J. Food Sci. Nutr.
Vol. 4, No. 2, p. 145~151 (1999)

Comparison of Surface and Core Peptide Fraction from Apo B-100 of Human LDL (Low Density Lipoprotein)

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Abstract

Apolipoprotein B-100 (apo B-100) is an important component in plasma low density lipoproteins (LDL). It functions as the ligand for the LDL receptor in peripheral cells. The LDLs are removed from the circulation by both high-affinity receptor-mediated and receptor-independant pathways. LDLs are heterogeneous in their lipid content, size and density and certain LDL subspecies increase risk of atherosclerosis due to differences in the conformation of apo B in the particle. In the present study, surface and core peptide fraction of Apo B-100 have been characterized by comparing peptide-mapping and fluorescence spectroscopy. Surface fragments of apo B-100 were generated by digestion of LDL with either trypsin, pronase, or pancreatic elastase. Surface fractions were fractionated on a Sephadex G-50 column. The remaining core fragments were delipidated and redigested with the above enzymes, and the resulting core peptides were compared with surface peptides. Results from peptide-mapping by HPLC showed pronase-digestion was more extensive than trypsin-digestion to remove surface peptide fraction from LDL. Fluorescence spectra showed that core fractions contained higher amount of tryptophan than surface fractions, and it indicated that core fraction was more hydrophobic than surface fractions. A comparison of the behavior of the core and surface provided informations about the regions of apo B-100 involved in LDL metabolism and also about the structural features concerning the formation of atherosclerosis.

Key words: LDL (low density lipoprotein), apo B-100, core and surface fraction

INTRODUCTION

Apolipoprotein B-100 (apo B-100) is an important component in the system of plasma low density lipoproteins (LDL). It has functions as the ligand for the LDL receptor in peripheral cells. In humans, much of the apo B-100 of VLDL is transferred to IDL and subsequently to LDL. Recent studies in the rats (1-3) as well as in men have suggested that apo B exists in two forms : apo B-100 and apo B-48. Apo B-100 is synthesized by the liver and is an obligatory constituent of VLDL, IDL, and LDL (4,5).

The LDLs are removed from the circulation by both high-affinity receptor-mediated and receptor-independant pathways, the liver being the major organ responsible for LDL clearance (2). LDL are removed less efficiently by the hepatic receptors through binding to apo B-100, gradually gain access to extravascular compartments of various organs and tissues which contain LDL receptors, so that an appreciable fraction is taken up in extrahepatic tissues as well (4,5). The distribution of LDL to various tissues depends mainly on the rate of transcapillary transport and the activity of LDL receptors on cell surfaces.

Apo E as well as apo B-100 contain a recognition site for the LDL receptor. The human LDL receptor is a transmembrane protein of 839 amino acids (6,7). It's NH_2-terminal portion, which is rich in cystein residues, is composed of a 7-fold repeat, and which is an octapeptide sequence that contains three aspartyl residues and one glutamyl residue. These negatively charged regions presumably constitute the ligand-binding sites of the receptor which interact electrostatically with the positively charged region of the apolipoprotein ligands (8-11).

Although the primary structure of apo B-100 has been determined, its tertiary structure and conformation on LDL are still poorly understood. In the present study a number of apo B-100 core and surface peptides by enzyme digestion have been characterized by comparing with peptide mapping, fluorescence spectroscopy. A comparison of the behavior of the core and surface should provide information about the regions of apo B-100 involved in LDL metabolism and also about the structural features concerning the formation of atherosclerosis.

Purpose of this study was to chracterize the surface and core peptide fraction of apo B-100 which acts as the ligand for the LDL-receptor.

MATERIALS AND METHODS

Materials

Trypsin, pronase and elastase were purchased from Sigma chemical Co. (St. Louis, USA). Acetonitrile, methanol, HPLC grade water, and other solvents for HPLC were obtained from E. Merck (Darmstadt, Germany) and J. T. Baker (Phillipsburg, USA). DMPC was obtained from Sigma chemical Co. (St. Louis, USA).

