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Location of PRODAN in lipid layer of HDL particle. A Raman study

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Abstract

FT Raman spectroscopy has been applied to determine the location of PRODAN within HDL and to investigate its influence on the structure of the particle. The complex spectra of HDL and HDL labeled with PRODAN were divided into three regions according to the wave numbers, and adherent spectra were compared separately. Additionally, recorded spectra of protein and lipid fractions of HDL were used as a support for the assignment of particular vibrations in intact particles. *In high frequency region*, the shift in vibrational frequencies of CH_3 groups but almost negligible shift of CH_2 groups suggests that PRODAN is situated at the water/lipid interface in the vicinity of the protein. The statement is supported by the observed influence of PRODAN on particular lipid vibrations of phospholipids head-groups. *In the fingerprint region*, the influence of PRODAN is observed as the slight change in β-strand secondary structure of apolipoprotein and strongly reduced vibrations of the acyl chain in lipids. That additionally confirms that PRODAN mainly interacts with the lipid domain of the particle. *In the low frequency region*, the lack of change in Tyr Fermi resonance doublet and only slight differences in the pattern of CS and SS stretching vibrations in labeled HDL confirms that PRODAN has no influence on structure of apolipoprotein embeddd in lipid domain. The main conclusions drawn from the vibrational spectra of HDL with and without PRODAN clearly confirm that PRODAN induces negligible changes in HDL structure and hence is reliable fluorescent label for the structural analysis.

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Introduction

Fluorescence properties of hydrophobic probe PRODAN (6-propyionyl-2-(dimethylamino) naphthalene) have been widely used for the structural investigations of lipid bilayer (Kusube et al. 2005), phospholipids membranes (Krasnovska et al. 1993; Chong and Sugar 2002) and lipoproteins (Brnjas-Kraljević et al. 2003) ever since it was introduced by Weber and Farris (1979). The environmental sensitivity of the probe, ensured by its naphthalene ring, determines the type of useful information about the structure obtained from the fluorescence parameters (Parasassi et al. 1998). Obviously, the site of PRODAN embedment in the system is very important. The hydrophobic PRODAN molecules participate in an apolar lipid bilayer as well as in a polar water environment. The participation depends on the conformation of the phospholipid phase. It is also important to stress that the lipid bilayer is highly polar with structured H-bonding at the water/lipid interface, while the hydrocarbon tails have low polarity. On the basis of the fluorescence experiments the group of Krasnowska (2001) proposed that fluorescent moiety of the probe resides along lipid axis, in a way that its dimethylamonium group is interacting with the lipid head groups (Hsieh and Wu 1995). On the other hand, based on his fluorescence studies Chong (1988) proposed different alignment of PRODAN. He suggested the positioning of the naphthalene ring at the water interface perpendicular to the lipid chain, allowing the interaction between dimethylamonium group and phospholipid head group, while the ketone carbonyl group is in the lipid layer parallel to the hydrocarbon chain. Nevertheless, PRODAN will be sensitive to all structural changes near the water/lipid interface, regardless which of the two positions is correct, but with different information.

Lipoproteins are object of extensive research for last 40 years due to their active role in atherogenesis (Nofer et al. 2002; Barter et al. 2003). The spherical particles consist of polar lipid monolayer which encapsulates apolar lipids (Gotto 1987). The apolipoprotein is mostly

embedded in the lipid monolayer. Two lipoprotein classes are of higher interest: LDL (low-density lipoprotein) as the promoter of atherosclerosis and HDL (high-density lipoprotein) as the protector against the atherosclerosis (Barter 2004). The structural changes of lipoprotein particles, induced by risk factors, alter their biological functioning. The environmental sensitivity of PRODAN fluorescence behavior has been used to study the influence of ethanol on HDL structure. The results indicate the substantial changes in the particle structure particularly at the protein/lipid interface (Brnjas-Kraljević et al. 2003). However, the interpretation of the experimental results is partially limited by the lack of knowledge about the changes that the probe itself induced in the structure of the particle.

The possibility to study the influence of PRODAN on lipid systems by FT-IR and Raman spectroscopy (Chong et al. 1989) prompted us to use FT Raman to establish the binding site of PRODAN to HDL and to investigate possible resulting changes in the structure of the particle.

