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FT-IR SPECTROSCOPY OF LIPOPROTEINS – A COMPARATIVE STUDY

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FT-IR spectra, in the frequency region 4000 – 600 cm^{-1}, of four major lipoprotein classes: very low density lipoprotein (VLDL), low density lipoprotein (LDL) and two subclasses of high density lipoproteins (HDL_2 and HDL_3) were analyzed to obtain their detailed spectral characterization. Information about the protein domain of particle was obtained from the analysis of amide I band. The procedure of decomposition and curve fitting of this band confirms the data already known about the secondary structure of two different apolipoproteins: apo A-I in HDL_2 and HDL_3 and apo B-100 in LDL and VLDL. For information about the lipid composition and packing of the particular lipoprotein the well expressed lipid bands in the spectra were analyzed. Characterization of spectral details in the FT-IR spectrum of natural lipoprotein is necessary to study the influence of external compounds on its structure.

Key words: Lipoprotein; Apolipoprotein; Lipid; FT-IR spectroscopy

1. Introduction

During last four decades many groups have been involved in studies of lipid metabolism because the malfunction in the lipid transport is connected with atherogenesis [1]. Due to the hydrophobic character of lipids and sterols, their direct transport in plasma is not possible. Their transportation is enabled by the particles known as plasma lipoproteins [2]. Several classes of serum lipoproteins differ in size and lipid / protein composition according to their different roles in metabolism [3].

Human plasma lipoproteins studied here (VLDL, LDL, HDL_2 and HDL_3) have very different size, but similar general structure. They are particles with hydrophobic core, consisting of triglycerides and cholesterol esters, and outer phospholipids' monolayer with incorporated molecules of free cholesterol. The ratio of triglycerides and cholesterol esters in the core varies within lipoprotein classes, reflecting their role in transport of hydrophobic molecules. This ratio is about 3 for VLDL, but only 0.2 for LDL and about 0.7 for HDL particles. The composition of monolayer is also different. In HDL_2 and VLDL, the number of cholesterols is higher than the number of phospholipids, while in LDL is lower. In HDL_3 there is four times more phospholipids than cholesterols. The part of the surface of monolayer is occupied with apolipoproteins which are partly embedded into the lipid monolayer. The major protein in HDL is apo A-I and in lesser extent apo A-II. These two apolipoproteins are much smaller than the major apolipoprotein, apo B-100, in LDL and VLDL. The protein content in VLDL (8%) is low, somewhat higher in LDL (21%) and twice higher in HDL_2 (41%) and HDL_3 (55%).

Intensive clinical studies have produced a lot of evidence about changes in lipoprotein metabolism induced by external factors like alcohol, nicotine, oxidative compounds, vitamins or some drugs [4]. The intention of our group is to study the

interactions of those external molecules with lipoproteins by FT-IR and FT Raman spectroscopic methods. The prerequisite for such an investigation are well defined spectra of intact particles, in the whole frequency region, and their relevance to the structural details. The search of literature showed that the majority of papers on optical spectroscopy of lipoproteins were limited to the analysis of apolipoprotein domain of the particle. However, in the paper by Liu *et al*, the spectrum above 1400 cm^{-1} of lipid part of LDL and HDL was used to discriminate the two types of particles for the possible clinical application [5].

Guided by the well known fact that the external compounds interacting with lipoprotein and influencing its function are mainly embedded into the lipid monolayer we specially paid attention to the spectral analysis of lipid bands in the spectrum.

2. Experimental and calculations

Human plasma lipoproteins were isolated by the sequential ultracentrifugation of pooled plasma from normolipidemic donors [6]. The cut-off densities: 1.020 g cm^{-3} for VLDL, 1.063 g cm^{-3} for LDL, 1.125 g cm^{-3} for HDL_2 and HDL_3 were adjusted by adding KBr. Each centrifugation procedure was performed at 50,000 rpm for 48 hours at 10°C in a Beckman 70 Ti rotor. EDTA (1g/L) was added in the solution through all preparation steps to protect lipoprotein against peroxidation. Before spectroscopic measurements, the lipoprotein solutions were dialyzed against degassed 0.01 M phosphate buffer, pH 7.4 and stored at 4°C. The purity of isolated lipoprotein solutions was checked by gel-electrophoresis as described elsewhere [7]. The concentration of solutions, in μg of protein per μl: 0.323 for VLDL, 2.785 for LDL, 0.892 for HDL_2 and 4.225 for HDL_3, was determined by the standard Lowry method.

Liposome samples were prepared by mixing solutions of phosphatidylcholine (PC), sphingomyelin (SM) and cholesterol (C) dissolved in chloroform-methanol (2: 1, v/v). The compounds were purchased by Sigma. The aliquots were combined to obtained the molar ratio of PC : SM : C as 2 : 1 : 0.3. The solvent was slowly removed from the lipid solution under a stream of nitrogen and additionally under vacuum for 1 hour. The film obtained by this procedure was further dispersed in phosphate buffer by shaking for 5 minutes in vortex. Sonication of the resultant liposomal solution led to formation of unilamellar vesicles.

For spectroscopic measurements, the lipoprotein solution (50 μL) was placed on ZnSe window and dried under nitrogen for 10 minutes to obtain thin film. FT-IR spectra were recorded with the PerkinElmer Spectrum GX spectrometer equipped with a DTGS detector in absorption mode with 50 co-added scans in the region 4000 – 600 cm^{-1}, with 4 cm^{-1} resolution, at room temperature. The spectrometer was continuously purged with nitrogen to remove the water vapor from the detector and sample compartment. The liposome samples were treated in the same way and recorded at same conditions, separately.

The decomposition of amide I band was performed in the region 1700 – 1600 cm^{-1}. Prior the curve fitting procedure [8] the linear baseline correction was made. The curve fitting procedure was performed with the linear combination of Lorentzian and Gaussian profiles, allowing positions, widths, heights and shapes to be free. The initial positions were determined by Fourier self-deconvolution (FSD) and 2nd derivative method. The difference of the measured spectrum and fitting curve was calculated as an internal control of the success of curve fitting process.

