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Amino acids studied by Surface Enhanced Raman Spectroscopy

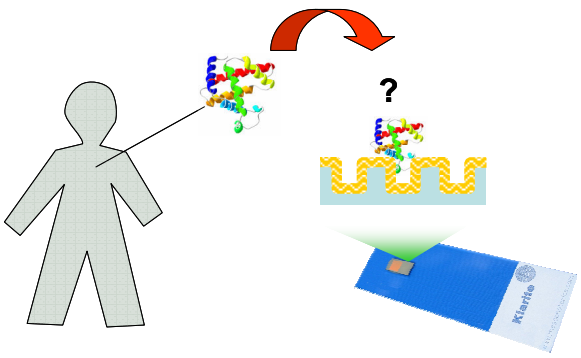
mesphotonics

Key Words

- Amino acids
- Surface enhanced
- Raman
- Life science
- Proteomics

Introduction

One of the fundamental aspects of life science is to understand how the elemental blocks are assembled in living organisms and how they modify under certain conditions such as diseases or new drugs. Proteins, which carry out the body's life functions, are composed of amino acid molecules, which are strung together in long chains. These chains loop about each other, or fold, in a variety of ways. The key to understand the protein functions and their role in health and disease is to unravel their sequence and structure.



How Klarite® SERS substrates can help

Possessing an analytical tool that allows access protein and amino acid structural information in high throughput mode is one of the greatest needs of the biochemist community.

Klarite Surface Enhanced Raman Scattering (SERS) substrates provide an innovative and powerful approach to protein and amino acid studies. Based on engineered nano-textured metal surfaces[1]. Klarite offers great advantages such as access to molecule composition and structural information thanks to the unique spectral fingerprint. Other important advantages offered by SERS technology are:

- use of small quantity of sample (<1 microlitre)
- detection of very low concentrations (ppm-ppb)
- use of low laser power (100µW-10mW)

Up until now these have been the limiting factors to the applicability of Raman spectroscopy in the life science field.

The objective of this note is to demonstrate the detection capability of non-functionalised SERS substrates for very low concentration of amino acids solutions. This allows faster and cheaper tests suitable for all those applications requiring non-specific early detection or diagnosis of physiological concentrations.

The binding and alignment of molecules onto a engineered SERS surface provide access to a larger number of molecular vibrations, unlike conventional Raman. This allows extra molecular structural and compositional information to be investigated and molecular databases generated to uniquely identify each amino acid. Klarite reproducibility over large areas is a fundamental tool in order to obtain repeatable and unambiguous results.

Structure of amino acids

The general structure of amino acids consist of a carboxylic acid (-COOH) and an amino functional group ($-NH_2$) attached to the same tetrahedral carbon atom (α-carbon) (Fig.1). Distinct R-groups, that distinguish one amino acid from another, also are attached to the α-carbon. The fourth substitution on the tetrahedral α-carbon of amino acids is hydrogen.

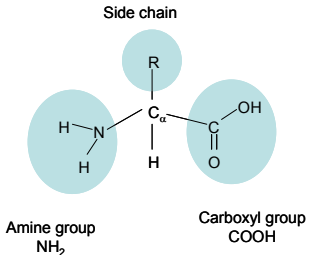


Figure 1 - Structure of a α-amino acid

The amino acid content dictates the spatial and biochemical properties of the protein or enzyme. The amino acid backbone determines the primary sequence of a protein, but it is the nature of the side chains that determine the protein's properties.

SERS of three different amino acids

The SERS study on the L isomers of Alanine, Phenylalanine and Cysteine is reported. Those molecules were selected in order to study how the molecular binding to nano-textured metal and SERS is affected by the molecule size and the side chain type. The properties of the selected amino acids are summarised in Table 1. Samples were prepared by using a drop coat technique optimised for proteomics analytes [2, 3].

Table 1 - Properties of selected amino acids.