Experimental

Human serum HDL, in the density range of 1.063-1.210 g cm^{-3}, was isolated by the sequential ultracentrifugation of fresh-drawn plasma from two normolipidemic female donors as described earlier (Brnjas-Kraljević et al. 1997). The purity of isolated lipoprotein samples was checked by electrophoresis and the concentrations of lipoproteins solutions were determined by spectrometric method (Lawry et al. 1951). For Raman experiments, the final concentration of HDL stock solution in 0.01 M phosphate buffer (PB) at pH 7.4, was 1.359 mmol/L.

Molecular probe PRODAN (MW 227.31) was purchased from Invitrogen, Eugene, Oregon, USA. The probe was dissolved in ethanol to obtain 10 mmol/L stock solution. Prior to mixing with HDL, 1.17 mL stock solution of PRODAN was evaporated, and then the remaining solid compound was dissolved in 4 mL stock solution of HDL. By that procedure the obtained ratio of PRODAN against lipids was 1:30. Although, this ratio is higher than that usually used in fluorescence measurements: 1:100 (Brnjas-Kraljević et al. 2003), 1:500 (Massey et al. 1998) we purposely used it in order to investigate whether PRODAN in such high concentration will affect the structure of the particle. In that way the applicability of PRODAN as a probe for fluorescence methods will be verified.

The lipid and apolipoprotein fractions of HDL and labeled HDL solutions were separated by lipid extraction procedure. After centrifugation for 10 minutes at 1000 g, well-defined separation of two phases was achieved. Further treatment differed for each phase. The lipid phase was further centrifuged to discard remaining water, evaporated with nitrogen flow and than the sediment was dissolved in acetone. The lipid solution was than washed with equal volume of buffer and filtered through a small column of celite type 535 from Serva. On the other hand, the protein fraction was stored in the appropriate buffer.

Raman spectra were recorded with the PerkinElmer Spectrum GX FT-Raman spectrometer at room temperature. For the excitation a Diode Pumped Nd:YAG (DPY) near

infrared laser (1064 nm) was used with 200 - 500 mW power at the sample. The spectrum for the analysis was an average of 500 scans at 4 cm^{-1} resolution. HDL and labeled HDL samples were recorded in liquid accessory, while lipid and protein samples were recorded in powder and solid accessories, respectively. Before analysis spectra were preprocessed using Spectrum v.5.0.1. software supplied with the instrument. Difference spectra of liquid samples were obtained by subtraction of PB spectra recorded under same conditions.

Results and discussion

Raman difference spectra of labeled HDL solution (A), HDL solution (B), of labeled lipid fraction (C) and lipid fraction (D) are presented in Fig. 1 together with the Raman-spectrum of PRODAN powder (E). By the first inspection it is difficult to detect any changes in the HDL spectra induced by PRODAN. Therefore, for more detailed analysis, 2nd derivatives of difference spectra were calculated and studied separately in three distinctive frequency regions: the high frequency region of group vibrations (3400 – 2800 cm^{-1}), the fingerprint region (1800 – 1500 cm^{-1}) and the low frequency region of deformation and skeletal modes (900 – 400 cm^{-1}). The second derivatives of spectra of HDL solution with (A) and without (B) PRODAN in these regions are presented in Fig. 3, Fig. 4 and Fig. 6. The spectra of lipid fraction of HDL and labeled HDL solutions in fingerprint region are presented in Fig. 5.

It should be emphasized that the intensities of lipid vibrations are generally much lower than the intensities of protein vibrations and therefore in the HDL spectra they could be identified mainly in the high and low frequency region.

Comparing the spectra of lipoprotein particle (Fig. 2A) with the spectra of its apolipoprotein fraction (Fig. 2B), it is obvious that protein vibrations are dominant in the whole frequency interval studied and therefore could be recognized in the spectra of HDL particles. When the spectra of HDL and labeled HDL were compared the difference in amino acid vibrations were observed. However, the comparison of spectra of apolipoprotein fraction of these samples did not demonstrate any difference. These findings confirm that PRODAN is not embedded in apolipoprotein moiety but resides at lipid/protein interface. Therefore, it is expected that vibrations from amino acids in the vicinity of interface could be shifted.