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Isolation of low density lipoproteins

The blood from healthy donors was used, and aprotinin (0.055 Units/ml), 0.05% EDTA and 0.05% NaN_3 were added to plasma. The LDL, 1.025 < d < 1.055 g/ml, were isolated by sequential ultracentrifugation using a KBr gradient at 40,000 rpm for 15 hrs at 4°C, followed another run for 24 hrs under the same conditions (4,5). The protein concentration of the LDL was determined by the method of modified Lowry et al. (12). Purified LDL was dialyzed against 0.1 M ammonium bicarbonate (NH_4HCO_3), pH 8.0 buffer with a No. 2 dialysis bag (Spectra/por). The purification of LDL was identified by 5~14% SDS-PAGE and the result was identified if there was only one protein band at the position of apo B-100.

Enzyme digestion

Enzymatic digestion of LDL was performed with trypsin, pronase, and elastase (Fig. 1). The enzyme/substrate ratio was 1:150 (w/w). The mixture was stirred at room temperature for 8 hrs. The enzyme hydrolysate was applied to a Sephadex G-50 (2.6×40 cm) column at a flow rate of 4 ml/10 min. Each 4 ml fraction was monitored by ultraviolet absorbance at 220 nm (13,14). Surface fractions of apo B-100 generated by digestion of LDL with trypsin, pronase or elastase and the remaining core fragments were pooled and lyophilyzed. The lyophilyzed core fragments were delipidated by being extracted three times with 50 ml of ether/ethanol (3:1, v/v). Each extraction was carried out by adding the solvent to the samples by vortexing and placing the sample in a freezer (-20°C) for 1 hr, and then centrifuging at 2,000 rpm and removing the solvent by aspiration. The core fragments were redigested with the trypsin, pronase, and elastase (enzyme/substrate ratio was also 1:150). Fifty μl of surface and core fractions were used for the identification with 5~14% gradient SDS-PAGE.

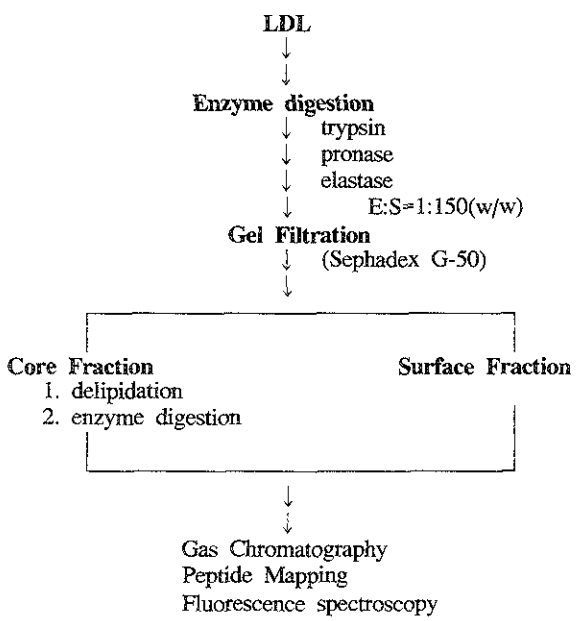

Fig. 1. Separation of surface and core peptide fraction from apo B-100 of human LDL.

Gas chromatography

Gas chromatography analysis was performed with a Hitachi model 163 equipped with F.I. detector (15-18). A 2 m×3 mm (ID) glass column containing GP 3% SP-2310/2% SP-2300 on 100/120 chromosorb was used for the GLC analysis. The temperature was as follows; injector, 220°C; column, 130°C for 1 min, and detector, 220°C. The flow rates were: nitrogen, 39 ml/min; hydrogen, 36 ml/min; and air, 500 ml/min. The sensitivity and sample size were 8×102 afs and 1 μl, respectively. Chart speed was 10 mm/min (15,17).

