Adsorption of vapreotide on gold colloids studied by surface enhanced Raman spectroscopy

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Abstract. Surface Enhanced Raman Spectroscopy (SERS) has been used to investigate the somatostatin (SST) analogue Vapreotide (VAP) in gold colloids. The optimum conditions to detect SERS signals of VAP have been studied. The observed SERS bands correspond to different vibrational modes of the peptide; being the most dominant SERS signals the ones derived from the aromatic amino acids Tryptophan (Trp), Phenylalanine (Phe) and Tyrosine (Tyr). Changes in enhancement and wavenumber of the proper bands upon adsorption on gold colloid are consistent with VAP adsorption, primarily through Tryptophan residues.

1. Introduction

Vapreotide (VAP) is a synthetic Somatostatin (SST) analogue that comprises eight amino acids (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2) and bears an internal disulphide bridge in its backbone (Cys^2-Cys^7) (see Figure 1).

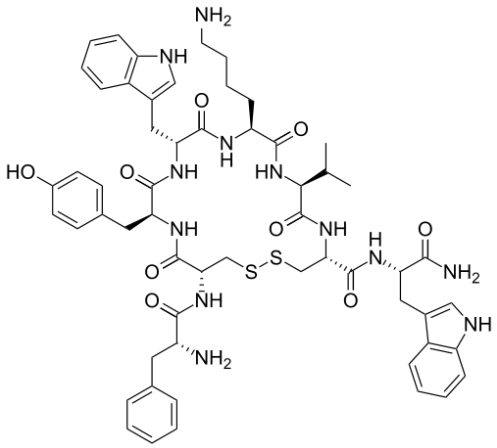

Figure 1. Molecular Structure of VAP.

SST is a tetradecapeptide which acts as a physiological inhibitor of growth hormone secretion. It also inhibits the secretion of a wide variety of stimulatory gastrointestinal hormones and decreases gastrointestinal motility and blood flow [1]. SST binds to specific receptors (sst) of which five principal subtypes have been identified: sst1, sst2, sst3, sst4 and sst5. SST receptors are well known to be overexpressed in a broad range of tumour cells. However, SST has a short half-life in vivo due to its rapid degradation by various peptidases [2].

In recent years, a variety of SST analogues have been prepared and studied because of their potential in diagnosis and clinical treatment: octreotide, lanreotide, vapreotide, somatuline, etc. [3]. Some of these analogues have longer plasma half-lives than SST and, therefore, their therapeutic effects are extended throughout the time. These peptides can be used in the treatment of some tumours (acromegaly, thyrotropin secretory and non-secretory pituitary adenomas and carcinoid tumours). VAP, specifically, is a widely studied SST analogue with anti-neoplastic properties. It has a higher binding affinity to SST receptor subtype 2 (sst2) than native SST. Additionally, it is up to 500 times more potent than octreotide and somatuline in inhibiting the synthesis/release of hormones from SST-expressing pituitary and malignant neuroendocrine cells [2].

The main motivation to use VAP arises from its biological function as synthetic SST analogue which specifically targets SST receptors. Despite the biological importance of the aforementioned peptide analogue, limited information regarding its structure or the manner in which it interacts with its receptors can be found in the literature. An improved understanding of the adsorption mode of this peptide on a specially prepared gold colloidal surface will enable future predictions about the interaction between a peptide (with a known amino acid sequence and structure) and a given gold surface.

2. Experimental

Gold nanoparticles were prepared by chemical reduction of chloroauric acid with sodium citrate as reducing agent [4]. In brief, 50μL of $HAuCl_4 \cdot 3H_2O$ (2wt%) was heated to boiling and 500μL of $Na_3CH_5O_7 \cdot 2H_2O$ (1wt%) solution were added under stirring. After addition of sodium citrate solution, stirring continued until solution turned brilliant red. Gold colloid was aged 24 hours prior to use. This solution was stored at 4°C for further use.

Raman and SERS measurements were registered with a LabRam HR high resolution Raman spectrometer (Horiba Jobin-Yvon) equipped with a microscope and a cooled (-70°C) CCD camera and using a 600grooves/nm holographic grating. The signal was calibrated by using the 520cm^{-1} line of a Si wafer and a 50X objective. As an excitation source, a 785nm irradiation from a diode laser with a power of 25mW was used. Spectral scanning conditions were chosen to avoid sample degradation. No spectral changes that could be associated with peptide decomposition were observed during these measurements.