Since PRODAN spectrum of solid sample (Fig. 1E) consists of strong bands they should be observed in spectra of HDL and its fractions. However PRODAN bands were detected only in the spectrum of lipid fraction of labeled HDL (Fig. 1C) resenting the distinctively different

pattern from that of lipid fraction of intact HDL (Fig. 1D). This is an evidence for PRODAN localization in the lipid monolayer.

The high frequency region - stretching group vibrations

Fig. 3 presents the spectra of labeled HDL (Fig. 3A) and HDL (Fig. 3B), together with the spectral segments of their lipid fractions (Fig. 3A' and Fig. 3B'). The sticks and dots indicate the position of group vibrations from amino acids and from lipids, respectively. It is obvious that NH_2 and NH stretching bands above 3200 cm^{-1}, presumably from apolar and basic amino acid residues, are sensitive to PRODAN presence. However, in this region most information is comprised in CH, CH_2 and CH_3 vibrations.

Aromatic CH vibration bands attributed to Phe, Tyr, Trp and His (Jenkins et al. 2005) detected around 3060 cm^{-1} are in labeled HDL slightly shifted to higher frequencies. Antisymmetric and symmetric CH_3 and CH_2 stretching vibrations (2970-2870 cm^{-1}) are attributed to amino acids side chains and to hydrocarbon lipid chains (Parker 1983). The assignment of these bands was made by comparison with spectra of lipid fraction as indicated by dots in Fig. 3A' and Fig. 3B'. For CH_3 modes, addition of PRODAN changes the band positions and the distance between antisymmetric and symmetric stretching bands. On the contrary, CH_2 vibrations are only slightly shifted (within resolution limit) without the change in their distance, implying that these groups are further away from PRODAN than CH_3 groups. Taking into account these observations, CH_3 stretching vibrations are attributed to amino acid residue close to the lipid/protein interface and to those groups in cholesterol and phospholipids which are close to that area (Parker 1983). CH_3 lipid vibrations could also originate from hydrocarbon chain tail but in that case CH_2 vibration should be affected by PRODAN presence and that was not observed.

Except CH_3 and CH_2 stretching vibrations, two other distinctive bands were observed in lipid fractions of labeled and intact HDL (Fig. 3A' and Fig. 3B'). The band at 3045 cm^{-1} is attributed to the choline methyl antisymmetric stretching mode (Tantipolphan et al. 2006) and is shifted to 3052 cm^{-1} in the sample with PRODAN. The aromatic CH vibration detected at the 3015 cm^{-1} in lipid fraction and at 3011 cm^{-1} in lipid with PRODAN is attributed to the cholesterol (Kraft et al 2005; Bresson et al 2004). This change is in agreement with the finding that cholesterol content promotes the participation of PRODAN in lipid monolayer since it prefers the cholesterol vicinity (Bondar and Rowe 1999). The significant change in the choline head vibrations confirms that PRODAN locates in the vicinity of polar region of phospholipids.

The fingerprint region

This spectral interval between 1600 cm^{-1} and 1750 cm^{-1} (Fig. 4) is dominated by amide I band. It is composed mainly of stretching C=O mode and in smaller extent of in plane bending NH vibrations of the peptide bond (Parker 1983). This band is suitable for the study of induced changes in secondary structure within β-strand, α-helix and β-turn part of apolipoprotein (Herzyk et al 1988). The α-helix bands were not influenced by the presence of PRODAN. There is a lot of evidence (Brouilette et al. 2001; Edelstein et al. 1979; Segrest et al. 1999) that the helix structures of HDL apolipoproteins apo-AI and apo-AII are embedded in lipid moiety. Although PRODAN is located near the protein/lipid interface its influence is not strong enough to induce changes in the α-helix secondary structure. Addition of PRODAN, shifts the first two of well resolved lines attributed to the β-strand structure at 1712 cm^{-1} and 1694 cm^{-1} to lower frequencies, 1704 cm^{-1} and 1685 cm^{-1}, respectively. The β-strands of apolipoproteins are sticking out into the water environment and, therefore, could

come close to PRODAN which is hooked at the phospholipids head region. The observed change is probably not the consequence of direct interaction of PRODAN and apolipoprotein, but of the induced changes in H-bridging at the lipoprotein surface.

