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Probing the role of Cys-78 in dihydropteridine reductase (DHPR) using Raman Spectroscopy

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Dihdyropteridine reductase (DHPR) catalyzes reduction of the unstable quinonoid dihydropteridine to the active tetrahydropteridine form through the oxidation of NADH. DHPR deficiency or any blockage in biosynthesis of tetrahydropteridine results in development of phenylketonuria (PKU), a progressive neurological illness which does not respond to dietary treatment. It has been speculated that DHPR or metabolites associated with it may have antioxidative properties. In another study, DHPR has been shown to have NADH-ferric reductase activity. This activity is postulated to have an important role in dietary iron uptake. There is an emerging role, though mechanistically unclear at this point, for BH4 in maintaining nitric oxide synthase (NOS) activity. NOS produces the signaling agent nitric oxide (•NO) from L-arginine. In the light of these emerging roles there is a growing need to completely understand how DHPR works.

The mechanism of catalysis in DHPR is not fully understood. Previous studies have suggested the involvement of the thiol group of a cysteine residue in DHPR. Using Raman spectroscopic techniques, the effect of the inhibitors/substrate and/or cofactor on the protonation state of the thiol group is investigated. Raman spectroscopy is particularly effective because of a unique peak at ca. 2500 cm-1 due to the S-H stretch and another one at ca. 900 cm-1 due to the C-S bond. Peak shifts and modulation in intensities are monitored and analyzed in terms of its mechanistic implications.

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Fabrication of SERS Active Gold Nanoarrays for the Study of Adsorbed Phospholipids

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In this study Surface-enhanced Raman scattering (SERS) was employed to study phospholipid molecules adsorbed on nanometre-sized Au structures. Highly ordered gold nano-rod arrays were produced using anodized aluminum oxide templates and electrochemical deposition. The nanorods were imaged with scanning electron microscopy and had an aspect ratio of 10. Raman spectra of 4-aminothiophenol on the prepared substrates showed an enhancement of 102 when compared with a smooth gold surface. Lipids were deposited on the nanoarrays and peaks at 2850 and 2927 cm-1 in the Raman spectra were identified as CH2 and CH3 stretches respectively. It has been demonstrated that nano-rod textured electrodes are excellent substrate for SERS of biomolecules at metal surfaces.

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FTIR Spectroscopic Studies on Protein Migration in the Retina due to Light Exposure

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Phototransduction, the initial event in vision, starts in rod and cone photoreceptors and continues across the entire retina. Some of the chemical processes that are triggered by light involve the migration of proteins to and from distinct compartments of the photoreceptors cells. In this work, we analyzed the protein migration process by imaging thin mouse retina slices using FTIR spectromicroscopy, both with conventional and Synchrotron Radiation (SR) source. We studied protein migration with IR spectroscopy, since it is a label-free technique, not dependent on antibodies of specific proteins. By using a Focal Plane Array (FPA) IR-bidimensional detector coupled to a FTIR microscope, we acquired in one shot 4096 spectra, from which we obtained the distribution of chemical components. From the analysis of Amide bands, we identified the protein location following light exposure of increasing duration and followed their translocation. We used SR from the light source of ELETTRA in Trieste (Italy) to obtain better quality spectra at the same spatial resolution from specific regions of the retina.

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Determination of Simvastatin Induced Variations in Sciatic Nerve by ATR-FTIR Spectroscopy

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Simvastatin, a lipophilic statin derived drug, is commonly used drug for treating lipid and cardiovascular disorders. It is well known that the long term usage of this drug leads to peripheral neuropathy, mononeuropathy and memory problems. Moreover a possible treating agent in central nervous system disease like Alzheimer has been reported. The results that were obtained from simvastatin and nervous system are clinically unclear and controversial. Therefore the current study was aimed to clarify the possible effects of simvastatin on the content, composition, dynamics and conformation of macromolecules in rat sciatic nerve using ATR-FTIR Spectroscopy. Experimental male adult rats were divided two groups as control (n=10) and simvastatin-treated group (n=10). 50 mg simvastatin/ kg was given to treated group by oral gavage for 1 month. In the FTIR spectra, the shift in peak positions, the change in bandwidths and the intensity/area values of the bands were determined and compared in between control and treated groups. The results revealed that there is a significant decrease in amount of unsaturated lipid in simvastatin treated sciatic nerve, which indicates an increase in lipid peroxidation. Moreover, with simvastatin treatment a reduction in the saturated lipid, protein, glycogen and nucleic acid content was found showing degradation or decrease in the synthesis of these molecules. Protein secondary structure was found to be changed as a decrease in α-helix and β-sheet content and an increase aggregated β-sheet and random coil content in treated samples implying protein denaturation. The outcomes of this study show that high dose simvastatin application leads to structural and molecular variations in sciatic nerve by affecting its macromolecular composition.

Keywords: sciatic nerve, simvastatin, statin, ATR-FTIR spectroscopy, protein secondary structure.

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Using Difference Infrared Spectroscopy to Investigate the Effects of pH on PGK-Substrate Complexes

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Yeast phosphoglycerate kinase catalyzes the reversible phosphate transfer in the reaction: ADP +1,3-bis-phosphoglycerate ↔ ATP + 3-phosphoglycerate. Prior research indicates a hinge-bending mechanism occurs during catalysis to bring the substrates into closer proximity. Domain closure is only initiated in ternary complexes, in which both substrates are simultaneously bound to the enzyme. The activity and conformation of PGK is directly influenced by substrate and salt concentrations as well as pH. For example, activity assays confirm that PGK activity increases from pH 6.5 to 7.5. To determine the effects of pH on the conformational changes of PGK, we used difference Fourier transform infrared spectroscopy (FTIR) in conjunction with caged nucleotides. Difference infrared data associated with nucleotide (ATP or ADP) binding to PGK or PGK-3PG complexes was compared at pH 5.5, 6.5 and pH 7.5. Circular dichroism was also used to study PGK secondary structure at the aforementioned pH conditions. Comparison of the difference FTIR data allowed the isolation of pH dependent vibrations that arise from protein conformational changes induced by substrate binding. We have identified multiple vibrations that are associated with the PGK ternary complex and are influenced by pH. Difference FTIR studies resulted in the identification of specific changes within amino acid side chains and protein secondary structures that are altered by pH and associated with ternary complex formation.

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Resolving the Complications Induced by the Side Chain Carbonyl of Glutamine

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IR spectroscopy is a widely used technique for structural studies of proteins which focuses on the amide I mode, an efficient marker of secondary structure. However, the use of IR for studying the glutamine based proteins associated with diseases such as Huntington's disease and Spinobulbar muscular atrophy is limited due to the additional complications introduced by the side chain carbonyl of glutamine. The two carbonyls will both vibrate in the amide I region, giving rise to multiple amide I modes; these must be resolved and unequivocally assigned if one is to understand the IR spectra of proteins containing glutamine. Furthermore, there is the opportunity for coupling between the two carbonyls through an electrostatic interaction known as transition dipole coupling (TDC). TDC causes a delocalization of the amide I vibrational wavefunction across oscillators that vibrate at similar frequencies (e.g. the backbone carbonyls of a protein or the two carbonyls of glutamine) and affects the frequency and transition strengths of the observed IR bands. TDC is dependent on the proximity and alignment of the carbonyls and thus is affected by the secondary structure of the protein as well as the side chain arrangement.

Here we report a fundamental study of the IR spectra of glutamine that will ultimately guide the spectral assignment of the many amide I bands observed in the IR spectra of long stretches of polyglutamine residues (pQ32 and pQ43). We explore the hypothesis that the side chain of glutamine can form a hydrogen