



# Surface-enhanced Raman spectroscopy of tridehydropeptides adsorbed on silver electrode



Mariusz Gackowski<sup>a,b</sup>, Kamilla Malek<sup>a,\*</sup>

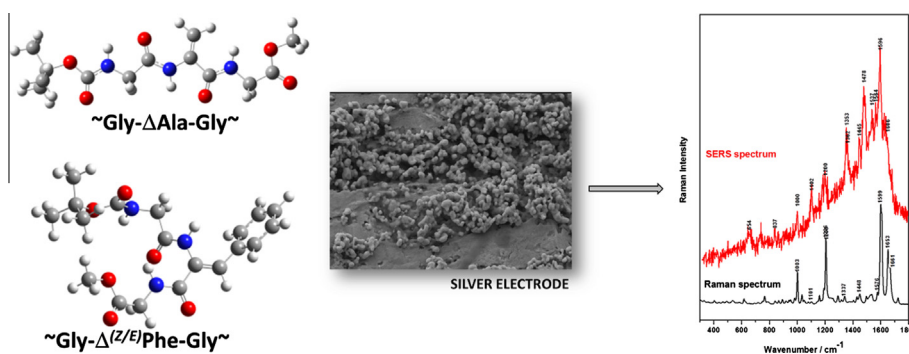
<sup>a</sup> Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Krakow, Poland

<sup>b</sup> Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences, Niezapominajek 8, 30-239 Krakow, Poland

## HIGHLIGHTS

- Peptides containing dehydroalanine and two isomers of dehydrophenylalanine are studied.
- The structure of the peptides is investigated by using IR, Raman and SERS techniques.
- The adsorption mechanism of the peptides on the silver electrode is proposed.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 14 January 2014

Received in revised form 25 February 2014

Accepted 25 February 2014

Available online 11 March 2014

### Keywords:

Dehydroalanine  
Dehydrophenylalanine  
Tripeptides  
SERS  
Ag electrode

## ABSTRACT

Surface-enhanced Raman spectroscopy (SERS) was used to characterise interactions between six tridehydropeptides and the silver electrode. Boc-Gly-X-Gly-OMe and Boc-Gly-X-Gly-COOH (X = dehydroalanine ( $\Delta$ Ala), dehydrophenylalanine ( $\Delta^{(Z)}$ Phe and  $\Delta^{(E)}$ Phe)) were studied in this work. The type of the rigid dehydroamino acid residue and the isomer of  $\Delta$ Phe have a strong impact on the adsorption mechanism of the peptides. The respective vibrational assignments were proposed by the analysis of FTIR and FT-Raman spectra of solids enabling the evaluation of SERS spectra. Generally, the most intensive SERS bands relative to those in the bulk-phase spectra are associated with vibrations of the dehydroamino acid moiety, i.e. the C=C bond and the phenyl ring. Only, in the case of the peptides containing the  $\Delta$ Ala and  $\Delta^{(Z)}$ Phe residues and ionised carboxylate group, the molecules interact with the silver electrode *via* the peptide backbone. In most cases of the peptide containing  $\Delta$ Phe the aromatic ring is almost perpendicular to the metal surface.

© 2014 Elsevier B.V. All rights reserved.

## 1. Introduction

Dehydroamino acids are formed by changing  $sp^3$  hybridization of the  $C_\alpha$  carbon. The formed double  $C_\alpha C_\beta$  bond promotes the steric hindrance and imposes conformational restrictions in peptides. In addition, this leads to  $\pi$ -electron conjugation between the  $C_\alpha C_\beta$

bond and amide groups. This modified amino acid residues have been found in a number of naturally occurring peptides like in nisins [1] or thiopeptide antibiotics [2] and their action is also connected with biocatalysis [3,4]. The most common in nature dehydroamino acids are dehydroalanine ( $\Delta$ Ala) and ( $Z$ )dehydrophenylalanine ( $\Delta^{(Z)}$ Phe). The  $E$  form of  $\Delta$ Phe shows the lower thermodynamic stability than the  $Z$  analogue and is less common in natural peptides. It is expected that both the isomers possess different conformational structures, and therefore they often serve

\* Corresponding author. Tel.: +48 12 663 2064; fax: +48 12 634 0515.