For the reasons mentioned above, lipid assigned vibrations were studied in the spectra of separated lipid fraction from HDL (Fig. 5A) and labeled HDL (Fig.5B). PRODAN vibrations, indicated by asterisk, are recognized in the bottom spectrum. Changes in lipid vibrations, labeled by sticks, induced by PRODAN in this region are the decrease in intensity of bending methyl bands around 1270 cm^{-1} and methylene scissoring bands at 1451 cm^{-1}, both reflecting the dynamics of acyl chains.

The low frequency region

The frequency region below 1100 cm^{-1} is very rich in spectral bands and hence difficult for analysis and assignment. Fig. 6 presents the spectra of labeled HDL (Fig. 6A) and HDL (Fig. 6B), together with the spectral segments of their lipid fractions (Fig. 6A' and Fig. 6B'). Tyr Fermi resonance doublet at 853/830 cm^{-1} is indicated in HDL spectra. It is considered to reflect the H-bonding network around the tyrosine residue, and in this case it is not affected by the presence of PRODAN. In addition, the bands attributed to CS and SS stretching vibrations (Parker 1983) are not affected by PRODAN. From these findings it is obvious that the structure of apolipoprotein embedded in lipid monolayer is conserved.

The bands in the interval 770 – 650 cm^{-1} observed in spectra of separated lipid fractions are attributed to O-P-O stretching vibrations, labeled by dot, and to bending vibration in choline head group, indicated by stick diagram, (Fig. 6A' and Fig. 6B'). The stretching of phosphate group is also observable in spectra of whole particles (Fig. 6A and Fig. 6B). In the original lipid spectra its intensity is much higher in the presence of PRODAN

while the intensities of the vibrations of the choline head are much lower. These changes in intensity indicate the positioning of PRODAN in the vicinity of polar region of phospholipids. In conclusion the differences observed in Raman spectra of HDL and labeled HDL solutions give the strong evidence for the localization of PRODAN in the lipid monolayer. Shift of aromatic CH vibration, attributed to the cholesterol head region, confirms the statement that PRODAN prefers cholesterol vicinity. On the other hand, the fact that the shifts of antisymmetric and symmetric CH_2 stretching bands are not observed, confirms PRODAN anchoring within the phospholipids head group region. Differences observed in the amide I band, can be explained only if PRODAN is near a protein/lipid interface trapped at the particle surface. The observed changes induced in Raman spectra of HDL by PRODAN confirm that it causes negligible alternations in lipoprotein particle structure. Hence, PRODAN is a good probe for monitoring the risk factor induced structural changes in lipoproteins.

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Figure captions

Fig. 1

Raman difference spectra of PRODAN labeled HDL solution (A), HDL solution (B) and the spectra of solid lipid fraction of labeled HDL (C) and HDL (D); Raman spectrum of solid PRODAN sample (E)

Fig. 2

Raman difference spectrum of HDL solution (A) and its solid protein fraction (B)

Fig. 3

Raman second-derivative spectra of PRODAN labeled HDL solution (A) and HDL solution (B) and the spectra of the lipid fraction of labeled HDL (A') and lipid fraction of HDL (B') in high frequency region. The particular stretching vibrations from amino acids are indicated by sticks and those from lipids by dots. CH_3 vibrations are indicated by upper dots and CH_2 by lower. Asterisk indicates CH vibrations attributed to cholesterol.

Fig. 4

Raman second-derivative spectra of PRODAN labeled HDL solution (A) and HDL solution (B) in the region of amide I band. The vibrations of different apolipoprotein structures are indicated by stick diagrams.

Fig. 5

Raman spectra of lipid fraction of HDL (A) and labeled HDL (B) in fingerprint region. The positions of PRODAN vibrations in (B) are labeled by asterisk. The sticks indicate the position of the bending methyl bands and methylene scissoring bands.

Fig. 6

Raman second-derivative spectra of PRODAN labeled HDL solution (A) and HDL solution (B) and the spectra of the lipid fraction of labeled HDL (A') and lipid fraction of HDL (B') in low frequency region. The vibrations of the Fermi resonance of tyrosine ring and CS and SS

stretching in apolipoprotein are indicated by sticks. The vibrations from phosphate group and choline head in lipids are indicated by dots.

Fig. 1.