Peptide mapping

Core and surface fragments of apo B-100 were fractionated on a Waters HPLC system equipped with a variable wavelength detector. The column temperature was set at 50°C. For fractionation, a Vydac C18 reverse phase column (1.0 ×25 cm) was used with a trifluoroacetic acid (TFA) buffer system at a flow rate 1.5 ml/min, buffer A: 0.1% TFA in water (v/v), buffer B: 0.08% TFA on 95% acetonitrile and 5% water (v/v/v). A linear gradient of buffer B at 1% B per minute was applied. The eluted fractions were monitored at 220 nm.

Fluorescence spectroscopy

The measurements of the tryptophan (Trp) fluorescence emission were used to monitor core and surface fragments of apo B-100. Fluorescence studies were performed at 25°C using SFM-25 spectrofluorimeter using 1 cm cuvette. Each fraction (trypsin core, trypsin surface, pronase core, pronase surface, elastase core and elastase surface) was dissolved in 0.1 M NH_4HCO_3 (pH 8.0) buffer. Total fluorescence emission spectra were recorded from 320 nm to 450 nm with excitation at 300 nm (13,15,19,20).

RESULTS AND DISCUSSION

Isolation of LDL

LDL was separated by sequential ultracentrifugation and apo B-100 is the only one apoprotein of LDL. The concentration of pure LDL was 13.2 mg/ml when protein concentration was quentitated at 280 nm. The purification of LDL was identified by 5~14% SDS-PAGE and the purity was identified if there was only one protein band at the position of apo B-100.

Enzyme digestion of LDL

To remove surface fraction from lipid-containing core fraction, LDL was digested with trypsin, pronase and elastase (Fig. 1). Two nonspecific cleavage enzymes, pronase and elastase, were chosen to compare the effect of trypsin, a specific-cleavage enzyme. The trypsin is a pancreatic serine protease with substrate specificity based upon positively charged lysine and arginine side chains. But, the pronase is a nonspecific endogenous and exogenous cleavage of apo B-100 polypeptide. Proteolytic hydrolysis of apo B-100 by either trypsin or pronase generated peptides with mass from approximately 20-70 KD. Therefore, pronase-digested peptides showed much smaller size than trypsin-digested peptides. The first group of

peptides was labelled as trypsin-surface (TS) or pronase-surface (PS), and the second group as trypsin-core (TC) and pronase-core PC). Also, apo B-100 peptides digested by trypsin, pronase and elastase were compared with HPLC peptide mapping on a Vydac C_{18} column and TFA buffer system (Fig. 2). The peptide mapping of trypsin, pronase and elastase-digested peptides showed the lipid-associated core fraction, in the range of the retention time from 55 min to 70 min. Surface peptide fractions are seen from 10 min through 45 min. Differences were shown on surface peptide fractions between trypsin-igested and pronase-digested apo B-100 peptides. Surface fraction of trypsin-digested peptides appeared from 18 min to 45 min, while that of pronase-digested peptides were distributed between 12 min and 40 min. On reverse-phase chromatography, the more non-polar protein sample components interact more with the relatively non-polar column packing (3,4). Thus, polar and smaller-size peptides elute earlier than non-polar and larger peptides. Therefore, pronase-digested peptide was smaller than trypsin-digested peptide.