3. Results

The spectra in high frequency region, 3500 - 2700 cm^{-1}, presented in Fig. 1, mainly consist of OH stretching band (A) of water and the quartet (B) of asymmetric and symmetric methyl (*) and methylene (#) stretching vibrations from lipid chains.

Fig. 1

The pattern of group vibrations is essentially the same in all four lipoprotein samples. The CH_2 asymmetric stretching at 2927 cm^{-1} and symmetric stretching at 2854 cm^{-1} give intense bands, while asymmetric CH_3 stretching at 2954 cm^{-1} and symmetric stretching at 2866 cm^{-1} bands are seen as shoulders. However, the intensity ratios: $r_1 = I_{2927} / I_{2866}$ and $r_2 = I_{2854} / I_{2866}$ could be measured from spectra. The r_1 parameter could be considered as the measure of environmental polarity as it increases with the polarity of lipid chains environment [9]. On the other hand r_2 correlates with the looseness of lipid chains packing [9]. Both parameters are higher in VLDL then in other lipoproteins.

The OH stretching band is attributed to the water molecules which remain within the particle, at the protein-lipid interface, shielded by apolipoprotein, after drying procedure. Therefore, the intensity of this band should be proportional to the surface occupied by apolipoprotein, i.e. in some extent, depending on the conformation of particular apolipoprotein, proportional to its content in the particle. It was not possible to compare the intensities of OH bands among different samples. Therefore, we chose lipid CH_2 asymmetric stretching band as a referent signal, because it is essentially the same in lipoprotein spectra. The intensity ratio of OH band and this lipid band in each spectrum was calculated separately. The calculated values, 0.2 for VLDL, 0.4 for LDL, 0.5 for HDL_3 and HDL_2 reflect the increase of apolipoprotein content of the particle. The evidence for the protein shielding is confirmed by the difference of peak position in

the spectra of lipoprotein particles containing different apolipoproteins: at 3294 cm^{-1} for HDL, and at 3282 cm^{-1} for LDL and VLDL.

The fingerprint region 1800-1500 cm^{-1} is presented in Fig. 2.

Fig. 2

The band A is known as C=O stretching band from ester bond in phospholipids [10]. In phospholipids, it is composed of two vibrations from ester groups in two acyl chains. In lipoproteins, in addition to phospholipids, ester bond is also present in triglycerides and cholesterol esters in the particle's core. In the spectra of both HDL samples, LDL sample and liposome (LS) the peak of C=O band is located at 1737 cm^{-1}, while in VLDL it is at 1744 cm^{-1}, reflecting the difference in the surrounding and dynamics of C=O groups.

The broad band B, 1700 – 1600 cm^{-1} is amide I band [8], containing mainly C=O stretching vibrations of protein peptide bonds. The apolipoprotein in HDL_2 and HDL_3 is predominantly apo A-I, while in LDL and VLDL it is apo B-100.

The third band in this spectral region, denoted by C, at 1548 cm^{-1} is amide II band [10] composed of NH bending and CN stretching vibrations of the peptide bond. This band is of much lower intensity than amide I band but of similar shape.

The decomposition of the amide I band, presented in Fig. 3, was performed in order to extract the information about the secondary structure of apolipoproteins and to compare it with the secondary structure data of apo A-I [11] and apo B-100 [12] obtained by other methods. This band has significantly different shape for apo A-I, spectrum (a), solid curve, than for apo B-100, spectrum (b), solid curve. The six component bands, presented as dashed curves, were used to fit the profile of amide I band, presented by dotted curve. The lipid bands in this region are too weak in comparison to the amide I band to be considered in the fitting procedure [13].

Fig. 3

The results of decomposition for apo A-I are presented in Table 1 and for apo B-100 in Table 2.

Table 1

Table 2

The predicted positions of the component bands of particular secondary structure and their contribution to the overall amide I band are compared with the data from literature [13, 14] obtained from FT-IR and Circular Dicroism (CD) measurements. It is known that the results from CD are usually in some discrepancy with FT-IR data, due to methodological reasons [13]. According to the results of decomposition, the dominant contribution in amide I band of apo A-I is α helix. This is in agreement with the data from literature [11] that apo A-I consist of ten α helices, eight of them are 22-mers and two are 11-mers. They are connected by the short segments of β strands and turns. The results for the decomposition of amide I band for apo B-100 demonstrate the high contribution of β sheets and turns to the amide I band. It is known [12] that apo B-100 has a pentapartite structure: NH_3-α_1-β_1-α_2-β_2-α_3-COOH with significant proportion of β sheets.

The intensity ratio of the lipid ester band (A) and amide I band (B) in Fig.2 widely varies in different classes of lipoproteins, reflecting the different content of lipid and protein moiety in the particles. This ratio is the smallest for HDL_3 with 55% of apolipoprotein content and the highest for VLDL with only 8% of apolipoprotein content.

Spectra in frequency region 1500 – 1300 cm^{-1} are presented in Fig. 4. The dominant band, A, with the peak at 1467 cm^{-1} is attributed to bending (scissoring) vibrations of the lipid CH_2 groups in acyl chains [15, 16].

Fig. 4

The band has a shoulder at approximately 1458 cm^{-1}, which is more pronounced in HDL_3 than in other three particles. The low intensity band at 1443 cm^{-1} is attributed to the asymmetric bending of CH_3 group [5].

The band B at 1400 cm^{-1}, which was not present in the liposome (LS) spectrum, is attributed to the COO^- symmetric stretching vibration from Asp and Glu residues [17]. This band is very strong in HDL_3, weaker in HDL_2 and hardly visible in LDL and VLDL. It is known that Asp and Glu residues are exposed toward the polar environment [12]. HDL_3 is the smallest particle of four lipoproteins and has two molecules of apo A-I or apo A-I/ apo A-II wrapping the lipid monolayer. That enables the large number of COO^- group vibrations arising from the residues at the parts of protein surface to be in contact with the polar surrounding. HDL_2 is larger particle, with less protein content and therefore the band B is smaller. Due to the completely different conformation and positioning of apo B-100 in LDL and VLDL, the spectral band of COO^- vibrations is very weak.