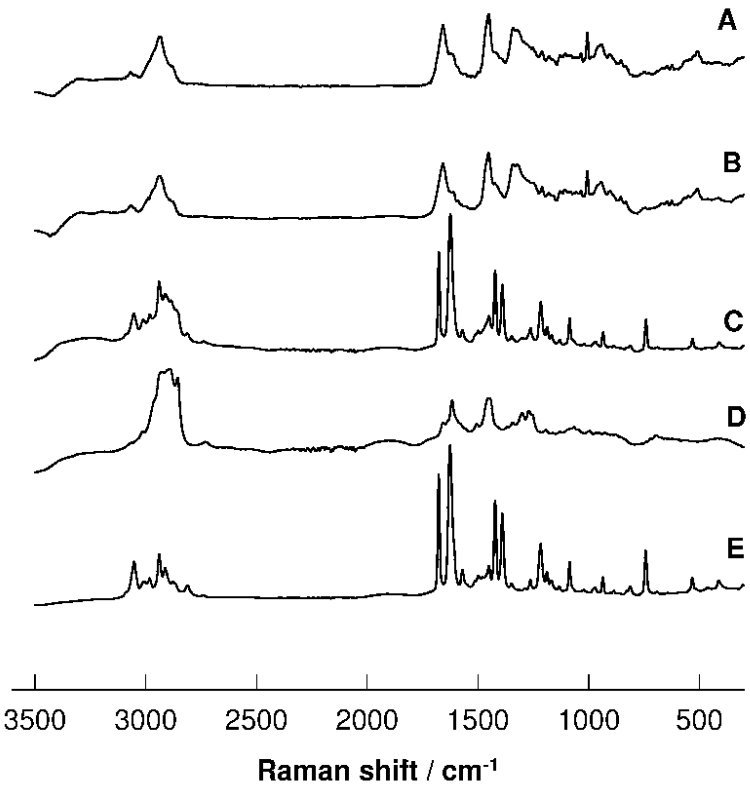


Fig. 2.

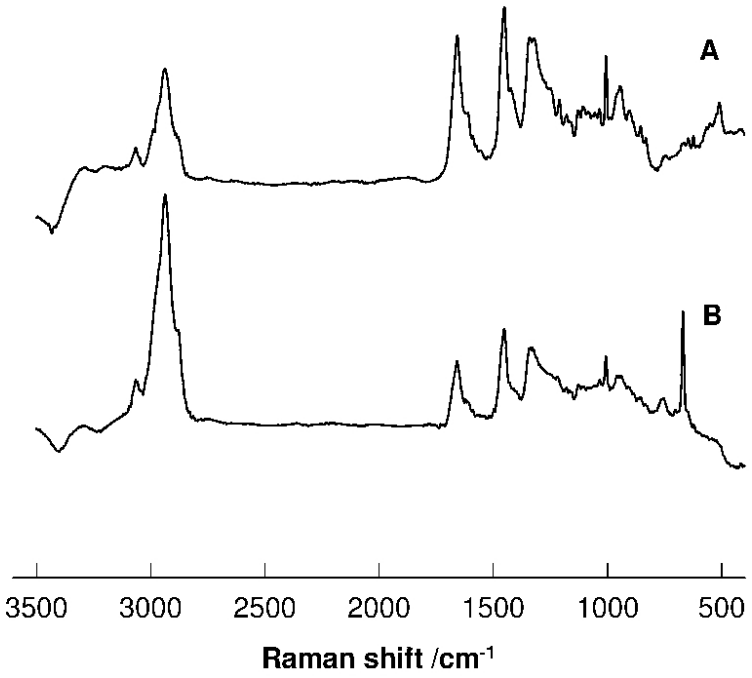
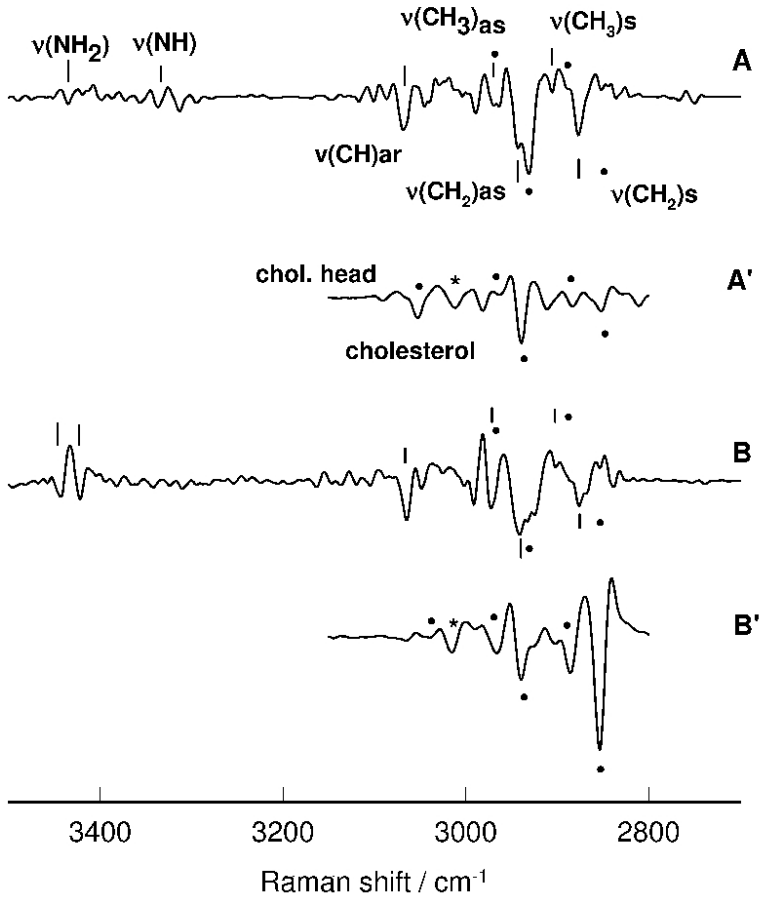