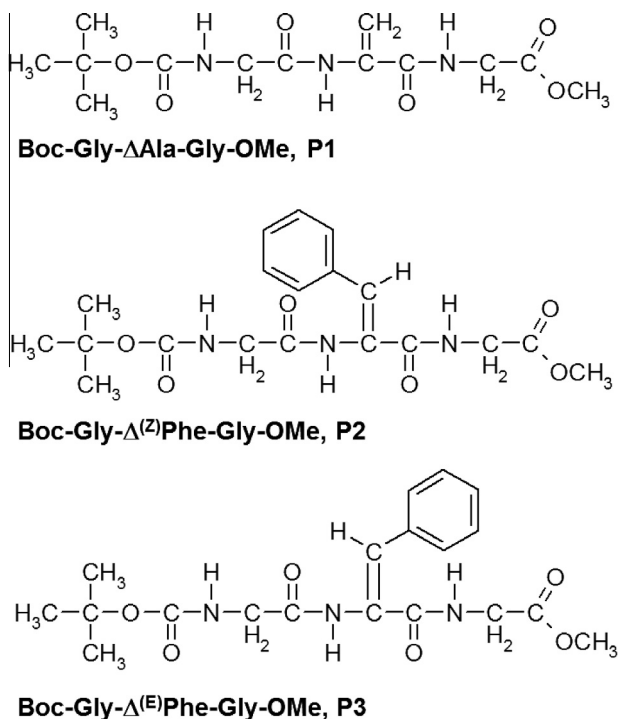
E-mail address: [malek@chemia.uj.edu.pl](mailto:malek@chemia.uj.edu.pl) (K. Malek).

as pharmacophores [5]. However, details on mechanism of their pharmacological action have not been yet recognised.

Fourier transform infrared absorption (FTIR) and Raman scattering (RS) spectroscopies are well known as a powerful tool in studies on the secondary structure of peptides and proteins in the solid state and solutions by investigating the spectral amide I, II and III regions sensitive to the peptide backbone structure. Several experimental [6-11] and theoretical [6,8,11] studies on molecular structure of peptides containing  $\Delta$ Ala and  $\Delta^{(E/Z)}$ Phe have been reported. But they have been mainly focused on the analysis of IR [6-9], circular dichroism [7,10] and NMR spectra [11].

The SERS spectra of various kinds of peptides have been also reported up to now [12-16]. However, to our best knowledge, a tentative Raman and SERS study of dehydropeptides of various number of dehydroresidues and terminal groups has not been reported except of our recent study [17]. In this work we reported IR, Raman and SERS spectra of three dipeptides Boc-Gly-X, where X is  $\Delta$ Ala,  $\Delta^{(Z)}$ Phe and  $\Delta^{(E)}$ Phe. SERS spectra were collected by using Ag sol as a metal substrate. In this case, the dehydropeptide-metal interactions mainly occur due to the deprotonation of the terminal carboxylic group. The adsorption process strongly affects the appearance of SERS bands in the region of 1500-1650  $\text{cm}^{-1}$ , indicating possibility of  $\pi$ -electron resonance between the phenyl ring and the peptide backbone. SERS technique was also used in studies on pentapeptides containing  $\Delta$ Phe [18]. This investigation showed that this type of peptides is promising a capping agent for gold nanoparticles exhibiting properties of drug delivery vehicles.

In the present study, we focus on an evaluation of SERS features of trihydropeptides, in which a rigid central moiety ( $\Delta$ Ala,  $\Delta$ Phe) is surrounded by two flexible glycine residues. The following peptides are chosen as model compounds: Boc-Gly- $\Delta$ Ala-Gly-OMe (P1), Boc-Gly- $\Delta^{(Z)}$ Phe-Gly-OMe (P2), and Boc-Gly- $\Delta^{(E)}$ Phe-Gly-OMe (P3) (Boc, *t*-butoxycarbonyl; OMe, methoxy), see Scheme 1. In addition, we investigate SERS profile of their structural analogous, in which the OMe group is substituted by the COOH group (P1', P2', and P3', respectively) since it is well known that this group exhibits the high affinity to the metal surface [17,23].



Scheme 1. Structures of the tripeptides P1-P3.

Despite this the chosen molecules exhibit a variety of conformational preferences in the solid state that may be reflected in their ability to adsorb on the metallic support. Thus, we also discuss FTIR and Raman profile of the solids to give an insight into molecular structures of the peptides. The main aim of this work is to determine the nature of the interaction of these peptides with the solid silver surface as well as to determine how the type of dehydroamino acid residue and the C-terminal functional group affect peptide ability to adsorb on the silver electrode.

## 2. Experimental

Compounds were synthesized according to the procedure described in [19]. Briefly, peptides were synthesized in condensation reaction between trifluoroacetate (TFA) amide of alanine or phenylalanine and  $\alpha$ -keto acid (pyruvic acid or phenylpyruvic acid) in benzene. The reaction is catalysed by *p*-toluenesulfonic acid. In the case of TFA-Gly- $\Delta$ Phe-Gly, both isomers (Z and E) are formed in ratio of 4:1. Then, the TFA group is substituted by the Boc group. Yield for all syntheses: 50-80%. The purity of the compounds were tested by standard analytical methods (elemental analysis and NMR).

For FT-Raman measurements, a few milligrams of each solid sample were measured on metal discs directly. Spectra were accumulated from 256 scans, with a spectral resolution of 4  $\text{cm}^{-1}$ . Spectra were recorded on a MultiRAM FT-Raman Spectrometer (Bruker), equipped with a germanium detector cooled with liquid nitrogen. Each of the samples was illuminated from a Nd:YAG laser ( $\lambda = 1064 \text{ nm}$ ) at an output power of 100 mW. ATR FTIR spectra of the solid samples were collected using a ALPHA Bruker spectrometer equipped with a 1-reflection ATR diamond crystal. Spectra were collected in the range of 375-4000  $\text{cm}^{-1}$ , with spectral resolution of 4  $\text{cm}^{-1}$ . 128 scans were co-added, and then extended ATR correction was employed.

For SERS measurements, solids were dissolved in ethanol/water in volume ratio of 1:1 to prepare  $1 \times 10^{-3} \text{ M}$  solutions. After dissolving pH of solutions was ca. 6. Since, protonation constant of the COOH group in dehydropeptides is found in the range of 3-4, this group should be deprotonated in P1'-P3' during collection of SERS spectra [20]. The silver electrodes were prepared by electrochemical roughening, using five positive/negative cycles in 0.1 M KCl solution from -0.3 to +0.3 V (vs Ag/AgCl) and the potential close to the end of the last negative cycle was held for 30 s. A platinum electrode was used as a counter electrode while Ag/AgCl in 1 M KCl was used as a reference. The silver electrodes were immersed in  $1 \times 10^{-3} \text{ M}$  solutions of each peptide, then SERS spectra were recorded after a few hours since no SERS signal was observed immediately after immersing the electrode in the solution. From SEM pictures one can deduce that the electrode is not roughened equally on its whole surface (Fig. 1A and B). Roughness of the silver surface also varies in thickness. The SERS measurements were conducted on a LabRam 800 Raman Spectrometer equipped in a confocal microscope, a CCD detector and a He-Ne laser excitation line (632.8 nm). The spectra were collected through an air objective with the magnification of 50 $\times$  with the use of 600 g/mm grating. For each SERS spectrum 60 scans were collected with integration time of 1 s. Output laser power was set from 0.154 to 3.4 mW. Laser power for each measurement of SERS spectrum was adjusted in such a way to use a minimal power and to obtain spectrum showing a good signal to noise ratio.

## 3. Results and discussion

Figs. 2-4 show FT-Raman spectra of the peptides P1-P3 and their analogues P1'-P3' in the solid state along with SERS spectra collected on silver electrodes.

Download English Version:

<https://daneshyari.com/en/article/1409346>

Download Persian Version:

<https://daneshyari.com/article/1409346>

[Daneshyari.com](https://daneshyari.com)