The third band in this region, C, is the composite band of symmetric bending of methyl groups at 1378 $cm^{-1}$and CH_2 wagging vibration at 1367 cm^{-1} [16, 18]. The CH_3 vibration is partly overlapped by the protein band B in the spectra of HDL samples. Three observed lipid bands in this region are also present in liposome spectrum.

Three well resolved lipid associated bands could be observed in the frequency region 1300 – 1000 cm^{-1}, Fig. 5.

Fig. 5

The strong band A at 1241 cm^{-1} is usually attributed to P=O stretching vibration [10, 16] or asymmetric PO_2^- stretching [5, 19]. The band B at 1171 cm^{-1} with the shoulder at about 1140 cm^{-1} is attributed to the stretching vibrations of the ester *sn*-1 and *sn*-2 CO-

O single bonds [19]. The composite band C consists of PO_2^- symmetric stretching at 1089 cm^{-1} [5,10] and ester C-O-C symmetric stretching vibration at 1068 cm^{-1} [5] for two HDL particles and at 1064 cm^{-1} for LDL and VLDL particles. In addition to the differences in the position, the overall shape of the band is not the same for different particles.

There are several bands in the region of skeletal vibrations, but only three will be mentioned here, Fig. 6.

Fig. 6

The band A at 970 cm^{-1} is $N^+(CH_3)_3$ asymmetric stretching vibration from the choline group in phospholipids [19], and the band B at 925 cm^{-1} is a skeletal C-C or C-O stretching vibration, from the phospholipids' head [10]. These two bands are similar in all lipoproteins, differing slightly in the relative intensities. The complex band C with the peak at 720 cm^{-1} is rocking band of methylene groups in lipid chains [15, 16]. This band has three components (737 cm^{-1}, 721 cm^{-1}, 700 cm^{-1}) in HDL particles, but only two (721 cm^{-1}, 700 cm^{-1}) in LDL and VLDL. However, the intensity of the vibration at 737 cm^{-1} is higher in HDL_3 than in HDL_2.

4. Discussion

Two apolipoproteins, predominant in lipoproteins in our study, are apo B-100 in LDL and VLDL and apo A-I in HDL_2 and HDL_3. The difference in their conformation and secondary structure is apparent in the different shape of strong amide I band in two sets of particles. Amide I band is commonly used for the analysis of the protein secondary structure. The predominance of α-helix in apo A-I results in characteristic shape with pronounced peak (Fig.2 band B). Opposite to that, amide I band from apo B-100 has two peaks which reflect the presence of α-helix and β-sheets as major

components of this apolipoprotein. The results of decomposition and curve fitting of amide I band, Table 1 and Table 2, showed the presence of some other component bands, attributed to β turns, β strands and random coil structure. These assignations are proposed according to the literature. It has to be mentioned that the same vibration is sometimes attributed to the different secondary structure. There is a possibility that the band at 1639 cm^{-1} could be also assigned to α-helix, because two sets of helices of different length are found in apo A-I [11]. The composition of apo B-100 is more complex. Amphipathic β-sheets in β_1 and β_2 domains, parallel to the surface of the particle, are in close contact with core lipids [12]. It is supposed that there is a variability of length of individual strands within the sheets. Globular $\beta\alpha_1$ domain at N-terminus consists of amphipathic α-helices and several β sheets. This domain is not in contact with the core. It is possible that the different surrounding of β sheets in particular domain is responsible for the presence of several component bands from this structure. Amphipathic α-helices in α_2 and α_3 domains, covering about 20% of the surface, are in contact with polar lipids. There is evidence that β sheets mainly determine particle's size while the surface covered by α-helices increases with the decrease of particle. We expect to observe the changes in apo B-100 conformation in an investigation of the LDL samples with selected particle size.

Some information about the dimension of the particle and lipid composition of four classes of lipoproteins is presented in Table 3.

Table 3

The size of the lipoprotein particles, but even more, the ratio of the particle surface (A_{part}) and the core surface (A_{core}) are significantly different. HDL_3 hardly possesses any core, while in VLDL the core is predominant. Consequently, molecular volume of lipids is almost equal to the volume of the tiny core in HDL_3, but only about half of core

volume in HDL_2 and in LDL, and, even less, only one third in VLDL (Table 3). That implicates the much higher freedom of motion for core lipids, triglycerides and cholesterol esters, in VLDL and very tight packing of lipids in HDL_3 core. On the other hand, the occupancy of phospholipids and free cholesterol in monolayer is about one third for HDL_3 and LDL and about one half for HDL_2 and VLDL. From the last two columns in Table 3 it is evident that in LDL and VLDL the proportion of lipid chains is higher in the core than in the monolayer while for HDL particles is the opposite. These data are important for the explanation of the observed spectral differences in lipid bands of different lipoproteins.

The measured intensity ratios of particular group vibrations, r_1 and r_2, demonstrate the difference between VLDL and other three classes of lipoproteins. In both cases, the values for VLDL are higher (7.4 and 3.7) than for the other three lipoproteins (5.7 and 2.6). This is in agreement with the higher chain mobility in VLDL roomy core, where most of the lipids are stored. The high proportion of free cholesterol in VLDL monolayer, Table 3, has influence on the distance between the lipid chains and enables better contact of polar heads with the polar environment. In other three particles the number of cholesterol molecules is equal or less than the number of phospholipids which increases the chain packing.

The position and structure of C=O band from ester bonds (Fig. 2 band A) is the valuable information for the differentiation of VLDL from other particles. In second derivatives of the spectra (not shown), two distinct components are observed: at 1737 cm^{-1}, predominant in HDL particles and LDL, and a broad band at about 1747 cm^{-1} predominant in VLDL, but much weaker in other three lipoproteins. This component shifts the position of overall ester bond in VLDL to higher frequency. The difference in the position of the band can be explained by lipid composition of VLDL. Triglycerides,

with three ester groups predominate in the core, while the number of phospholipids in the outer layer is much smaller, Table 3. The position of ester band at 1742 cm^{-1} was found in Raman spectra of single optically trapped VLDL [20]. Chemically different surrounding and conformation around ester groups in triglycerides could result in the shift of the band position to higher frequency than in other particles. Additionally, the number of phospholipids covered with apolipoprotein in VLDL is much smaller than in other particles. The effect of cholesterol on the position of ester band in VLDL is also possible. It was found that cholesterol forms hydrogen bonds with P=O group in some lecithin cholesterol dispersions [10] and that bonding shifts phosphate group bands to the lower frequency. However, its influence was not observed in the bands arising from phosphate group at 1241 cm^{-1} and 1089 cm^{-1} (Fig. 5 bands A and C*). These bands are at same position in all lipoproteins, despite the different content of cholesterol in the monolayer. Cholesterol molecule in lipoproteins is probably positioned further away from the polar domain of phospholipids, with possible influence on ester domain but not on phosphate group.