Fig. 3.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ν(NH)
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ν(CH3)s
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ν(CH)ar
ν(CH2)as
ν(CH2)s
chol. head
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cholesterol
B
B'
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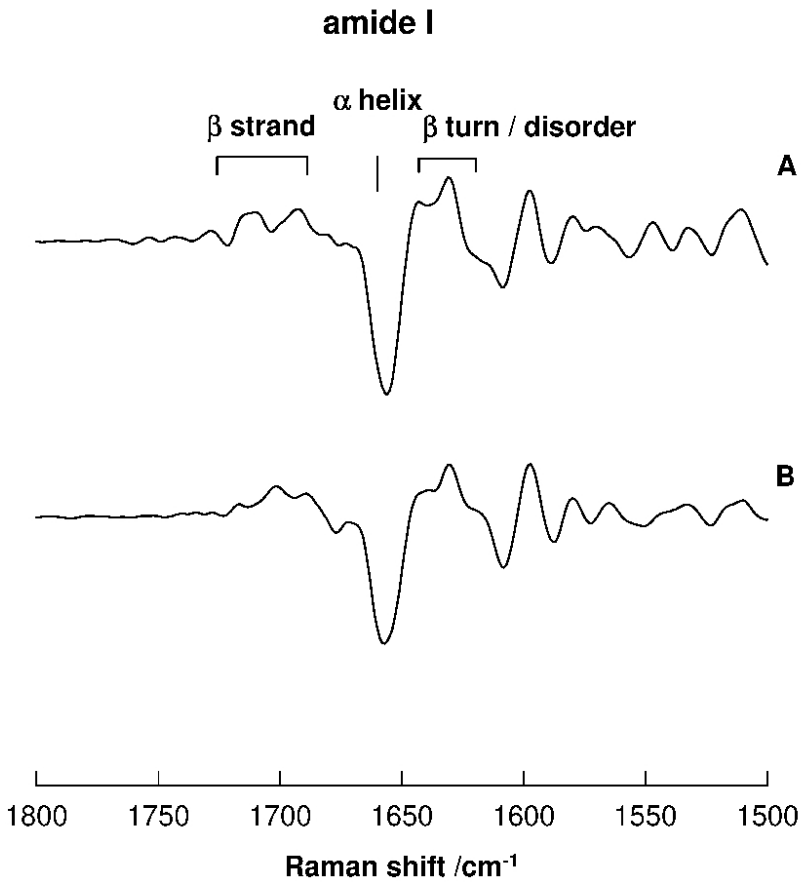
3200
3000
2800
Raman shift / cm-1