3. Results and discussion

Figure 2 presents the Raman and SERS spectra of VAP in the range of 400-1800cm^{-1}. The spectral analyses were carried out on the basis of early Raman and SERS band assignments of the amino acid residues in the peptide and some VAP analogues or shorter peptides with similar sequences [5-12].

It is evident that the Raman signals are mostly associated with the characteristic vibrational modes of Phe, Trp and Tyr residues present in the peptide amino acid sequence. This was not surprising in light of previous studies that have shown that modes due to aromatic amino acid vibrations dominate the Raman spectra [5].

3.1. Raman spectra of VAP

The most intense band at 1002cm^{-1} corresponds to the aromatic ring breathing mode in Phe. At the higher frequency side of this band, around 1030, 1181, 1204 and 1604 cm^{-1}, several characteristic Phe bands appear. These bands are due to in-plane CH bending of the aromatic ring, the combination of C-H in-plane bending mode with a stretching vibration of the phenyl ring [6], the stretching of the aromatic carbons in Phe and the ring stretching mode of benzene ring in Phe [7], respectively. On the other hand, in the lower frequency region of these spectra, only one Phe mode appears at 620cm^{-1}, and it is due to the in-plane ring deformation vibrations.

For VAP, the Phe modes are accompanied by the Raman markers of Trp and Tyr residues. Specifically, these bands are observed at around 757, 877, 1009, 1338, 1359, 1549 and 1577cm^{-1} for Trp and 641, 827, 851, 1181, 1204, 1604 and 1617cm^{-1} for Tyr. These bands are related to the

vibrations of the indole ring breathing, indole ring H-scissoring and a N-H deformation, indole ring breathing, Trp Fermi doublet (which arises from a Fermi resonance between a fundamental mode at 134cm^{-1} and a combination of two out-of-plane modes involving the benzene and pyrrole rings), indole ring vibration mainly contributed from the C=C stretch [8] and benzene and pyrrole ring skeletal stretching [9], respectively for Trp. Likewise, for Tyr the bands come from aromatic ring deformation, Tyr Fermi doublet (rise to a pair of relatively strong vibrations, assigned to the ring breathing mode and the first overtone of an out-of-plane ring deformation of the phenolic ring, respectively [10,11], C-H in-plane bending, symmetric stretching of the carbons in the para-substituted aromatic ring, ring stretching mode of benzene ring and C=C stretching mode.

