Components for Raman Spectroscopy

In addition to the spectrograph and CCD detector used in the research above, Roper Scientific™ offers a wide range of high-performance spectrometers, detectors, and spectroscopy accessories designed for Raman. The Acton Research SpectraPro monochromators and spectrographs feature automated multiple grating turrets and a 32-bit microprocessor scanning controller with automated self-calibration. The digital cameras combine exclusive front- or back-illuminated CCDs, deep-depletion silicon, ultra-low-noise amplifiers, and a choice of thermoelectric or cryogenic cooling to provide unparalleled sensitivity. Roper Scientific also offers complete analytical Raman packages. The Acton Research SpectraVIA™ for Raman incorporates the SpectraPro 300i spectrometer and a SpectraVIA™ GS CCD. It is supplied with an InPhoTechs probe for use with a 755 nm laser. The SpectraVIA for Raman system can be outfitted with other versions of the standard probe for 514-, 532-, and 670-nm laser-excitation wavelengths. It can also be configured using the SpectraPro 500i spectrograph for high-resolution requirements with shorter wavelength traces, and either SpectraVIA HP or SpectraVIA UK series CCDs for weak or low-concentration Raman samples. The SpectraVIA for Raman system comes standard with Acton Research SpectraScope™ spectral-acquisition and data-treatment software. A full line of sources and sampling accessories are also available from Roper Scientific.

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BALF Research with Raman Spectroscopy

The development of extremely sensitive CCD detectors and holographic-notch filters is fueling a resurgence in the field of Raman spectroscopy, particularly in the study of organic structures. Traditionally, using Raman spectroscopy to investigate living systems was hampered in two ways: its inability to detect very low-level signals (<10^-9/10^-12) from the excitation source, and the high stray-light levels in monochromators that masked low-frequency components in proximity to excitation wavelengths. With the more powerful and precise equipment available today, Raman spectroscopy has overcome these problems and is well suited even for the study of complex organic compounds. This note describes the landmark use of Raman vibrational spectroscopy to compare bronchoalveolar lavage fluid (BALF) of normal and alveolar proteinosis (AP) lungs.
The goal at Southern BioGene was to investigate how the biochemical structure of the lipid-protein complex facilitates host defense and surface tension reduction at a molecular level in the TLL. The development of replacement surfactants for respiratory therapy depends on understanding this process. Southern BioGene examined the lipoprotein complex of TLL and multilamellar structures, paying particular attention to the interaction of the lung apoproteins SP-A, SP-B, and SP-D with defined phospholipids. Using Raman spectra obtained with the equipment and methods detailed below, they compared the BALF from normal lungs to that of AP patients to investigate possible differences in constituent apoproteins and phospholipid makeup.

Research Equipment and Methods

Southern BioGene has put together a state-of-the-art registration system based on the Acton Research Spectroscopy® 150/IV spectrophotometer, with a macroparticulate linear dispersion of 5 pm/μm for 1200-μm grating, and a thermoelectrically cooled 1024 x 256 detector (the Acton Research SpectraPro:GS256 detector). The CCD has a 7 x 25-mm imaging area with 24 x 24-μm pixels. By using a set of gratings with different groove densities (600 and 1200 lines/mm), they were able to cover both 60- and 125-mm spectral windows. A grating with a low groove density was utilized to get a full Raman spectrum at 1220-wavelength relative to the excitation wavelength. To analyze different regions of the Raman spectrum, the high-power excitation wavelength (514 nm) was adjusted to a power level of 50 mW. The laser-excitation source was a TEM00, linearly polarized HeNe laser (λ = 632.8 μm) with 10-mW output power (Plantech, San Jose, CA). For sample excitation, the sample from Southern BioGene placed the laser 5 mm from the sample and used first-order reflection of the laser beam from a 1000-μm-diameter grating. Several spectrums were used to control the beam path. These steps were taken to clean up the spectrum of the HeNe laser and prevent spectral lines of H2O and NO gas discharge in the laser tube from entering the spectrophotometer. As a result, the spectral region of interest was virtually clear of parasitic lines. The output power of the first-order reflected beam was about 3 mW at 632.8 μm. Thermal effects during sample excitation were avoided by using low-power excitation. This also prevented thermal damage of this sample.