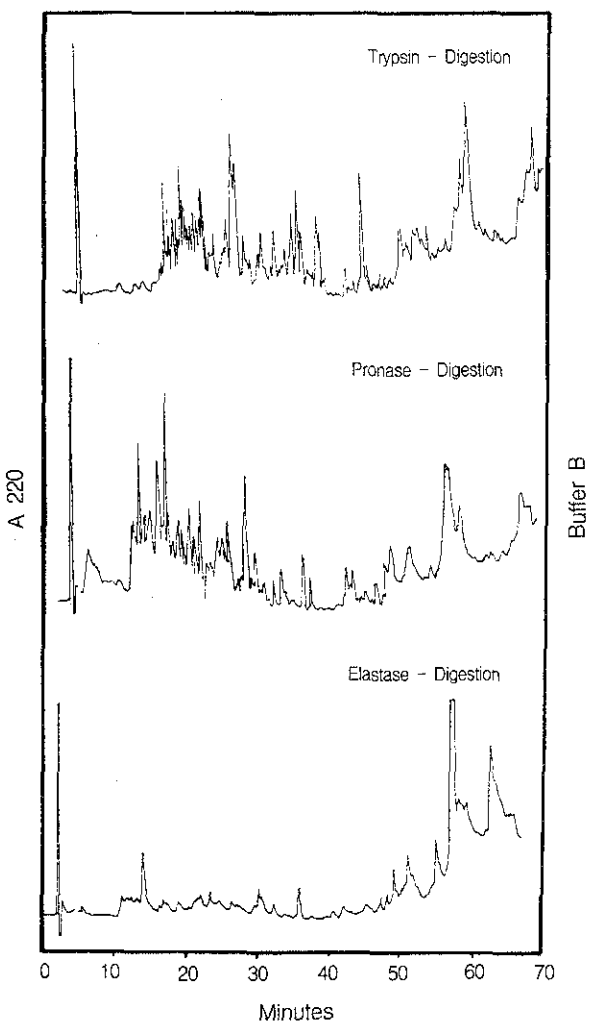

Fig. 2. HPLC separation of apo B-100 digested with trypsin, pronase and elastase. Apo B-100 peptides digested by trypsin, pronase and elastase were compared with HPLC peptide mapping on a Vydac C_{18} column and TFA buffer system.

It is evident from these results that pronase digestion was more extensive to remove exposed surface protein fraction from LDL compared to trypsin digestion. However, elastase digested peptide did not show any significant peaks on HPLC peptide mapping. It might be due to the structural properties of elastase. Elastase hydrolyzes peptide bonds on the carboxyl side of small uncharged side chains. And, binding sites of elastase have pocket which have valine and threonine residues present large apolar side chains from fitting. Therefore, large size of LDL could not be fitted to the binding sites of elastase for hydrolysis. Apo B-100 polypeptide in LDL could be divided into five domains based on the susceptibility of the region to trypsin digestion release (3,4). We performed hydrolysis with pronase and elastase as well as trypsin, because tertiary and conformation on LDL are poorly understood. Also, we have studied the characterization of surface and core fraction in order to search for physical features of inside and outside of apo B-100.

Separation of surface and core peptide fraction

Digested surface peptide fraction is separated from lipid-associated core fractions by passing through Sephadex G-50 gel permeation chromatography (Fig. 3). The amount of surface peptide fractions by three enzymes was well accorded with the degree of enzyme hydrolysis activity. PS showed the largest amount of surface peptide fraction compared to TS and ES, which means pronase acts as an nonspecific enzyme on LDL. Small amount of ES was same as the result from the peptide mapping of elastase digestion. Lipid-containing core fraction was delipidated with ether/ethanol (3:1, v/v) and redigested with the enzyme to compare with surface-peptide fraction.

Fatty acid composition of LDL

Fig. 4 shows an extraction pattern of fatty acids from LDL. For characterization of fatty acid composition, the extracted lipid-moiety was separated into two subclasses, first extraction and second extraction. The major fatty acids were palmitic (16:0), oleic (18:1) and linoleic (18:2) acid, which amounts approximately 60% of the total fatty acid content. Palmitoleic (16:1) and stearic (18:0) were constituted of the remaining 10%. From the result above, the major fatty acids of LDL were palmitic and linoleic acid. These findings are very similar to Sattler et al. (21) who reported that the major fatty acids present in LDL are palmitic (16:0), oleic (18:1) and linoleic (18:2) acid, amounting to approximately 85% of the total fatty acid content. However, the amount of oleic acid showed higher value, 20% compared to the result from Fig. 4, 8.9%. It might be due to the differences in the diet pattern between Koreans and Westerners.

