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Full Length Research Paper

Laser raman and infrared spectrum analysis on low-density lipoproteins purified from hen egg yolk

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Diversified proteins were separated from hen egg yolk by ammonium sulphate rapid fractionation, and pure low-density lipoprotein (LDL) was obtained after filtrating through Sephadex G-200 chromatography. After the qualitative detection of Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), we discovered that LDL consists of five major apoproteins. The results show that: (1) the CH_2 presents asymmetric stretching and symmetric stretching mode, (2) the derivative of cholesterol C=C stretching vibration is at 1652.07 cm^{-1} in the Raman spectrum, and (3) α-helix bands in apolipoprotein is at 1655.12 cm^{-1} in the infrared spectrum. The results also suggest that laser raman and infrared spectrum analysis on LDL can provide useful information for studying the LDL structure.

Key words: Low-density lipoprotein (LDL), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), C-H stretching vibration, infrared spectrum, laser raman spectrum.

INTRODUCTION

Atherosclerosis is the main cause of death in many countries, so any contribution to finding out the pathogenesis of atherosclerosis is potentially of major interest (Liu et al., 2002). Most study of atherosclerosis focuses on the character of lipoproteins. Plasma LDL comprises more cholesterol than any lipoprotein in the body. The main function of plasma LDL is transporting cholesterol to the liver to synthesize bile acid. We have found that LDL plays an important role by studying the mechanism of disease, for example, if there are a large number of LDL in the arteries, most of them may accumulate in arterial intimae, which is easy to cause atherosclerosis. Many examinations on the pathogenesis of atherosclerosis have focused on the importance of plasma LDL. Eggs are an important food for human beings. Low density lipoprotein is one of the major components in hen egg yolk. However, there are few literatures which have reported the links between structure and function of low density lipoproteins. Furthermore, it is not yet known whether the yolk LDL has some influence on atherosclerosis. In this paper, we extracted pure low-density lipoprotein from hen yolk eggs, and studied the structure of yolk LDL by laser raman spectroscopy and near infrared spectroscopy, and identified the simulation and differences between yolk LDL and plasma LDL.

There were a large number of studies about the composition of egg yolk low density lipoprotein. LDL accounts for two-third of the dry weight of yolk. LDL density is 0.982 g/ml and the lipoproteins are water soluble (Martin et al., 1964). LDL consists of 11 to 17% protein, 83 to 89% lipid, with a lipid core surrounded by a monolayer of apolipoproteins, phospholipids and unesterified cholesterol (Martin et al., 1964). LDL's surface-embedded structure has also been studied by electron microcopy and X-ray diffraction (Holdsworth and Finean, 1972). In this structure, lipids and proteins occupy distinct areas and do not interact tightly together. Recently, there has been several researches towards LDL, concerning the emulsifying properties of its border membrane, and there are few literature which describes

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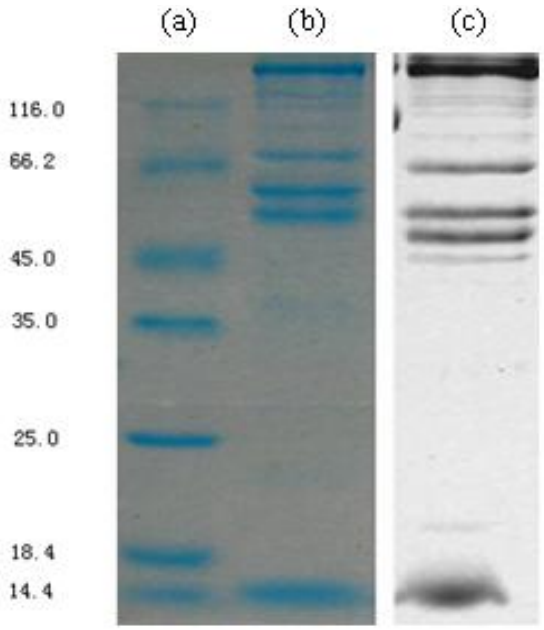

Figure 1. SDS-polyacrylamide gel electrophoresis. (a) marker, (b) gel eletrophoresis of LDL solution, (c) gel eletrophoresis of Anton et al.(2003).

spectral detecting LDL.