Two additional bands arising from the vibrations in lipid ester group observed in the spectra are better for the differentiation within lipoproteins. Ester CO-O stretching band at 1171 cm^{-1} is present not only in phospholipids (Fig. 5 band B), but also in triglycerides and cholesterol esters. That is reflected in variation of intensity ratio of phosphate and ester CO-O band in different lipoproteins: 0.7 for VLDL, 1.4 for LDL, 2.2 for HDL_2 and 1.7 for HDL_3. That is in accordance with the different number of phospholipids in comparison with the number of all lipids, in individual particles, Table 3. The difference in the position of other ester C-O-C stretching band (Fig. 5 band C#) was observed in different lipoproteins. It is at higher frequency (1068 cm^{-1}) for HDL particles, than for LDL and VLDL (1064 cm^{-1}). That difference could not be explained

by the influence of cholesterol, because the phospholipids' and cholesterols' content are different between HDL_2 and HDL_3 on the one hand, and between LDL and VLDL on the other hand. Various positions of the band are the result of difference in lipid distribution, which is much higher in the core than in the monolayer for LDL and VLDL, but not so for HDL particles, Table 3.

Vibrations from phospholipids' heads are seen in two stretching bands in low frequency region: the stretching of choline group at 970 cm^{-1} (Fig. 6 band A) and of nearby CO or CC bond at 925 cm^{-1} (Fig. 6 band B). Both bands are quite similar in all lipoprotein samples and are not affected by the composition of monolayer.

The information about the dynamics of acyl chains could be obtained from two deformation bands. The structure of CH_2 rocking band (Fig. 6 band C) should be similar to the structure of CH_2 scissoring band (Fig. 4 band A) because both are determined primarily by the lateral packing interaction of the hydrocarbon chains [16]. This is confirmed in the spectra, because the difference between HDL_3 sample and other three lipoproteins is expressed in both these bands. The studies of the organization of hydrocarbon chains in crystalline lipid assemblies and films [16] explained the splitting of the methylene scissoring and rocking bands as the consequence of the interaction between the chains with different orientation, i.e. the different transition moments. The difference in the band pattern in HDL_3 should be connected with the distribution of hydrocarbon chains in this particle. The total number of hydrocarbon chains in the core and monolayer of HDL_3 particle is almost the same. However, in the core they are tightly packed because they occupy 90% of core volume (Table 3), while in other particles they have more free space. On the other hand, the acyl chains in the monolayer are more separated, because the surface of the particle is almost 5 times larger than the surface of the core. This heterogeneity is similar to the different sub cells in the

crystalline complexes. In all other lipoproteins the packing of lipids in the core and monolayer is similar and the component at 1467 cm^{-1} dominates in the band structure. The same situation is reflected in the shape of CH_2 rocking band with three well resolved components in HDL_3 and in smaller extent in HDL_2, but only two components in other two particles. This band is even more suitable for the monitoring of chain packing because it is free of overlapping absorptions from the end methyl groups or vibrations from the polar head [16]. It is expected that these two bands will probably be sensitive to the incorporation of some external molecules into the lipid monolayer which could be used in further studies of that type.

The deformation band from the vibrations in the tails of acyl chains of phospholipids is readily observed in lipid spectra. It usually consists of CH_3 bending vibration, called methyl umbrella, and several deformation wagging vibrations from the chain CH_2 groups, in different conformers of unsaturated fatty acids. In our spectra only one wagging vibration was observed at 1367 cm^{-1} (Fig. 4 band C) and it is attributed to arise from kink and/or gtg conformers [16, 18]. There is indication in the spectra that some other vibrations of this type could be present but their intensity was too weak for the analysis. The deformation band is partly overlapped with COO^- vibration and the comparison of CH_3 bending vibration among the lipoproteins was not possible. Wagging vibrations should be sensitive to the number of kinks, i.e. to the content of unsaturated fatty acids in phospholipids. In our spectra, the position of CH_2 wagging vibration is the same in all lipoprotein samples, probably due to the very similar content of unsaturated fatty acids in these particles.

5. Conclusions

FT-IR spectroscopy gives useful information about the structural properties of complex supramolecular assemblies as lipoproteins. The differences in the size, core volume, lipid composition and dynamics, and apolipoprotein structure in particular classes of lipoproteins are reflected in the characteristic spectral bands of lipid and protein moiety or in parameters extracted from spectra. The results of spectral analysis of the intact particles will be used for recognition and description of the possible structural alterations induced by the interaction of lipoprotein with external molecules and to find the correlation to clinical studies.

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Table 1
Analysis of the composition of amide I band of apo A-I

apo A-I	wave number /cm^{-1}	FT-IR[a]	CD[b]
α helix	1655	40%	70%
β strand	1692, 1625	12%	10%
β turn	1681, 1670	32%	20%
random coil	1639	16%	

[a] present paper
[b] Nolte and Atkinson [14]

Table 2.
Analysis of the composition of amide I band of apo B-100

apo B-100	wave number /cm^{-1}	FT-IR[a]	FT-IR[b]	CD[b]
α helix	1658	19%	19%	33%
β sheet	1695, 1625	36%	44%	12%
β turn	1683, 1671	23%	19%	13%
random coil	1645	22%	18%	43%

[a] present paper
[b] Goormaghtigh et al. [13]