Peptide mapping of delipidated core peptides

The core fraction of LDL was delipidated and redigested with the above enzymes, and the resulting core peptides were compared with HPLC peptide mapping method (Fig. 5). Trypsin-digested core peptide showed the peak distribution from 15 min to 40 min, while elastase-digested core peptides

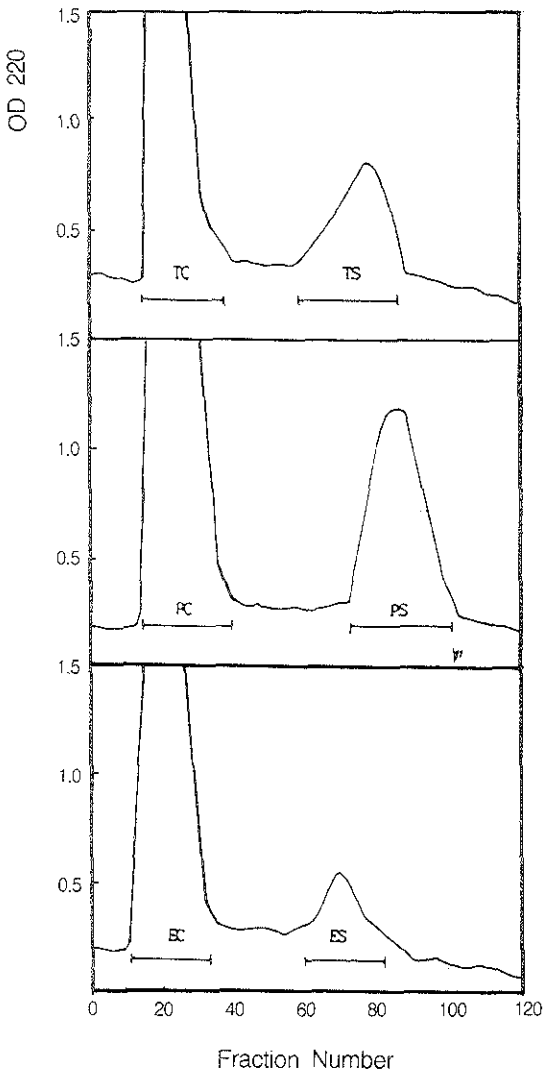

Fig. 3. Gel permeation chromatography of apo B-100 peptides digested with trypsin, pronase and elastase. Apo B-100 peptides digested by trypsin, pronase and elastase were separated with Sephadex G-50 chromatography. Each enzyme digested apo B-100 peptides was labelled as trypsin-surface (TS), trypsin-core (TC), pronase-surface (PS), pronase-core (PC) and elastase-surface (ES), elastase-core (EC).

showed only one major peak. It is proved from the above results that elastase is not a suitable enzyme for the hydrolysis of LDL. Pronase-digested core peptides were smaller than trypsin treated peptides, but showed very few peptides.

Fluorescence spectroscopy

Fluorescence spectra of the aromatic amino acids are shown in Fig. 6. The three aromatic amino acids contribute to the absorbance of peptides to the different extents. The fluorescence spectrum of phenylalanine (282 nm) was smaller by an order of magnitude than that of tyrosine and tryptophan, 303 and 360 nm, respectively. Fig. 7 shows the tryptophan fluorescence emission spectra of core and surface peptide fraction of apo B-100 digested by trypsin, pronase and elastase. Fluorescence emission spectra were scanned from 320 nm to 450 nm with excitation at 300 nm. The effect of surface and core upon conformation of apo B-100 was investigated by fluorescence techniques sensitive to the exposure of tryptophan residues. Comparison of fluorescence between surface and core peptide fraction of apo B-100 digested by trypsin, pronase and elastase showed in Fig. 7, exitation was 300 nm. Apo B-100 in ammonium bicarbonate buffer showed a maximum tryptophan emission wavelength at 365 nm (Fig. 8). The maximal emission wavelength for lipid-containing core and surface, 338 nm and 345 nm, respectively, indicating an exposed tryptophan to the solvent decreased to 338 nm was observed in the core fractions due to the more hydrophobic environment of tryptophan.