In this study, we extracted low density lipoproteins from hen yolk egg by ammonium sulphate rapid fractionation and column chromatography; just needing one or two days' experiment period, and the activity of lipoproteins can be well maintained at 4°C (Thomas et al., 2008). After extracting LDL, we used SDS-polyacrylamide gel electrophoresis to determine the composition and relative quantity of apoproteins from LDL. Electrophoresis image showed that apoproteins of LDL consist of 5 major polypeptides from 15 to 130 kDa, which is consistent with the results of foreign scholars (Anton and Gandemer, 1999). Pure LDL was dried 48 h in freeze dryer at low temperature, to form solid before spectrum scanning. We got characteristic a line through detecting dry low density lipoproteins by infrared and raman spectroscopy, then low density lipoproteins' specific functional groups was obtained after analyzing functional groups. The symmetric CH_2 and asymmetric CH_2 stretching vibration were detected by using spectrum analysis. The derivative of cholesterol C=C stretching vibration is at 1652.07 cm^{-1} in raman spectroscopy, α-helix bands in apolipoprote is at 1655.12 cm^{-1} in infrared spectra.

MATERIALS AND METHODS

Purification of LDL from hen yolk egg

We used the improved method of McBee and Cotterill's (1979) to purify LDL from hen yolk egg. Eggs were purchased from a local supermarket, and were manually broken, and then the hen yolk was rolled on a filter paper to remove chalazes and albumen adhering to the vitellin membrane. This membrane was perforated by clean tweezer to collect unspoiled egg yolk into a beaker cooled in iced water. Hen egg yolk was diluted with an equal volume of 0.17 mol/L NaCl solution, and this mixture was stirred with a magnetic stirrer for 1 h at 4°C. After stirring, the solution was centrifuged at 1.2×10^4 r/min for 45 min at 4°C and the supernatant (plasma) was separated from the sediment (granules). Supernatant was centrifuged again under the same conditions in order to remove sediment completely. The plasma was diluted with saturated ammonium sulphate to obtain 40% saturation and then the mixture was stirred with a magnetic stirrer for 1 h at 4°C. After rest for several minutes, the solution was centrifuged at 1.2×10^4 r/min for 45 min at 4°C. Precipitate was discarded and the supernatant was dialysed with distilled water for 24 h at 4°C (the bath being changed every 1.5 h), and the reserved suspension was centrifuged at 1.2×10^4 r/min for 45 min at 4°C. The resulting floating material containing LDL was pooled. The floating material was dissolved in 0.05 mol/L Tris-HCl buffer solution (pH 6.7) and applied to a glass column (2.5 × 100 cm, the Hai Qite Analytical Instruments Company Limited), which was filled with Sephadex G-200. The mixture was eluted with 0.05 mol/L Tris-HCl (pH 6.7) buffer solution, and automatic collector was used to collect filtrate. Absorbency at 236 nm was recorded. LDL samples eluted with 0.05 mol/L Tris-HCl buffer (pH 6.7).

SDS-polyacrylamide gel electrophoresis detected LDL:

SDS -polyacrylamide gel electrophoresis (stacking: 3.5% and resolving: 12%) was used to detect the composition and relative quantity of apoproteins from low density lipoproteins. Starting voltage was 80 V, and then increased to 120 V before the sample entered detached gel completely. Gel was kept with 25% isopropanol (containing 10% acetic acid) and the protein was stained with a coomassie blue solution (0.05% coomassie blue, 45% methanol, 10% acetic acid, 45% ultra pure water). Then, gel was discolored by acetic acid solution (10% acetic acid and 90% ultra pure water).

Infrared and raman spectroscopy

Pure low density lipoproteins were dried for 48 h at low temperature, 3 mg material was scanned by raman spectroscopy (the displacement range: 200 to 4000 cm^{-1}) with 532 nm wavelength, experimental measurement accuracy was 2 cm^{-1}. 1 mg material was mixed with KBr to make sample, which was scanned by infrared spectroscopy in the 4000 to 600 cm^{-1}.

RESULTS

Electrophoretic analysis of LDL

The pure low density lipoproteins were made by submitting the floating material extracted with ammonium sulphate rapid fractionation and gel filtration chromatography. The two processes allowed the separation of disruptors corresponding to α, β and γ livetins. We identified the molecular weight of apoproteins of LDL depended on protein standard (std), and the electrophoretic pattern showed that LDL consists of five major apoproteins, whose molecular weight was about 15, 60, 65, 80 and 130 kDa as shown in Figure 1.