Table 3
Lipid composition of lipoproteins

	diameter (nm)	A_{part} / A_{core}	V_{lip} / V_{core} (%)	V_{lip} / V_{layer} (%)	TG[a] + CE[a] in core	PL[a] + C[a] in monolayer
HDL_3	8	4.5	90	37	10+33	51+13
HDL_2	10-20	2.7	53	63	19+30	137+148
LDL	26	1.4	43	35	336+1600	724+536
VLDL	30-80	1.1	32	54	11529+3618	4466+6062

[a]TG – triglycerides; CE – cholesterol esters; PL – phospholipids; C – free cholesterol

Figure

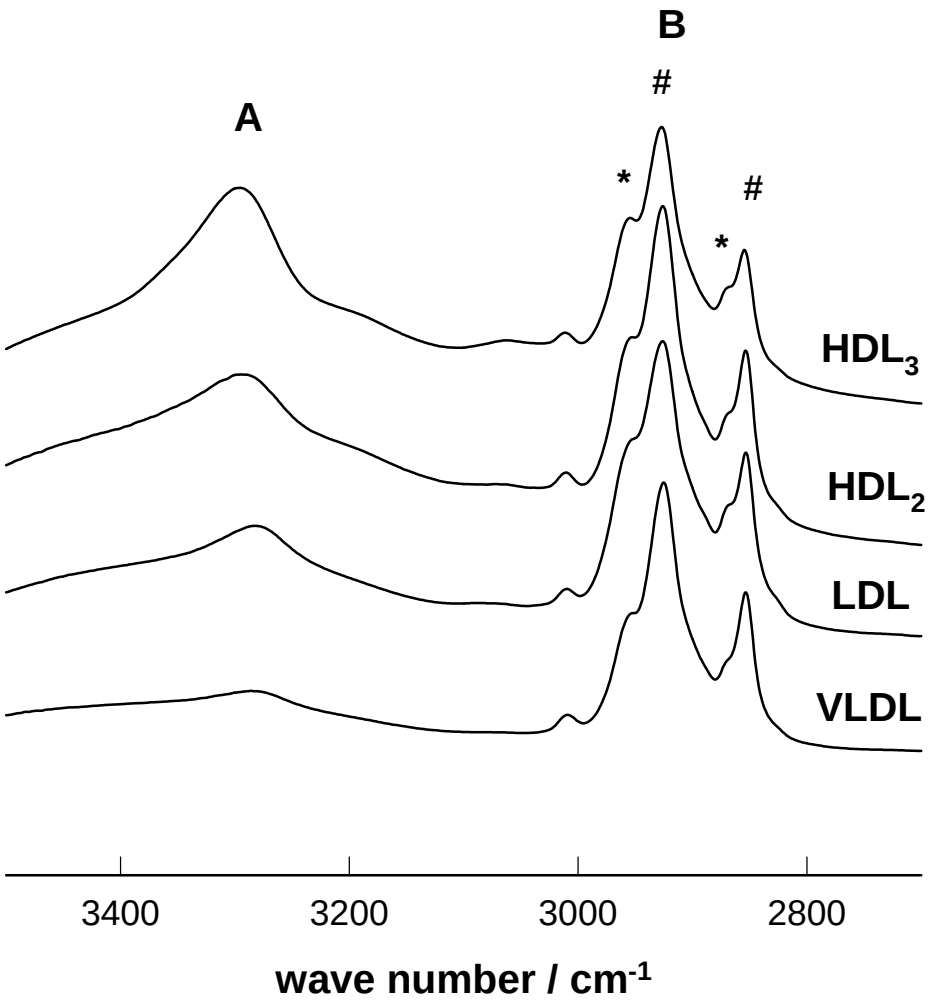

Fig. 1 FT-IR spectra of lipoproteins in the 3100 – 2700 cm^{-1} region. A: OH stretching from water, B: asymmetric and symmetric methyl (*) and methylene (#) stretching vibrations