In conclusion, apo B-100 is an impotant protein component in the system of plasma lipoproteins. It has functions as the ligand for the LDL-receptor in peripheral cells. And LDL is the major carrier of cholesterol in plasma, and its increased concentration is correlated with the development of atherosclerosis (22,23).

For the chracterization of the surface and core peptides of apo B-100 from human low density lipoprotein, LDL was digested with either trypsin, pronase and pancreatic elastase. Surface fractions were fractionated on a Sephadex G-50 column. And remaining core fractions were delipidated and redi-

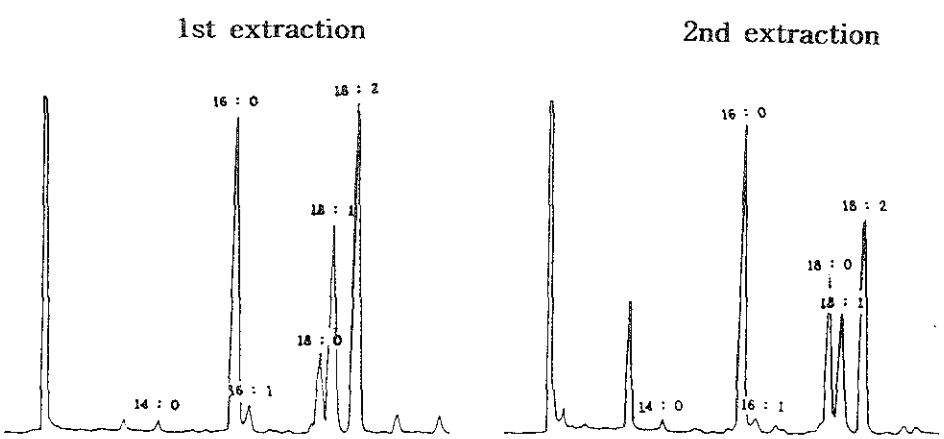

Fig. 4. Fatty acid pattern of LDL. Core fraction of LDL was extracted with ether and ethanol (3:1,v/v). For the characterization of fatty acid composition, the extracted lipid-moiety was separated into two subclasses, first extraction and second extraction.

gested, and compared with surface peptides.

Peptide mapping by ether SDS-PAGE or HPLC showed that pronase-digestion was more extensive than trypsin-digestion to separate surface peptides from LDL. And, results from the separation of surface peptides by Sephadex G-50 gel permeation chromatography showed the same phenomena ; PS showed the largest amount of surface peptide fraction compared to both TS and ES, which means pronase acts as an unspecific enzyme on LDL. Elastase was not a suitable enzyme for the hydrolysis of LDL because large size of LDL