Molecule	Side chain type	Side group	Mass
Alanine	aliphatic	CH3	89.09
Cysteine	sulfur containing	CH2 - SH	121.16
Phenylalanine	aromatic	CH2-C6H5	165.19

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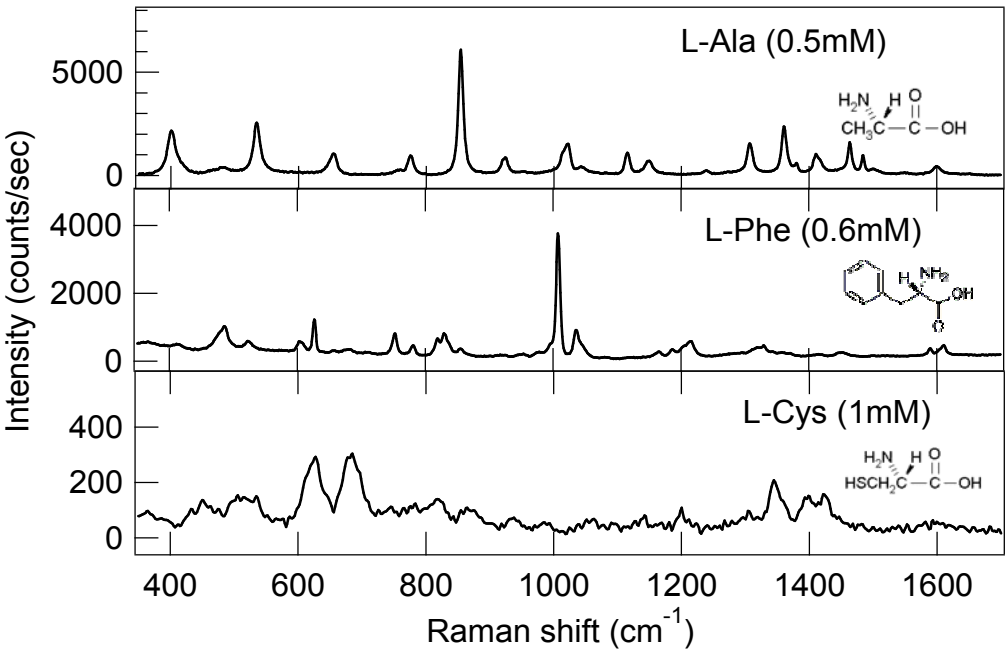

Figure 4 - (a) SERS spectrum of L-Alanine 0.5mM, (b) L-Phenylalanine 0.6mM and © L-Cysteine 1mM at 785nm on Klarite™ (laser power ~15mW).

Typical SERS spectra for the Alanine, Phenylanine and Cysteine are shown in Fig.4 (a), (b) and (c) respectively. The main vibrational modes of each analyte are clearly identifiable. The typical skeleton torsional and stretching modes of L-Alanine in the 400-1000 cm^{-1} range is shown in Fig. 4 (a). The phenyl ring stretch mode at ~1000cm^{-1} is distinctively detected for the L-Phenylalanine as shown in Fig. 4 (b). Many of the ring stretch and deformation modes are also clearly visible. The C-S main vibrational modes of L-Cysteine at 627 cm^{-1} and 684 cm^{-1} can be identified in Fig. 4 (c). Weak contribution from the different rotamers of the cysteine molecule can also be identified in the 700-1000cm^{-1} range.

The three amino acid solutions used had concentrations 0.5-1mM (~10-100ppm), this is in the range of typical physiological concentrations. The sensitivity of the Klarite substrate allows lower concentrations to be detected, depending on the analyte [4]. Due to the enhancement provided by the substrate low power lasers and fast acquisition times can be used. The results of Fig 4 have been obtained by using ~10mW of a 785nm laser. Typical exposure times are 2-10secs with 1 microlitre of sample.

Conclusion

The application to amino acids reported in this note highlight the many advantages provided by Klarite:

- Identification of different amino acids
- Chemical structure sensitivity
- Detection at physiological concentrations
- No requirement of surface chemistry
- Small sample volumes (<1microlitre)
- Short acquisition times (~1-10 seconds)
- Low laser powers (~1-10mW)

SERS technology make Klarite substrate suitable for many life science applications ranging from proteomics to dug discovery.

References
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[3] Klarite Applications Note 001
[4] Klarite Applications Note 008

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