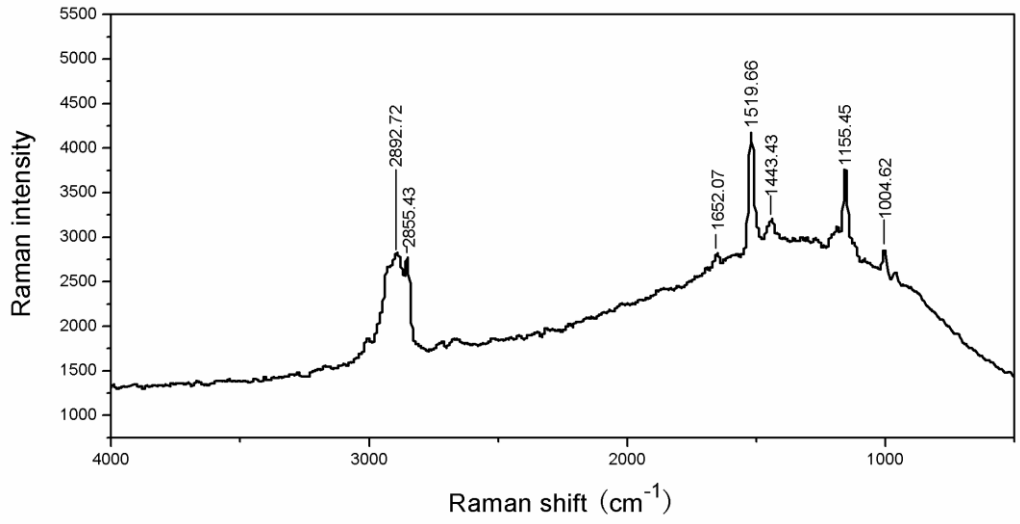

Figure 2. Raman spectrum of low density lipoproteins.

Raman spectroscopy of low density lipoprotein

Figure 2 shows the laser raman spectrum of low-density lipoprotein. In the range from 2700 to 3000 cm^{-1}, we can distinguish two study zones: the first spectral range is a high strength raman peak at 2892.72 cm^{-1}, the other is a weak peak at 2855.43 cm^{-1}, which is due to low density lipoproteins C-H stretching modes. In the range from 1600 to 1700 cm^{-1}, raman spectrum displays positive weak peak near 1670 cm^{-1} at 1652.07 cm^{-1}, and the strong peak is at 1519.66 cm^{-1}, which is because of alkyl chain C=C stretching vibration of cholesteryl ester nokia in low density lipoproteins, and C=C stretching vibration would be strengthened while the number of unsaturated double bond of alkyl chain was increased. In the range of 1430 to 1470 cm^{-1}, the CH_3 asymmetric bending vibration occurred, and the medium peak is at 1443.43 cm^{-1} as the result of CH_3 asymmetric bending vibration. In the range 1422 to 719 cm^{-1}, we observe a raise corresponding to CH_2 swing, at 1155.45 and 1004.62 cm^{-1}.

Infrared spectroscopy of LDL

Low density lipoproteins solid was mixed with KBr, and the mixture were scanned by IR spectra displayed in Figure 3. It showed slender band absorption at 3295 cm^{-1}, which is due to hydroxyl O-H stretching vibration. At the region of 2924.96 and 2856.29 cm^{-1}, CH_3 of lipid chain gave intense bands, while the CH_3 asymmetric stretching was at 2924.96 cm^{-1} and symmetric stretching band at 2856.29 cm^{-1}. In the range of 1500 to 1800 cm^{-1}, the unsaturated triglyceride C=O stretching band occured at 1744.92 cm^{-1}, amide-Iα-helices gave intense bands at 1655.12 cm^{-1}, and in amide-II (including peptide bond N-H and C-N) stretching vibration, medium bands were seen at 1544.20 cm^{-1}. At the region of 1459.22 cm^{-1}, we observed lipid acyl CH_2 shear bending vibration, which corresponded to the symmetric bending vibration of CH_3 in lipid and proteins at 1377.15 cm^{-1}. Vibration frequency of LDL Lipid chain is 1300 to 1000 cm^{-1} and P=O stretching bands appeared at 1237 cm^{-1}, PO^{2-} uniform stretching vibration was given at 1092.57 cm^{-1}, the band that was at 969.79 cm^{-1} [$N^{+}(CH_3)_3$ asymmetric stretching vibration] came from choline group of phospholipids, methylene C of low density lipoproteins occured with a rocking vibration at 720.97 cm^{-1}.