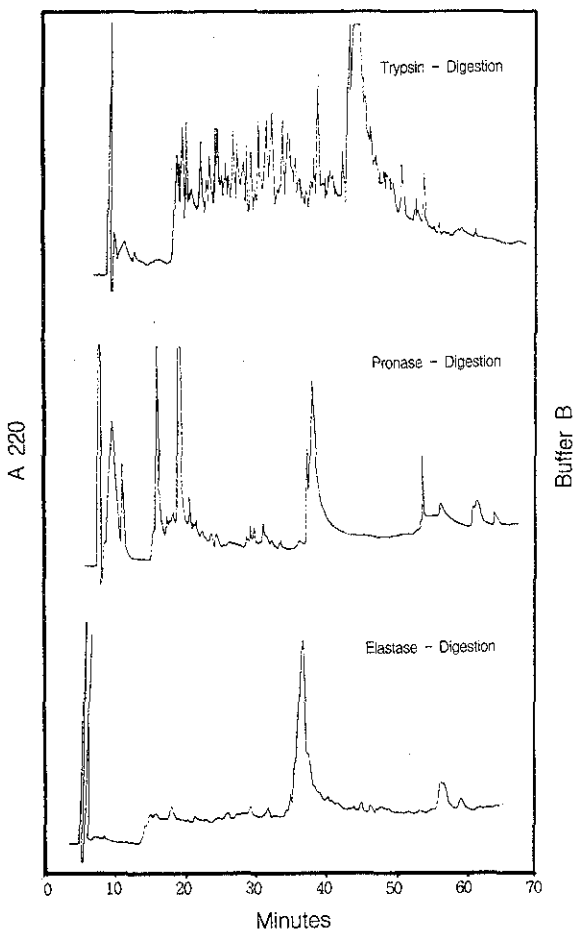

Fig. 5. HPLC peptide mapping of core apo B-100 peptide digested with trypsin, pronase and elastase. The core fraction of LDL was delipidated and redigested with the above enzymes, and the resulting core peptides were compared with HPLC peptide mapping method.

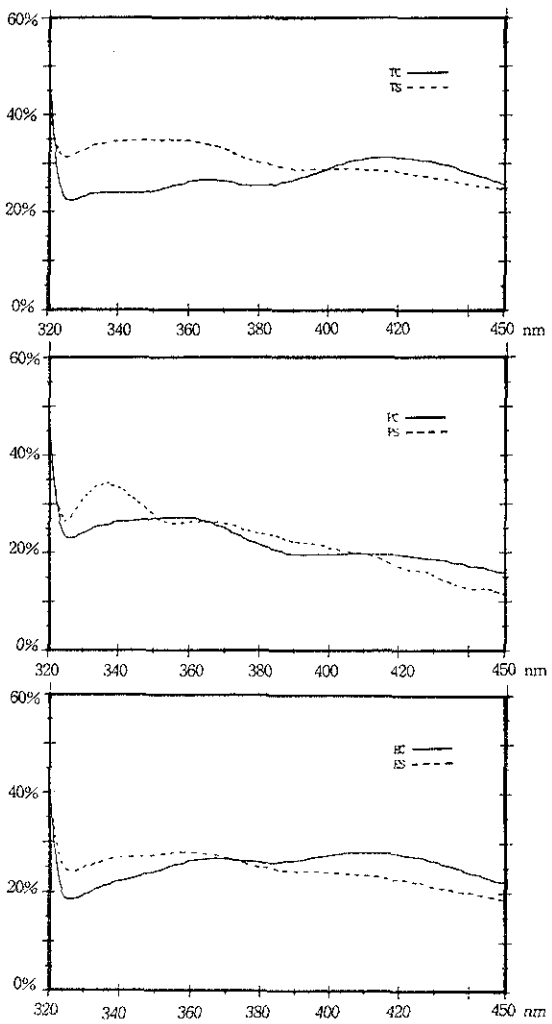

Fig. 7. Comparison of fluorescence emission spectra between surface and core peptides of apo B-100 digested by trypsin, pronase and elastase. Excitation wavelength was 300 nm.

