
Identifying Macromolecules

The authors review a surface enhanced laser Raman spectroscopy study of lung surfactant protein interaction with bacterial lipopolysaccharide.

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The molecular architecture of the surfactant lipoprotein system is important in understanding its interaction with the microbial world. It consists of 90% lipids — mainly phospholipids — and 5–10% specific lipoproteins consisting of four major apoproteins (SP-A, SP-B, SP-C, and SP-D). The importance of SP-A and SP-D for innate immunity of the lung is recognized now (1,2). The surfactant complex is secreted into the alveolar lumen (hypophase) in the form of multilamellar structures and transformed into tubular myelin lattices (TMLs), which form the precursor of the lipid monolayer to form a surface film for reducing the surface tension at the air–liquid interface during the expansion of the alveoli, and thereby limit their collapse during inspiration and expiration (3,4). Based upon the above structure, lung lipoproteins play a dual role in reducing the surface tension and, most importantly, provide host defense at the air–liquid interface (2,5).

The focus of our studies is to use the biophysical techniques of Raman spectroscopy to examine in detail the mode of interaction of these lipoproteins with defined phospholipids to form the TML and its role in innate immunity, especial-

ly the interaction of SP-A and SP-D with the bacterial and viral membranes. The biological application of biophysical techniques such as Raman spectroscopy has been well documented (6). Raman spectroscopy is well suited for the study of molecular interactions because the vibrational spectra provide a very sensitive and noninvasive means of probing the intermolecular and intramolecular interactions in surfactant lipoprotein matrix and the microbial pathogen membranes. In this study we report data on the molecular spectra of SP-D, lipopolysaccharide (LPS), and Lipid A and molecular interactions of SP-D with bacterial LPS using the surface enhanced Raman spectroscopy technique (SERS). The results indicate specific vibrational shifts in the Raman spectra that underlie changes in the conformational structure of the protein–LPS complexes.

Materials and Methods

Sample Preparation for Laser Raman Scattering Experiments. All samples were dissolved in PBS buffer. Two techniques were used for sample collection. In the first technique the samples were spotted onto a glass microscope slide using a pipette. In the second method,

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samples were spotted onto a roughened silver mirror. In both cases, the sample spots were allowed to dry. The samples then were washed with water to remove the buffer and redried.

Spectra were collected using the Ventuno Raman system (Jasco Inc., Easton, MD) equipped with an air-cooled CCD camera and

531.11-nm diode laser. Samples were imaged using the 20X lens of the Ventuno microscope, and then two accumulations were collected for each sample at 22 °C. For these experiments the laser power on the sample was 12 mW, and the exposure time was 30 s/accumulation. The grating used to collect this data was 1200 grooves/mm, and the slit widths were set at 150 μm .

The spectra were analyzed using Jasco Spectra Manager Software Version 1.53M. For the sample collected on the glass slide, the background Raman peaks from 1000 to 1200 cm^{-1} were subtracted out. The silver mirrored surface had much less interference in the areas of interest in the 900–1600 cm^{-1} range and was used throughout the study.

Surfactant Recombinant Proteins. Recombinant rat proteins (dodecamer and neck+CRD) were prepared by techniques as described by DeSilva et al (7) and used for the Raman spectral analysis.

Surfactant Protein–Ligand (LPS, Fatty Acid) Interactions. 100- μL aliquots of SP-D complexes were formed by the methods of DeSilva et al (7), dialyzed and freeze dried and Raman spectra taken.

Results and Discussion

The results of our work demonstrate the

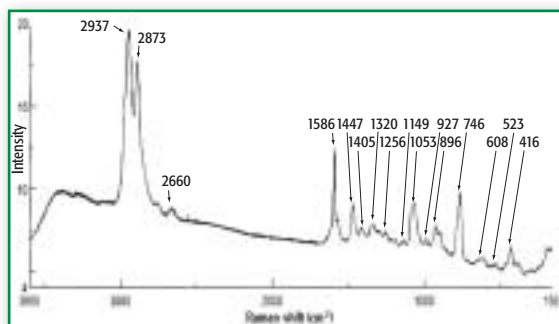


Figure 1. SERS spectrum of surfactant protein D (SP-D).

presence of specific Raman spectral characteristics for SP-D (Figure 1), LPS-B4 (Figure 2) and Lipid A. These results also are in agreement with our earlier studies (8). These data also support the notion that Raman spectroscopy could be extended to the study of single molecules, which is an enormous advance as vibrational spectra contain significantly more structural information and thus can be used to specifically identify macromolecules and their interactions with other binding molecules (9). In this study the SERS technique made use of a metal substrate (silver mirror) whose surface was electromagnetically and chemically modified to enhance the Raman scattering effect. Thus the Raman signal was highly specific and revealed subtle changes in the molecular structure of the compounds and the complexes formed between these compounds. The Raman signal was not affected by the water signal, unlike other optical methods currently in use. Thus the Raman spectral signature (RaSS) of SP-D (Figure 1), LPS (Figure 2) and Lipid A all show distinctive signatures. The detailed spectrum of each compound can be discussed in relation to specific Raman shifts (cm^{-1}) data. In Figure 1, SP-D, a dodecameric protein, is shown with

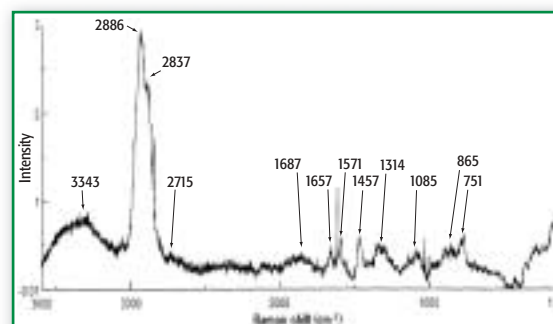


Figure 2. SERS spectrum of lipopolysaccharide (LPS-B4).