Southern BioGene investigated two BALF samples collected from AP patients and healthy subjects at the University Medical Center Hospital in Birmingham, Alabama. The samples were extracted from the lungs with 0.9% NaCl (saline) and stored at -180 °C until use. Sample were thawed at 4 °C for 2 hours to allow complete fluid of multiple layers. Immediately before analysis, each sample was carefully mixed in the Raman setup. The cuvette was positioned as shown in Figure 1 to prevent both the stray reflection of light from the cuvette’s top wall and the uncorrected Raman signal from the water layer.

Research Results

The data collected and analyzed by Southern BioGene were the results of careful subtraction of BALF extractant (0.9% NaCl in distilled water) and spectrums (correct wave) Raman spectrums. The specific frequency changes in the spectra of AP and normal BALF samples are mostly apparent in Figures 2 and 3.

Figure 1

Schematic diagram of Southern BioGene's experimental arrangement to measure Raman spectra of BALF.

The laser beam was focused into the sample by a 500-mm lens through a 45° mirror. Using a back-scattered scheme to collect the Raman signal, the scattered light from the cuvette’s top wall and the unwanted Raman signal from the water layer. The high-frequency area of the Raman spectrums contains the lipid chain stretching of MCL and CH-stretching modes. The Raman band 2850 cm⁻¹ reflects the CH groups’s (C=C, C=C) symmetric stretching vibrations, whereas 2880 cm⁻¹ reflects its asymmetric stretching vibrations. The 2925 cm⁻¹ feature represents a complex interval that contains spectral components from many interactions involving the chain methyl groups, and separately the CH=C=C symmetric stretching frequencies of the lipid’s chain methyl termini. The spectral intensity ratio ν = νCH/νMCL reflects the parallel chain-CH stretching intensities. The intensity ratio ν = 2880/2850 reflects the paralllel chain-CH stretching intensities as well, but these also contain contributions from vibrations of the CH chains' in the nitrogen matrix. Southern BioGene's results show that the value is the same (0.83) for both the AP and normal samples, whereas the value is significantly different (0.78 and 0.56, respectively). De Silva believes that this indicates a similar lateral chain-CH ordering for the samples, but different intrachain structures and, presumably, a different degree of perturbation in the various portions of the bilayer.

In the mid-frequency region, the absence of Raman lines 380-, 730-, 1220-, 1300-, and 1380 cm⁻¹ from the AP BALF sample indicates a difference in constituent phospholipids. This difference in constituent structure of diseased and normal lungs. De Silva theorizes that this could be due to the absence of functional groups specific for peptides and amino acids rather than for lipids. These data support the findings of Southern BioGene's earlier electron microscopy studies comparing the BALF structures of normal and AP patients. Based on these results, their conclusion is that the TML from AP patients contains no membrane interdigitation and is poorly developed in comparison to normal lungs.

Southern BioGene's use of Raman spectroscopy to show structural changes in constituent apoproteins and phospholipids that occur in the BALF of AP patients is an important step in understanding the molecular structure of TLL. Now that the technology exists, the biggest bottleneck remaining is simply the relatively few Raman spectrums of BALF studies that have been performed. According to De Silva, Southern BioGene is in the process of adding to their preliminary results in order to understand and develop new strategies for improving the TML from AP patients. The results from Southern BioGene continue to use Raman spectrums to study living systems, and correspondingly, additional detail will begin to emerge. This in turn will fuel the development and success of future drug and therapy design.
Introduction

Raman spectroscopy is a powerful and widely used technique for identifying and quantifying materials based upon their molecular and crystal-lattice bond vibrations. It is extremely sensitive, noninvasive, and unaffected by opaque matrices. Modern technology has taken this technique even further. High-performance CCD cameras, spectrometers, and filters deliver single-photon sensitivity, precisely focused high-energy-wavelength excitation, and greatly reduced stray light or radiation, thereby bringing an unique level of flexibility into experimentation.

