.

Fluorescent staining

Recently, there has been heightened interest in fluorescent staining due to its high sensitivity and compatibility with mass spectrometry. There are several fluorescent dyes used, such as Sypro dye. The mechanism of Sypro Ruby staining depends on the binding of ruthenium metal chelate and basic amino acids of proteins [47]. In spite of its high sensitivity and linearity, fluorescent stain procedures require special instrumentation. Furthermore, the expensive cost is considered as a major factor of this protocol.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 4 Staining methods in 2-DE [47]

Method	Detection mode	Detection limit (ng)	MS compatibility
Coomassie Blue-R	Colorimetry	8-10 [51] 50-100 [52]	+
Coomassie Blue-G (colloidal)	Colorimetry	8-10 [53] 10-20 [52] 30-100 [46, 51]	+
Silver nitrate (acidic methods)	Colorimetry	1 [51, 53] 3-5 [53]	+ (if without glutaraldehyde [52])
Silver ammonia (alkaline methods)	Colorimetry	<1 [52] 5-10 [52]	- +
Zinc imidazole	Colorimetry	10 [53, 54] 1 [55]	+
SYPRO® Ruby	Fluorescence	1 [52, 53] 1 [51]	+
SYPRO® Orange, Red, Tangerine Epicoccone	Fluorescence	4-8 [51] 30 [53]	+

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Liquid chromatography (LC)

LC separation can link directly to the mass spectrometer. LC coupled to mass spectrometry (LC-MS) is a powerful technology for proteins analysis. It combines the effective separation and identification techniques. Since proteins are digested with trypsin, they become a mixture of peptides. Separation is necessary in order to detect peptides in the mixture as much as possible. Liquid chromatography is a method used to separate the peptides according to their hydrophobicity. The mobile phase of LC usually contains water and a solvent such as acetonitrile (ACN). Acid (formic acid, trifluoroacetic acid or acetic acid) is often added to render the components of proteins and aid protonation of the sample molecules [56]. Since peptides have positive charges, they can be analyzed by mass spectrometry in positive mode.

Mass spectrometry (MS)

Mass spectrometry is a powerful technology used in proteomics for mass determination and can be applied to protein identification. The mass spectrometer consists of two major components including the ionization source and the mass analyzer. The principle of MS is based on ion formation of sample molecules. These ions can be analyzed according to their mass to charge (m/z) ratio. The molecule is ionized and transferred to the mass analyzer.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Ionization method

The ion source generates the gas-phase ions from sample molecules. Several ionization methods are used, such as electrospray ionization (ESI), matrix-assisted laser desorption ionization (MALDI) and surface-enhanced laser desorption ionization (SELDI). The most widely used technique coupled to LC is ESI.

Electrospray ionization (ESI)

It should be mentioned that ESI is often coupled with a separation method such as liquid chromatography. It requires the liquid-phase sample to flow into the ionization source. Ions from sample solution are produced and become gas-phase ions. The process involves the application of an electric field to a capillary tip from which the sample is flowing. The solution containing the analytes eluting from the chromatography column is transferred to a silica capillary. High voltage (>1.5 kV) is applied to create ions and induce the spray formation of charged droplets. Solvent is then evaporated from the charged droplet by application of the nebulizer gas. The analytes become single charged.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