Raman shifts around 523 cm^{-1} indicating disulfide bonds (S–S) and aromatic resonance around 746 cm^{-1} (C–S) pertaining to amino acid residues of Met, Tyr and Phe. Thus these bands around 900–1000 cm^{-1} could represent C–C–N stretching modes in SP-D and could be conformationally sensitive (see Figures 3 and 4).

These resonances are not evident in LPS and Lipid A. Most of the Raman shifts become more complex as we examine the 1000–2000 cm^{-1} region. As far as SP-D or SP-A protein is concerned, they show the basic protein spectra of the random coil around 1256 cm^{-1} , alpha-helix of 1320 cm^{-1} , and beta-sheet of 1447 and 1586 cm^{-1} , and so forth. There might be certain variations of these resonances depending upon the solution phase of different proteins. The amide III region is well represented as a measure of the secondary structure in SP-D as shown by the Raman shifts of 1256 and 1447 cm^{-1} . Part of these resonances also could be due to the methylene (CH_2) bending modes, due to the many methylene groups in the protein. Amide I region generally is assigned to the alpha-helical region of proteins and in SP-D is not well represented except for the 1588- cm^{-1} resonance which is lower than in other proteins. This might be

due to the dodecameric nature of SP-D with four CRD (each a trimer) regions confined to the corners of the cruciform structure. There are two alpha-helical regions for each trimer.

This same region in LPS and Lipid A is similar with certain resonances due to the lipidic nature of the two compounds. Because both

compounds contain several fatty acids chains, the Raman shifts around 1150–1200 cm^{-1} could represent vibrations of the all-trans hydrocarbon chains. The temperature dependence of these spectra was not studied and all spectra were run at 22 °C and therefore gel-crystalline transitions were not evident.

The resonances between 2000 and 3000 cm^{-1} were similar for all three compounds. These are due to the C–H stretching modes from the methylene and methyl groups for SP-D (Figure 1) and methylene and methyl groups for LPS (Figure 2) and Lipid A. In SP-D, three Raman shifts are evident, at 2660 cm^{-1} (minor band), and two major bands of 2873 cm^{-1} and 2937 cm^{-1} . However, in LPS only two major bands are evident, 2837 cm^{-1} and 2886 cm^{-1} , whereas in Lipid A, three bands are evident — 2673, 2860, and 2916 cm^{-1} . The lower resonances might refer to acyl chain methylene symmetric and asymmetric stretching vibrations, whereas the higher resonance might contain spectral components from Fermi resonance interactions involving C–H symmetric stretching modes of the chain methyl termini of the lipids.

In the molecular interaction study we used the carbohydrate recognition domain (Neck+CRD) of SP-D and fatty

acid, 3-OH-myristate (14:0). The spectra are shown in Figures 3 and 4. The CRD is only a trimer and the Raman spectrum although similar to the dodecamer (Figure 1), shows more complexity between 500 cm^{-1} and 1500 cm^{-1} . This could be attributed to the fact that the neck+CRD protein moiety is more exposed to the solution phase and more vibrations spectra could be derived. However, when the fatty acid is complexed to the protein, most of the Raman shifts occur around the 900 cm^{-1} and 1500 cm^{-1} regions. Of special note is the appearance of resonances such as 750, 1035, and 1579 cm^{-1} . It is possible that binding of such ligands to neck+CRD protein moiety brings about a conformational change as was shown with CD spectra (7).

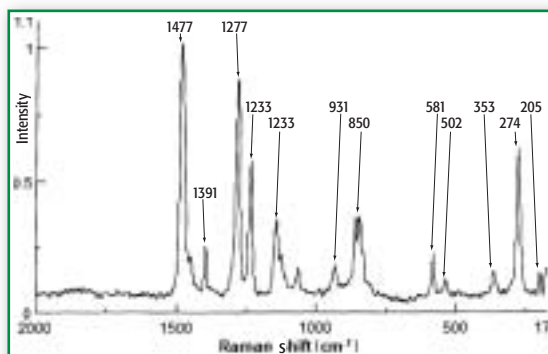


Figure 3. SERS spectrum of Neck+CRD of SP-D.

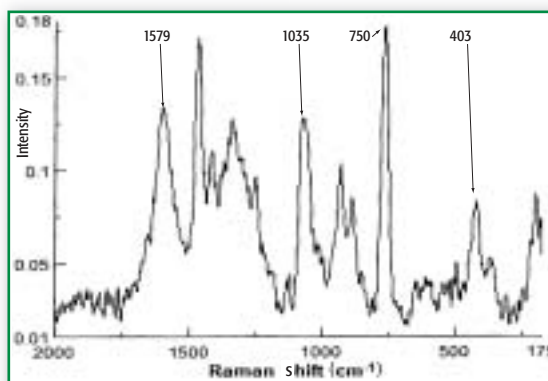


Figure 4. SERS spectrum of Neck+CRD complexed with 3-OH-myristate (14:0).

Conclusion

This study demonstrates that the SERS technique can be used to derive reproducible spectra of pure macromolecules such as SP-D, LPS, and Lipid A. Furthermore, the technique is amenable for studies of macromolecular interactions, especially protein-lipid interactions of surfactant lipoprotein and bacterial endotoxins such as LPS and Lipid A.

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