DISCUSSION

We obtained pure low density lipoproteins through ammonium sulphate rapid fractionation and gel filtration chromatography, and then detected its purity by SDS-polyacrylamide gel electrophoresis; the result showed that apoproteins of low density lipoproteins consist of 5 major polypeptides from 15 to 130 kDa. This appearance was consistent with results from the paper reported (Anton et al., 2003). There was γ-vitellin and apoproteins in LDL in the range of 60 to 70 kDa before gel filtration chromatography, during which the step allowed γ-vitellin filtration (Denmat et al., 2000). We could see blurred protein bands at 40 kDa, which were small amounts of β-vitellin (Moussa et al., 2002). We discovered the CH_2 symmetric and asymmetric stretching vibration by raman spectroscopy (Bresson et al., 2004) and fourier transform infrared (Li et al., 2009).

In the laser raman spectrum of LDL, the positive strong peak occurred at 1519 cm^{-1} and was consistent with the result from the study (Cacheux et al., 1996), and the CH_2 asymmetric bending vibration occured at 1443.43 cm^{-1} and was identical with Bresson et al. (2004). In the IR

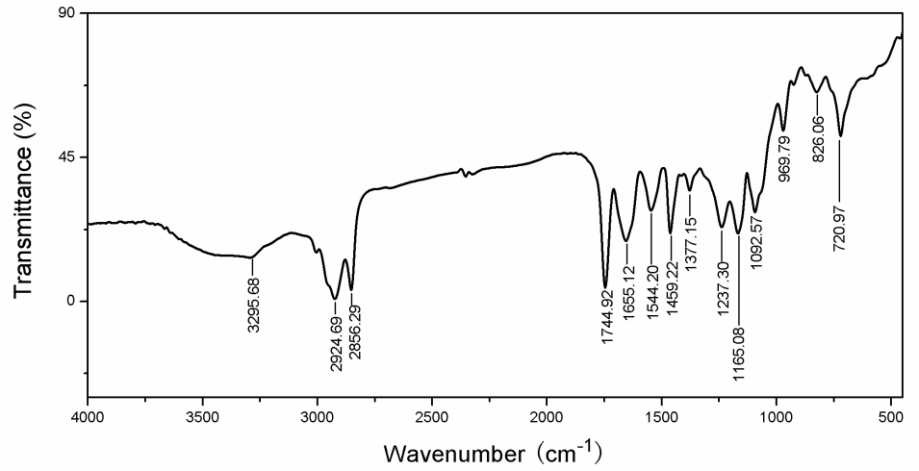

Figure 3. Infrared spactrum of low density lipoproteins.

spectra, our results showed the CH_2 asymmetric stretching band was at 2924.96 cm^{-1} and the symmetric one was at 2856.29 cm^{-1}, which was consistent with the article reported by Dubravka et al. (2009). The amide-II bands occurred in the region of 1700 to 1600 cm^{-1}, and was the same with the paper reported by Masayuki et al. (2002), and amide-Iα-helices gave intense bands at 1655.12 cm^{-1}, which was the same with the result from the study by . As well, amide-II (including peptide bond N-H and C-N) stretching vibration medium bands were seen at 1544.20 cm^{-1}, which was consistent with the paper reported by Liu et al. (2009). During our experiment, the raman spectrum peaks sometimes could not be completely detected, owning to the influence made by lipid of LDL because of incomplete vacuum, but infrared spectrometry could, which provided the supplement information of LDL structure. Through IR spectrum, we could see characteristic bands of cholesterol and triglyceride, and amide-Iα-helices bands characteristic of apolipoprotein. Simultaneously, both lipid chain P=O stretching vibration and $N^+(CH_3)_3$ asymmetric stretching vibration which comes from choline group of phospholipids were found.

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