Fig. 4.


amide I
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B
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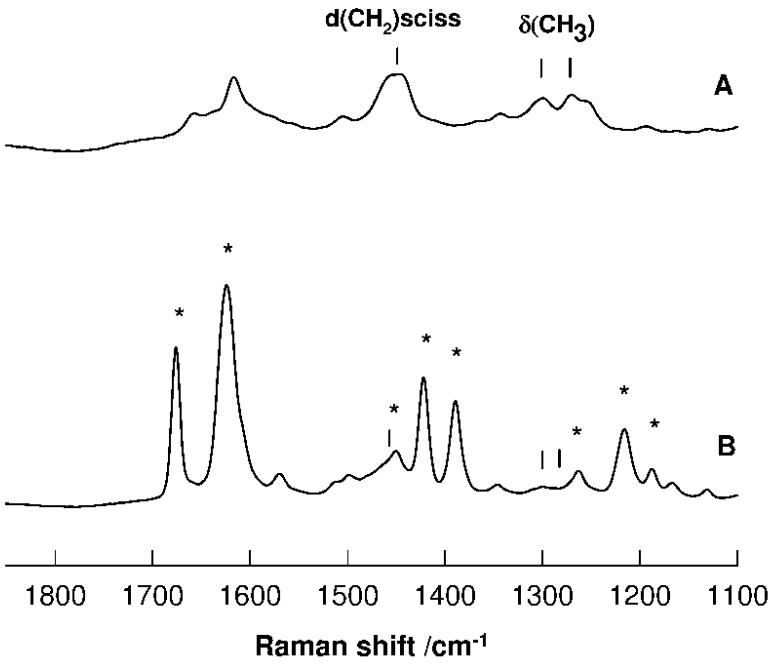
1550
1500
Raman shift /cm-1

Fig. 5.


d(CH2)sciss
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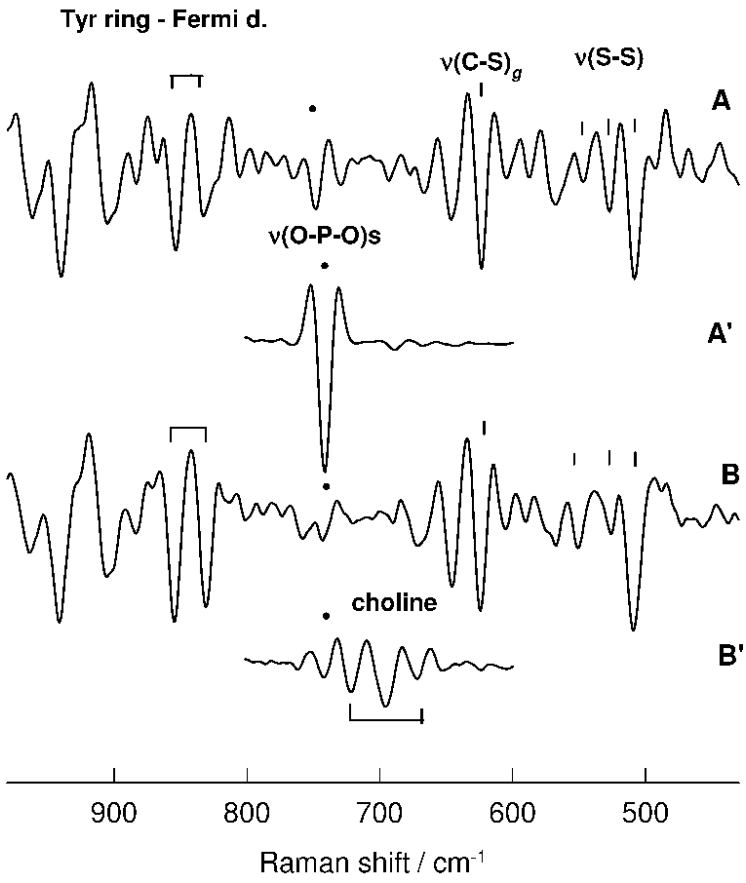
Raman shift /cm-1

Fig. 6.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ν(S-S)
A
ν(O-P-O)s
A'
B
choline
B'
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800
700
600
500
Raman shift / cm-1