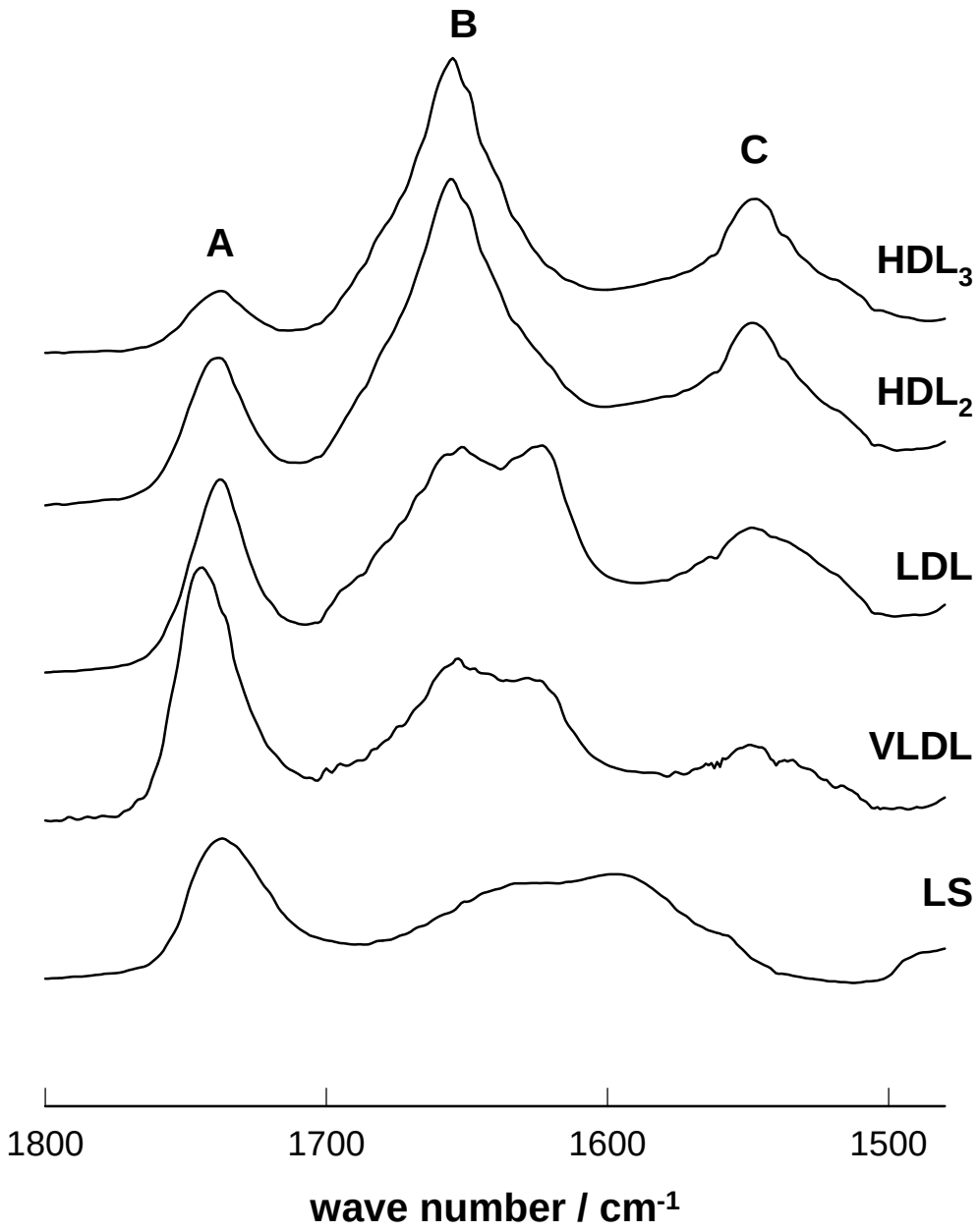

Fig. 2 FT-IR spectra of lipoproteins and liposome (LS) in the 1800 – 1500 cm^{-1} region.

A: C=O stretching band from lipid esters, B: amide I band, C: amide II band.

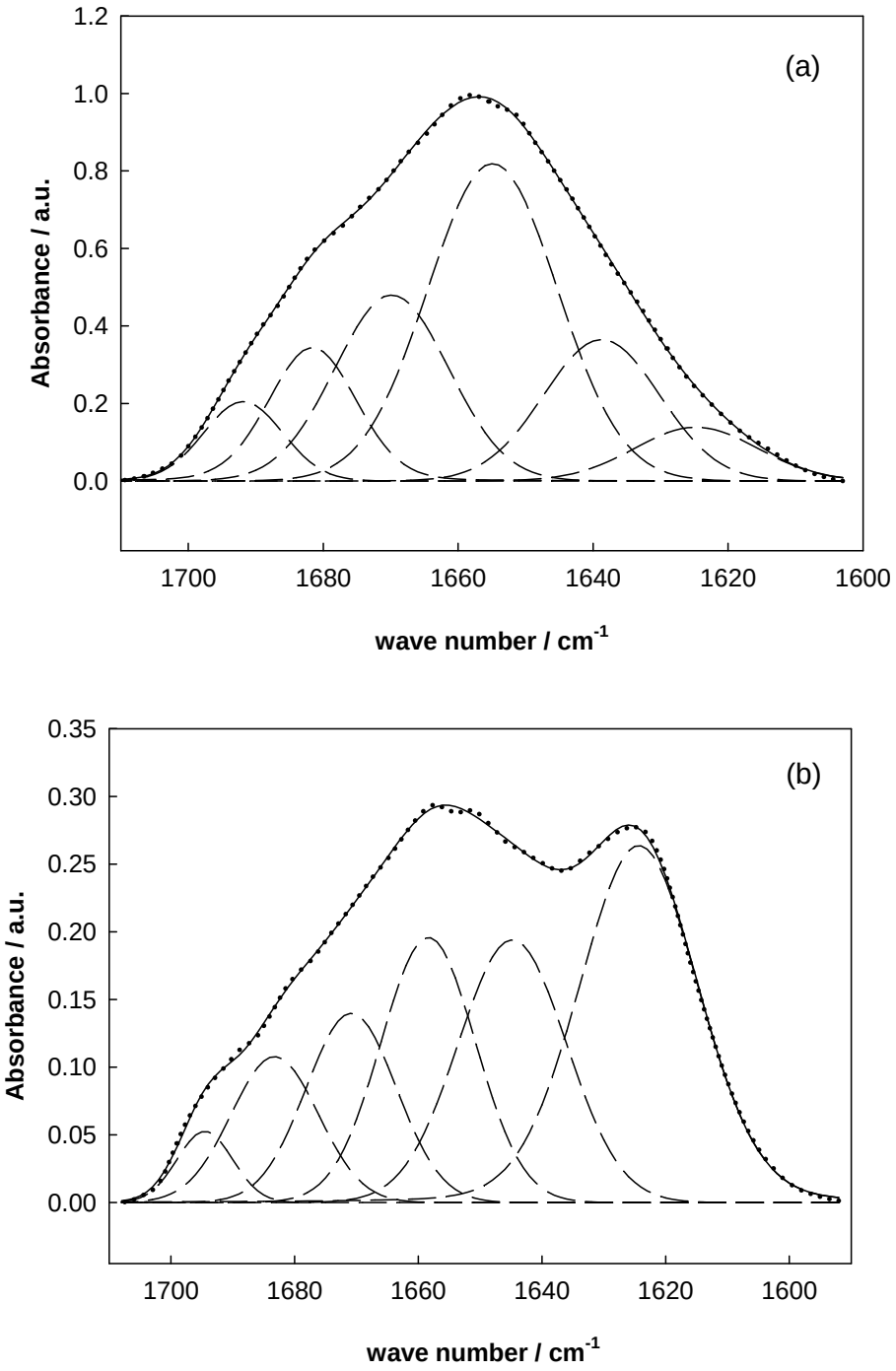

Fig. 3 Decomposition of amide I band, solid curve, of (a) apo A-I from HDL_3, (b) apo B-100 from LDL. Six component bands, drawn as dashed curves, were included in the fitting procedure. The simulated amide I band is presented by dotted curve. The positions of component bands are listed in Table 1.