Southern BioGene, located in Birmingham, Alabama, recognized the potential of using Raman spectroscopy for studying the intramolecular and intermolecular interactions involved in a bilayer lipoprotein matrix. In his September 1999 article, “Structural Changes in the Lipoprotein Complex of Bronchoalveolar Lavage Fluid Detected by Raman Spectroscopy” (Spectroscopy, vol. 14, #9, 21-20), Mr. S. De Silva discussed his groundbreaking use of this technique to measure spectra from bronchoalveolar lavage fluid (BALF) obtained from normal and alveolar pneumonia (AP) patient lungs. Their research, described in the following sections, shows that with the right equipment, Raman spectroscopy is an effective means for studying complex living systems.

Application Background

The surfactant lipoprotein system of the lung is made up of roughly 90% lipids (the majority of which are phospholipids) and 5 to 10% specific proteins (the apoproteins ... and contributes to the elastic properties of pulmonary tissue, thereby limiting collapse during inhalation and exhalation.

The goal at Southern BioGene was to investigate how the biochemical structure of the lipid protein complex facilitates host defense and surface tension reduction at a molecular level in the TLL. The development of replacement surfactants for respiratory therapy depends on understanding this process. Southern BioGene examined the lipoprotein complex of TLL and bilayer structures, paying particular attention to the interaction of the lung lipoproteins with SP-A, -A, -B, and SP-B.

Research Equipment and Methods

Southern BioGene has put together a state-of-the-art registration system based on the Acton Research Spectroscopy® 150 I/V spectrograph, with a monochromator linear dispersion of 5 mm/μm for 1200 grating, and a thermoelectrically cooled 1024 x 256 CCD camera (the Acton Research Spectroscopy® 150 I/V detector). The CCD has a 7 x 25 mm imaging area with 20 x 24-μm pixels. By using a set of gratings with different groove densities (600 and 1200 g/mm), they were able to cover both 60- and 125-nm spectral windows. A grating with a low groove density was utilized to get a full Raman spectrum at ≤ 2600 wavenumbers relative to the excitation wavelength. To analyze different regions of the Raman spectrum with a high spectral resolution (<4 cm−1), a grating with a high groove density was used. The laser beam was focused into the sample by a microscope objective and the uncorrected Raman signal from the water layer was eliminated.

Research Results

The data collected and analyzed by Southern BioGene were the results of careful subtraction of BALF extractant (0.9% NaCl in distilled water) and quartz (cuvette wall) Raman spectra. The specific frequency changes in the spectra of AP and normal BALF samples are readily apparent in Figures 2 and 3. De Silva believes this indicates a similar lateral chain-chain ordering for the samples, but a different intrachain structure and, presumably, a different degree of perturbation in the various portions of the bilayer.

In the mid-frequency region, the absence of Raman lines 380-, 730-, 1220-, 1300-, and 1380 cm−1 from the AP BALF sample indicates a difference in constituents that correspond to lipid and normal lungs. De Silva theorizes that this could be due to the absence of functional groups specific to peptides and amino acids other than for lipids. These data support the findings of Southern BioGene's earlier electron microscopy studies comparing the BALF structures of normal and AP patients. Based on these results, their conclusion is that the TLL from AP patients contains no membrane intercalations and is poorly developed in comparison to normal lungs.

Southern BioGene's use of Raman spectroscopy to show structural changes in constituent lipoproteins and phospholipids that occur in the BALF of AP patients is an important step in understanding the molecular structure of TLL. Now that the technology exists, the biggest bottleneck remains in simply the relatively few Raman spectra of BALF studies that have been performed. According to Dr. Silva, Southern BioGene is in the process of adding to these preliminary results to obtain meaningful and reproducible data for more detailed conclusions. As researchers like those at Southern BioGene continue to use Raman spectra to study living systems, oxidative data will begin to emerge. This in turn will fuel the development and success of future drug and therapy design.
Components for Raman Spectroscopy

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