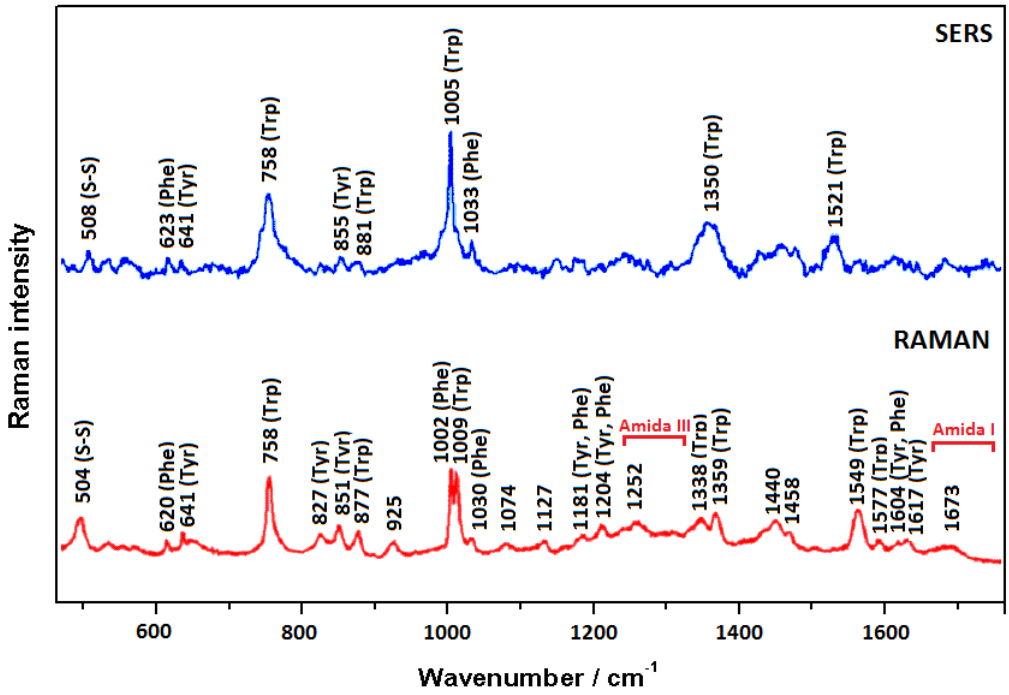

Figure 2. Raman (in red) and SERS (in blue) spectra of VAP. Measurement conditions: sample concentration, 20mM and 7.5x10^{-6}M, respectively. Excitation wavelength 785nm, power at laser output, 25mW.

There are three other noteworthy spectral characteristics. The first one is in the region from 500 to 600cm^{-1}, where a band at 504cm^{-1} is observed, assigned to the S-S stretching vibrations. The second one belongs to the vibrational modes corresponding the set of signals Amide III observed between 1310-1210cm^{-1}, that mostly come from N-H deformation modes due to the amide backbone of the peptide and contain information about the secondary structure of the biomolecules. And, finally, a band at 1673cm^{-1} in the region of the so-called amide I, corresponding to complex vibrations with contributions COO- asymmetric stretching modes (80%), CN (10%) NH stretching and bending (10%) [12].

3.2. SERS spectra of VAP

In order to examine the interaction of the gold nanoparticles with the peptide, an analysis of the changes in the intensity, width and shift in the bands between corresponding Raman and SERS spectra was carried out. Conclusions about the adsorption process of VAP on gold surface are drawn below.

The SERS spectrum of VAP is shown in Figure 2(in blue) compared to that of an aqueous solution (Figure 2(in red)). With no doubt, the medium-strong intensity bands due to the indole ring system of

Trp are observed at around 758, 881, 1005, 1338-1359 and 1521cm^{-1} in the SERS spectra of VAP. Particularly important are the Raman bands at 1009 and 1549cm^{-1} due to the out-of-phase benzene and pyrrole ring breathing and the pyrrole ring stretching vibration ν(C=C), respectively. These two bands appear at 1005 and 1521cm^{-1} in the VAP SERS spectrum (Figure 2, in blue). The first-mentioned spectral feature (at 1005cm^{-1}) increases in intensity and slightly shifts (Δ=-4cm^{-1}) from its position in the Raman spectrum, while the second one (at 1521cm^{-1}) shifts by 28cm^{-1} to lower wavenumbers. Additionally, the band width of these bands increases. Likewise, bands at 1138, 1359cm^{-1} are broader in the SERS spectrum and look like one single band. Hence, this could indicate that the Trp strongly binds to the gold nanoparticles.

On the other hand, the absence or very weak intensity of bands due to the Tyr or Phe vibrations in the SERS spectrum suggests that these side chain groups either lie parallel to the gold nanoparticle or are sufficiently far from the surface. Finally, among a variety of interactions in peptides and proteins, disulfide bridges are very important for the protein folding. The Raman and SERS spectra yield excellent information about disulfide bond conformations, based on the frequency of the S-S stretching vibration. The appearance at 508cm^{-1} a band assigned to the S-S mode suggests that the disulfide bond interacts with the metal surface without bridge cleavage.

4. Conclusions

The peptide vapreotide, a somatostatin analogue, has been vibrationally studied by Surface Enhanced Raman Scattering. Optimum conditions for the detection of a strong Raman signal have been found. SERS spectrum of VAP adsorbed on colloidal gold surface differs considerably from its Raman spectrum and exhibits features that can be used to identify the specific residues in the adsorbed peptide. The VAP SERS spectrum is dominated by signals coming from the tryptophan amino acid. Changes in the SERS spectra of VAP adsorbed on the gold surface showed that adsorption appeared to be stablished via the Trp side chain.

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