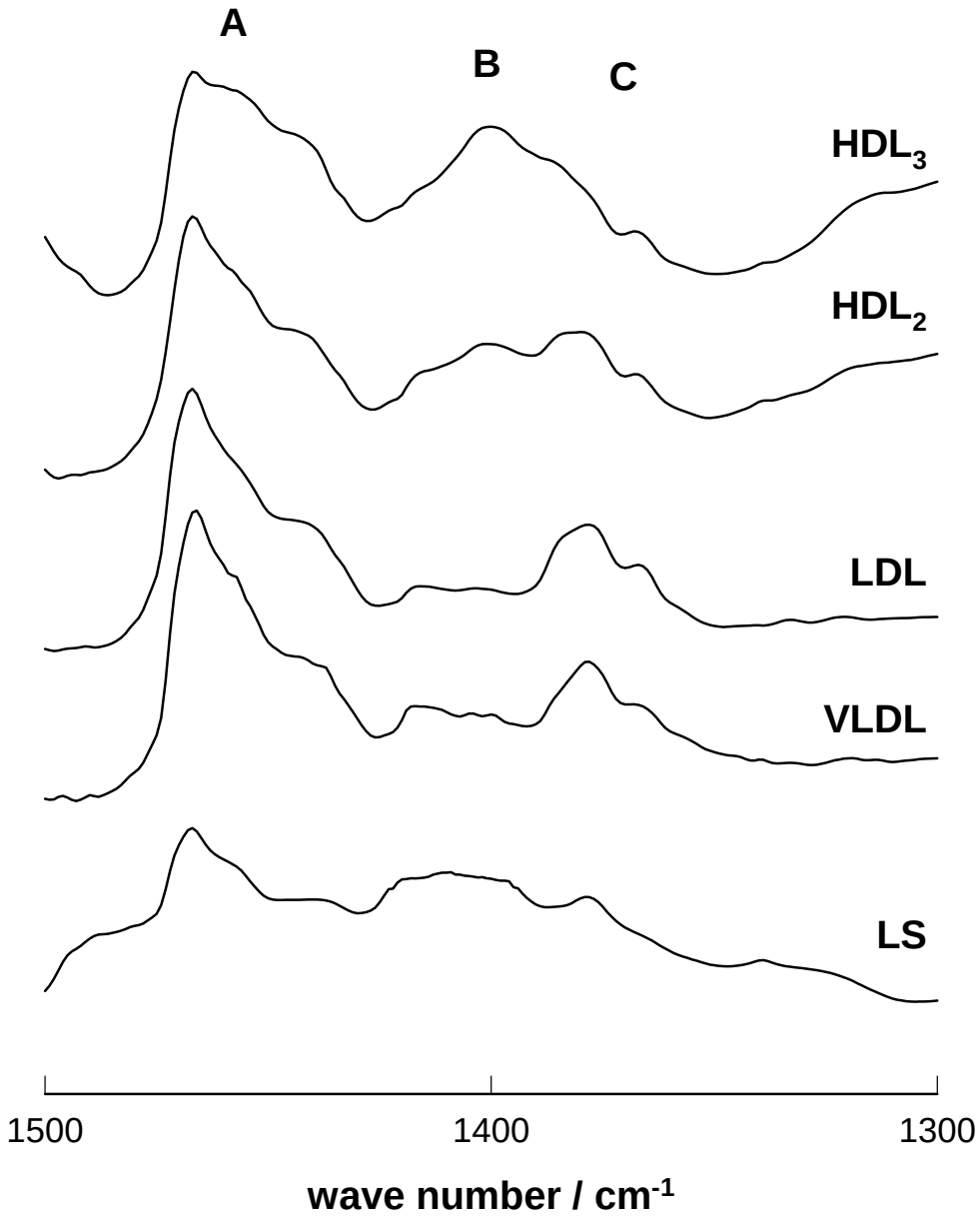

Fig. 4 FT-IR spectra of lipoproteins and liposome (LS) in the 1500 – 1300 cm^{-1} region. A: CH_2 deformation scissoring band from lipid acyl chains, B: COO^- stretching band from amino acid residues Asp and Glu, C: composed band of CH_3 symmetric bending and CH_2 wagging vibrations.