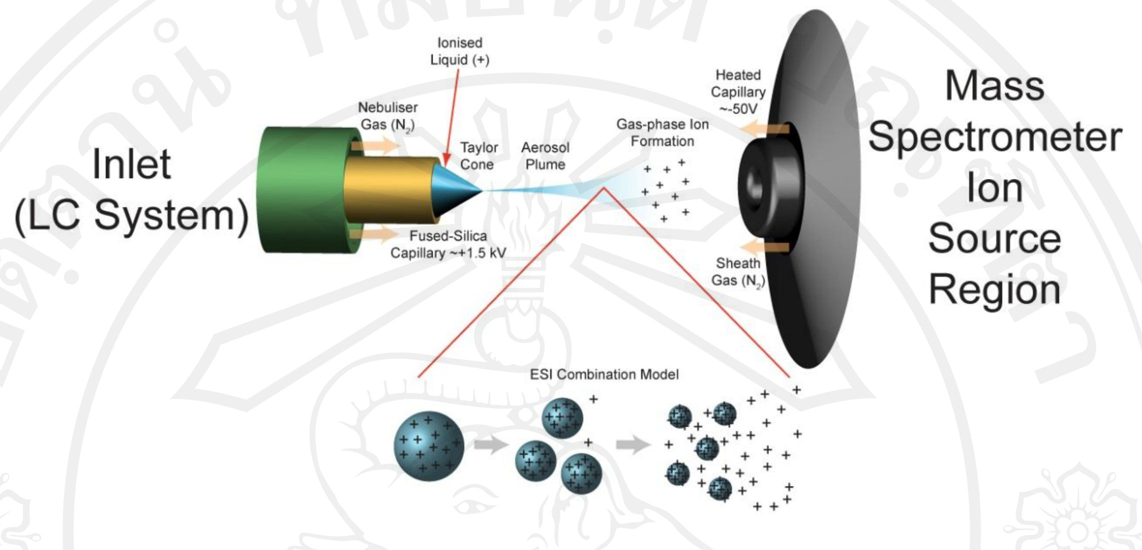

Figure 16 Schematic representation of electrospray ionization. It generates ions by introducing the solution of samples through a needle at high voltage. By application of nebulizer gas, the solvent is evaporated from each droplet. Droplets of one polarity are generated and transferred into the mass analyzer. (http://www.lamondlab.com/MSResource/LCMS/MassSpectrometry/electrosprayIonisation.php)

Mass analyzer

The mass analyzer is a device that measures the m/z ratio of gas-phase ions. Once the ions are formed, they are transferred to the mass analyzer to measure the m/z ratio. Several different types of analyzer have been developed, such as time of flight (TOF), the quadrupole, and ion trap.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Ion trap

The ion trap mass analyzer contains the three electrodes, including the ring electrode, the entrance endcap electrode and the exit endcap electrode. The principle of ion trap is based on trapping of ions in electric fields and manipulating them after they are trapped. The trajectories of trapped ions become unstable when the electric field is changed. Changing the electrode voltages selects which ions are trapped and ejected to a detector. The ions are trapped by the oppositely charged walls of the trap analyzer. Manipulation of the electrode voltage permits selected ions to leave the trap and move toward the detector. These ions exit the trapping field according to their m/z ratio. For MS/MS, specific ions are selected and undergo collision with helium gas, which result in fragmentation of precursor ions, and the voltages are adjusted to eject other ions from the trap [57-59].

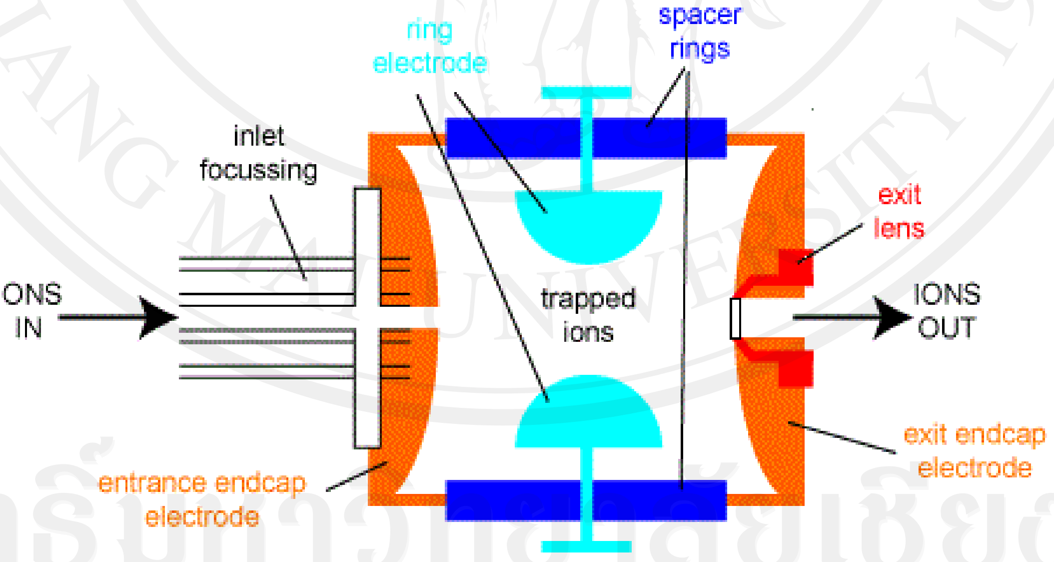

Figure 17 Structure of the ion trap

(http://www.chm.bris.ac.uk/ms/images/iontrap-schematic.gif)

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Tandem mass spectrometry (MS/MS)

The ions can be transferred into a tandem mass spectrometer. Peptides separated by LC are transferred to the first analyzer. The first stage displays the m/z ratio of parent peptide ions (MS1). This m/z data is not sufficient for identification of specific peptides. The next step, collision-induced dissociation (CID), is used to fragment selected parent peptide ions by breaking the peptide bonds. The fragmentation spectrum (MS2) of this stage can be analyzed for protein matching against theoretical spectra based on sequence data from genome sequencing project.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

1.3 Objectives

1.1.1 To develop techniques for identification of proteins of sdLDL and bdLDL

1.1.2 To compare protein profiles of sdLDL and bdLDL molecules using proteomic techniques

1.1.3 To identify novel proteins of LDL

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved