Applied Surface Science 257 (2011) 8246–8252

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Surface-enhanced Raman scattering studies on bombesin, its selected fragments and related peptides adsorbed at the silver colloidal surface

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ARTICLE INFO

Article history:
Available online 16 February 2011

Keywords:
Surface-enhanced Raman scattering
SERS
Colloidal silver surface
Bombesin
BN
BN-related peptides

ABSTRACT

SERS studies presented in this work on **BN$^{8-14}$**, **[D-Phe^{6},β-Ala^{11},Phe^{13},Nle^{14}]BN$^{6-14}$**, **[D-Tyr^{6},β-Ala^{11},Phe^{13},Nle^{14}]BN$^{6-14}$**, **BN** and its modified analogues, as well as **NMB**, **NMC**, and **PG-L** show that these molecules at pH 8.3 bind to a colloidal silver surface mainly through Trp^{8} and Met^{14} residues. Trp^{8} adsorbs at the surface almost perpendicularly. Met^{14} appears on the surface mainly as a P_C-G conformer. His^{12}, as is evident from the spectra, practically does not take part in the adsorption process. Substitution of L-leucine at the 13 position of amino acid sequence with L-phenylalanine does not change substantially the pattern of the adsorption mechanism; however, substitution of phenylalanine at the 12 position (instead of L-histidine) causes changes in the SERS spectra that show that Phe^{12} takes parallel orientation to the surface upon adsorption of **[D-Phe^{12}]BN**, while in the case of **[Tyr^{4},D-Phe^{12}]BN** this residue is perpendicular to the surface and influences the orientation of the bound Trp^{8}. On the other hand, substitution of Asn with Tyr in the 6 position in nonapeptide fragment causes changes in the adsorption mechanism. In this case, the discussed fragment binds to the silver colloidal surface by Tyr^{6}, Trp^{8}, and Met^{14}. The SERS spectrum of **NMC** is very similar to that of **BN**; although it differs by the binding orientation of the amide bond towards the surface. Appearance of Phe^{13} in **NMB** and **PG-L** causes that this residue competes successfully with Trp^{8} forcing it to take tilted orientation. As seen from the enhancement of the characteristic Phe vibrations this moiety in **NMB** and **PG-L** adsorbs on the silver surface in a tilted fashion. This arrangements cause that the 8–14 peptide chain in all these studied compounds takes almost a parallel orientation to the surface while the 1–5 fragment of the peptide chain is removed from the silver surface vicinity.

1. Introduction

Bombesin (**BN**), tetradecpeptide with the amino acid sequence of pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (where pGlu is 5-oxo-proline), is an endogenous neurotransmitter that was in 1971 isolated from the skin of the toads *Bombina bombina* and *Bombina variegate* [1]. Later **BN** was identified in mammalian brain, gut, and lungs [2]. In humans, **BN** binds to the GRP-preferring bombesin receptor (rGRP-R) [3], a member of the G protein-coupled receptor superfamily, and activates a complex signaling pathways and biological responses, including lung branching morphogenesis; proliferation of fibroblasts, lymphocytes, and cells of endothelial, epithelial, and mesenchymal origin [4,5]; and Type II cell differentiation [6]. A possible role of **BN** in inflammatory processes *in vitro* that include macrophage activation and phagocytosis [7], chemotaxis of macrophages and fibroblasts [6], vascular and other smooth muscle constriction [8], gastric secretion, and renal circulation and function was suggested. **BN** is the second, after cholecystokinin, most important source of negative feedback signals that stop eating behavior [9].

Numerous structure–function studies on **BN** and its analogues attempting to define its binding domain and requirements for receptor activation have been done [10,11]. These studies have demonstrated that the **BN** carboxyl terminal is the most biologically active molecular fragment of the molecule [12] and its heptapeptide (**BN$^{8-14}$**) is the minimal fragment that shows similar to **BN** biologic activity towards rGRP-R [13]. It has also been proposed that the active conformation of **BN** for interacting with rGRP-R is

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doi:10.1016/j.apsusc.2011.02.012

an antiparallel β-sheet structure in the *C*-terminal with a turn at position 10–13 and hydrogen bonds between the amide NH_2 of Met^{14} and C=O of Trp^8, C=O of Leu^{13} and N–H of Val^{10}, and between N–H of Leu^{13} and C=O Val^{10} [14–16]. Also, it has been shown that amino acids in positions from 7 to 10 of the *C*-terminal have different importance for rGRP-R and other bombesin receptor subtypes suggesting that the model of the **BN** active configuration for interacting with rGRP-R is not applicable to other receptors subtypes [17]. In addition, the carbonyl unit of the Gln^7 residue has been proven to play a key role for recognizing the receptor pathway in mammalian pancreatic acinar cells [18]; however, it has been proposed that rather the C=O moiety of the peptide bond between Gly^7 and Trp^8 than COOH of Gln^7 and its arrangement in the receptor binding domain is responsible for producing increase/decrease in rGRP-R affinity.

During the last three decades, several bombesin-related peptides have been characterized, including phyllolitorin and its Leu^8 analogue from *Phyllomedusa sauvagei* ([Leu^8]phyllolitorin, L-phenylalanine at the 8 position (Phe^8) of the phyllolitorin amino acid sequence substituted by L-leucine (Leu^8)), neuromedin C (NMC, $GRP^{18–27}$ – fragment from 18th to 27th amino acid in sequence of gastrin releasing peptide), neuromedin B (NMB), and peptide from *Pseudophryne guntheri* (PG-L) [2,19–23]. The amino acid sequences of these peptides are listed in Table 1. These peptides function as neurotransmitters in the central nervous system [2,6] and as regulators of numerous gastrointestinal functions [11,24]; additionally, they play an important role in normal lung development and in several pathologic conditions in the lung [25,26]. Furthermore, bombesin-like peptides are potent growth agents that cause proliferation of normal cells [27] and can accelerate growth of various tumor cell lines (small lung cancers, prostate, gastric, pancreatic, colon, and breast carcinomas) [28–32]. The use of **BN**-like antagonists as carrier biomolecules for targeting cytotoxic drugs to tumor cells has also recently been proposed [33]. However, little is currently known about the mechanism of action of these bombesin-like peptides.

Given the structural similarity among these peptides and their receptors [34,35], it can be hypothesized that small alterations in both the amino acid composition and tertiary structures of these peptides may play an important role in determining their affinity to the mammalian bombesin/GRP-preferring subtype receptor (rGRP-R), neuromedin B-preferring subtype receptor (rNMB-R), orphan subtype-3 receptor (hBRS-3R), and the subtype-4 receptor (fBB_4) [11,36–38].

In the present study we used surface-enhanced Raman scattering method, SERS, a simple and rapid method for probing different types of supramolecular architecture and studying adsorption phenomena at the peptide level [39–49]. This interaction is believed to be of great significance. That is due to the facts that in the presence of a solid surface, the process of protein adsorption is often energetically favorable; the adsorption of proteins does not affect their binding capabilities, implying that their structures are not strongly perturbed on the surface; and on a interface that is formed between biomolecule and metal surface proteins have regions, which directly interact with this surface. The amino acid composition and sequence of these regions usually determine the adsorption behavior of proteins onto given metal surfaces. Therefore, analysis of the SERS signal (enhancement, broadness, and wavenumber) coming from constituents' amino acids is useful for understanding possible ways in which a peptide can interact with the surrounding medium, such as how a peptide binds at solid/liquid interface [39–49].

The main concept of surface-enhanced Raman spectroscopy is based on the fact that in this method the bands that appear in the spectra are associated with vibrations of molecules or their fragments that adsorb at or are in close proximity (10–15 nm) to the metal surface. Thus, careful examination of such SERS behavior of these molecules can shed the light onto the mechanism of the adsorption taking into account that appearance of certain modes and their intensity enhancements are related to the orientation of proper molecular moieties at the surface. In this regard attention is devoted to the orientation of amino acids at the surface. Thus, for better understanding of readers, we will briefly discuss the influence of orientation of amino acids adsorbed at the metal surface on SERS spectral pattern, with special emphasis on aromatic amino acids orientations, since these molecules are the most important in understanding of adsorption mechanism of biomolecules studied in this work. As a result of a vast variety of evidence these molecules can be mainly oriented in end-on or edge-on manner taking perpendicular or slightly tilted geometry to the surface. However, in certain cases, they can take parallel orientation in which they π-system will interact with the surface, i.e., the aromatic ring lays flat on the surface. When the molecule takes perpendicular orientation to the surface, vibrational modes which are out-of-plane are either not present or very weak compared to in-plane modes of the adsorbed molecule due to SERS surface selection rules [50,51]. In such binding geometry a large number of the frequencies of SERS bands will decrease in comparison to Raman spectra. On the other hand, in general, out-of plane modes are strongly enhanced in comparison to in-plane modes that can be even missing from the spectra when molecule takes parallel orientation to the surface. In certain cases Raman forbidden (IR active) modes can be activated by the interaction of π-system with the surface. In addition, frequency shifts of molecules that adsorb *via* π-system are usually opposite to those observed for perpendicular adsorption.

Among amino acids the most complex binding is expected for histidine with the proposed strength of interaction with the surface arranged in the order: $N_{pyridine} < \pi$ bonds $< N_{pyrrole}$ despite binding to the metal surface by carboxyl group [52]. This order predicts that imidazole ring will be adsorbed *via* the pyridine nitrogen atom lone pair electrons resulting in a near vertical orientation. If so, the in-plane modes will be mainly enhanced in SERS spectrum. If histidine adsorbs through the pyrrole N lone electron pair the ring plane is expected to be tilted thus it will be oriented at the angle of approximately 60° from the surface normal. Thus, the out-of-plane modes in this orientation would be more enhanced but in-plane modes would still have some component normal to the surface. In other words, the observation of ν(NH) stretching mode in SERS spectra of this molecule indicates interaction of the pyridine N atom with the surface (perpendicular orientation of the ring) since if histidine is adsorbed *via* the pyrrole N atom, the N–H bond will be nearly parallel to the surface and the in-plane ν(NH) stretching mode will be not observed. Finally, a π bonded molecule would lay flat with the out-of-plane modes significantly enhanced relatively to the in-plane modes that often are not even observed in SERS spectra in such molecular arrangement. The best example of such binding is observation of δ(CH) mode at 1080 cm^{-1} that is one of the most intense band in the spectrum [52]. Keeping this in mind a tilted orientation is suggested by the enhancement of both in-plane and out-of-plane modes. The presence of in-plane modes that are enhanced together with those that are not enhanced is expected due to the fact that some of the in-plane modes might have components that are predominantly parallel to the surface causing the resonance enhancement of discussed modes.

Previously we showed [39,40,44,45,53,54] that adsorption mechanism of these species depends upon metal (Ag, Au and Cu), its surface rheology, and physicochemical properties of solution. The aim of the present work is to elucidate the nature of interaction of **BN**, its selected fragments and related peptides at the level that will allow understanding the nature of interaction of investigated molecules with the colloidal silver surface in terms of competition of certain structural components (amino acids) in adsorption pro-