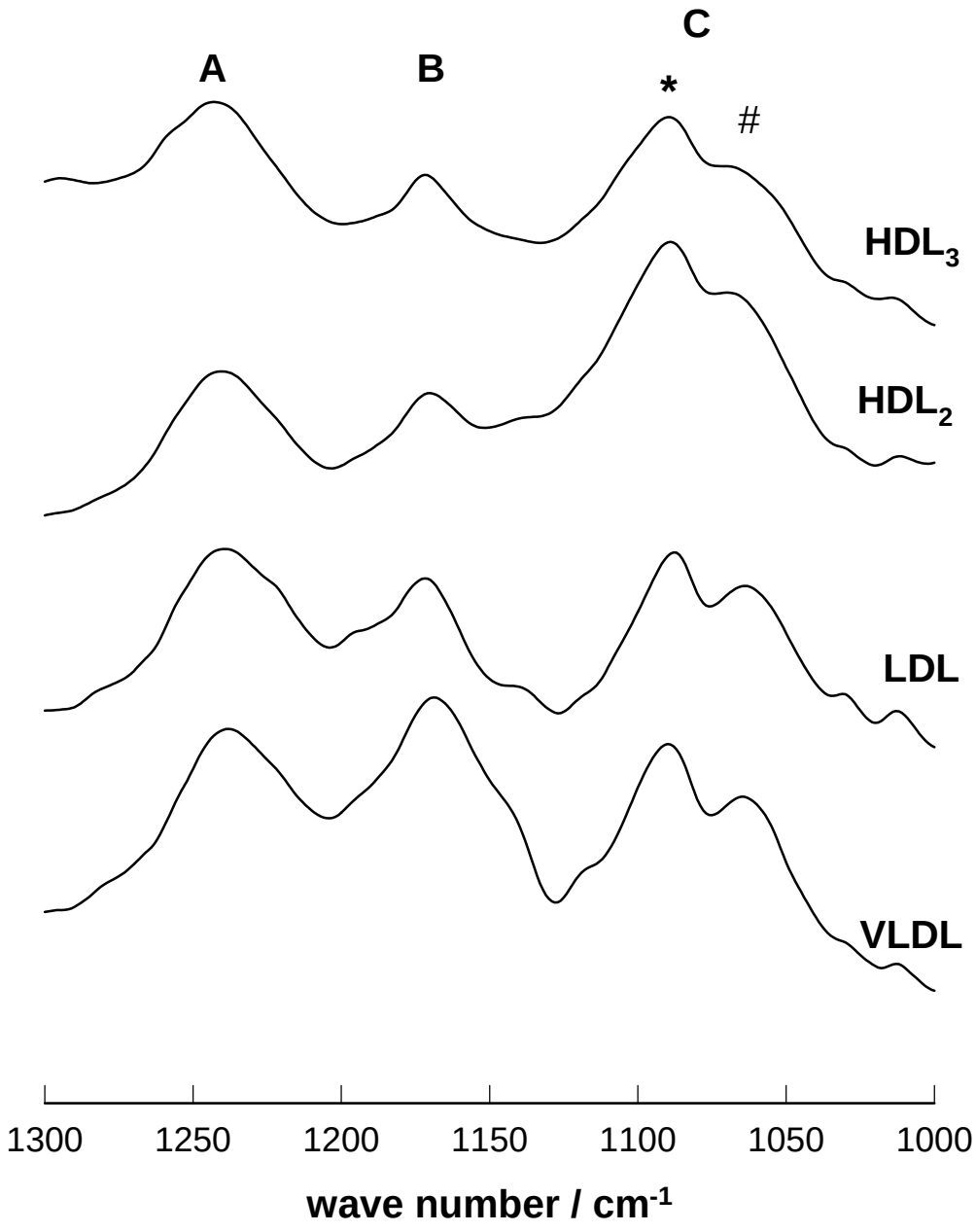

Fig. 5 FT-IR spectra of lipoproteins in the 1300 – 1000 cm^{-1} region. A: P=O or PO_2^- stretching band from phospholipids, B: stretching vibrations of ester CO-O single bonds, C: PO_2^- symmetric stretching (*) and ester C-O-C symmetric stretching (#) vibrations.

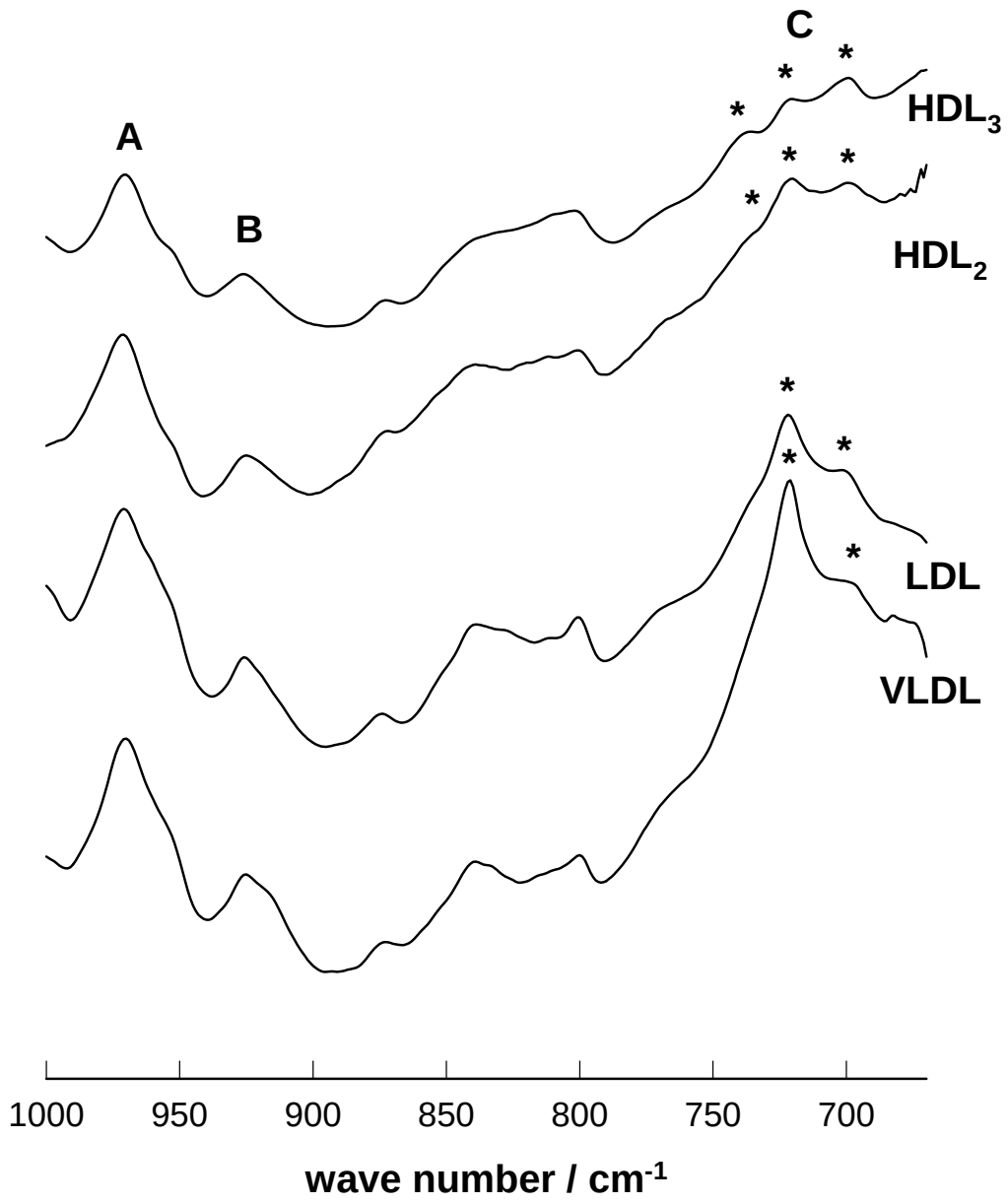

Fig. 6 FT-IR spectra of lipoproteins in the region below 1000 cm^{-1}. A: $N^{+}(CH_3)_3$ asymmetric stretching vibration from the choline group, B: skeletal C-C or C-O stretching vibration, in the phospholipids' head, C: CH_2 rocking from lipid chains, the individual vibrations are indicated by asterix.