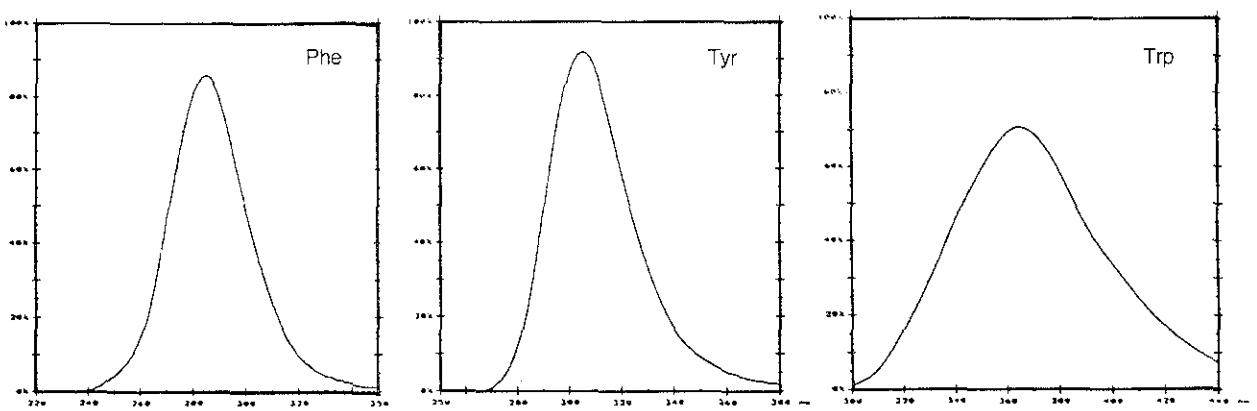

Fig. 6. Fluorescence spectra of aromatic amino acids.

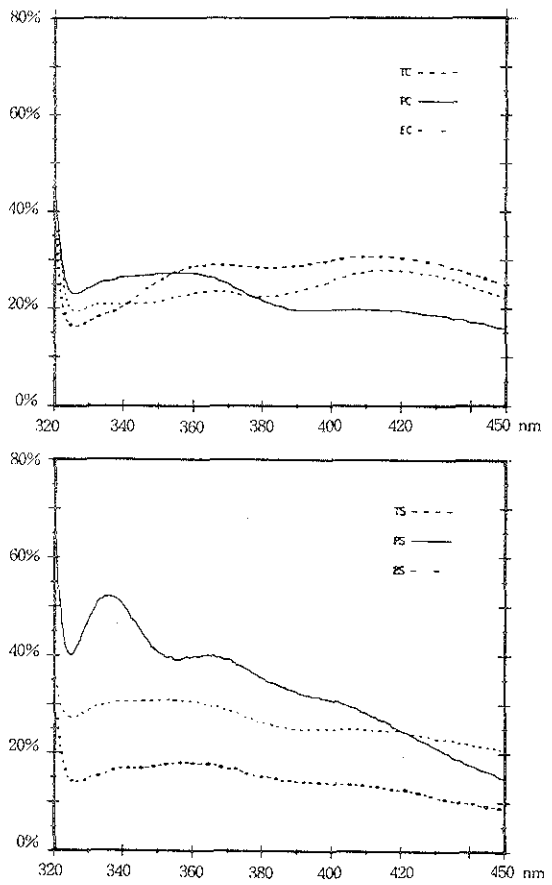

Fig. 8. Fluorescence emission spectra between surface and core peptides of apo B-100 digested by trypsin, pronase and elastase. Excitation wavelength was 300 nm.

could not be fitted to the binding sites of elastase.

Palmitic (16:0), oleic (18:1) and linoleic (18:2) acid were major fatty acids in LDL, approximately 60% of the total fatty acid content. Comparison of the tryptophan fluorescence emission spectra of surface and core peptides showed that TS fraction had higher amount of tryptophan than TC fraction whereas PS had smaller amount than PC.

The LDLs are removed from the circulation by both high-affinity receptor-mediated and receptor-independant pathways, the liver being the major organ responsible for LDL clearance (2,24). The distribution of LDL to various tissues depends mainly on the rate of transcapillary transport and the activity of LDL receptors on cell surfaces (25).

LDLs are also catabolized via a non-specific scavenger pathway involving tissue macrophages. The macrophages contains a receptor which binds and internalized LDL that has been altered by acetylation, or by acting with malondialdehyde (26). This receptor has very poor binding activity toward 'normal' LDL. LDL is heterogeneous in its lipid content, size and density and certain LDL subspecies may increase risk of atherosclerosis possibly due to the differences in the conformation of apo B in the particle (27,28).

Although the primary structure of apo B-100 has been determined, its tertiary structure and conformation on LDL are still poorly understood. A comparison of the behavior of the core and surface provides information about the regions of apo B-100 involved in LDL catabolism and also about the structural features concerning the formation of atherosclerosis. Physical and structural properties of apo B-100 has been known recently and, further studies should be continued to clarify and chracterize apo B-100 of LDL.

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(Received April 7, 1999)