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Adsorption of bombesin and its carboxyl terminal fragments onto the colloidal gold nanoparticles: SERS studies

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ABSTRACT

Surface-enhanced Raman scattering (SERS) is a Raman spectroscopy technique often used to study processes occurring at a solid/liquid interface. In this work, SERS was used to investigate the adsorption of bombesin (BN) and its *C*-terminal fragments adsorbed onto colloidal gold nanoparticles. Briefly, the SERS results demonstrated that (1) the elongation of the *C*-terminal peptide fragment causes movement of the Met (L-methionine) side-chain in the direction of the gold nanoparticles and the weakening of the interactions between the amide bond and gold; (2) the His (L-histidine) residue assists in the peptides interaction with the gold nanoparticles; and (3) Trp (L-tryptophan) dramatically changes the SERS spectral pattern on gold.

1. Introduction

Bombesin (BN) (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 found in many animals and humans. In humans, BN binds with the high affinity to the bombesin-preferred G-protein-coupled receptors (BRS-3 or BB_3) [1–3] and activates a complex network of signalling pathways and biological responses, including: hypertension, hyperglycemia, hyperinsulinemia, contraction of uterus, colon, or ileum, locomotor activity, and stimulation of gastric secretion. It also stimulates the growth of various tumor cell lines (*i.e.*, lung, prostate, stomach, pancreas, colon, and breast). Such a large variety of the biological functions of BN has caused interest in the understanding of the relationship between the BN function and structure [4–8].

The carboxyl terminal fragment of bombesin (Table 1), having 2–9 amino acid of the BN amino acid sequence, has been proved to be responsible for the BN biological activity. It has been also demonstrated that an antiparallel β-sheet structure in the *C*-terminal peptide end with a turn at positions 10–13 and hydrogen-bonds between the amino group (NH_2) of L-methionine (Met^{14}) and C=O of L-tryptophan (Try^8), C=O of L-leucine (Leu^{13}) and N—H of L-valine (Val^{10}), and between N—H of Leu^{13} and C=O of Val is the active conformation, in which BN binds to BB_3. Any perturbation of these hydrogen-bonds, which play an important role in the preserving of the BN β-sheet-folded structure, causes changes in the binding affinity of BN to its receptors [9–11]. Thus, the amino acids composition of BN at positions from 7 to 10 of the amino acid sequence has been demonstrated to be important for BB_3 and other bombesin receptor subtypes [12]. However, the minimal native BN fragment that shows the full biological activity towards BB_3 is BN^{8-14} (the 8–14 amino acids fragment) [13–15]. In addition, it has been suggested that the C=O moiety of the peptide bond between L-glycine (Gly^7) and Trp^8 rather than —COOH of Gln^7 (L-glutamine at position 7) and its arrangement in the receptor binding domain are responsible for the decrease/increase in the BN affinity to BB_3 [16,17].

The biological importance of BN motivated us to perform a number of spectroscopic studies on the native fragments of bombesin, such as: BN^{13-14}, BN^{12-14}, BN^{11-14}, BN^{10-14}, BN^{9-14}, and BN^{8-14} (X–14 fragments of the BN amino acid sequence) using vibrational spectroscopy methods and quantum-chemical calculations. Previously, we have shown the Raman and surface-enhanced Raman, in an aqueous silver sol, investigations and density functional theory (DFT) calculations of Raman wavenumbers and intensities with extended basis sets (B3LYP/6-31++G**) with the aim of providing the definitive band allocations to the normal coordinates [18]. This paper presents the surface-enhanced Raman scattering (SERS) results for BN and its aforementioned carboxyl terminal fragments immobilized onto the colloidal gold nanoparticles. This is because the morphology of the adsorbed species in monolayer and submonolayer coverage of

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Table 1
Wavenumbers and bands assignment for the SERS spectra of BN and its *C*-terminal fragments adsorbed onto the colloid gold surface.

	Wavenumber [cm^{-1}]						
	BN^{13-14}	BN^{12-14}	BN^{11-14}	BN^{10-14}	BN^{9-14}	BN^{8-14}	BN
ν(C—H)$_{ring}$	–	–	–	–	–	3004	–
ν_{as}(C—H)$_{CH3}$	–	–	–	–	2976	–	–
ν_{as}(C—H)$_{CH2}$	2925	2914	2913	2907	2912	2919	–
ν_s(C—H)$_{CH3}$	–	2854	2875	2853	2852	2875	–
Amide I	1628	1628	–	–	1640	–	–
ν_{as}(COO^-)	1578	1572	–	–	–	–	–
His [ν(ring) + $\rho_r(N_1H)$]	–	1558	1570	1576	1570	–	–
W3 [pyrrole $\boldsymbol{\nu}$($C_2 = C_3$)*]*	–	–	–	–	–	1567	1540
Amide II	1535	1519	1521	1536	1524	–	1519
W6 [pyrrole $\boldsymbol{\nu}_s(N_1C_2C_3) + \delta(N_1\text{-}H)$ *and phenyl* $\delta(CH)$*]*	–	–	–	–	–	1483	–
$\delta/\rho_b(CH_3)$, δ/ρ_b(C-CH_3)	1460	1465	–	1471	1461	–	–
W5 [phenyl]	–	–	–	–	–	–	1448
His [$\delta(C_2H) + \delta(N_1H)$]	–	–	1411	–	1418	–	
ν(COO^-)	1375	1380	–	–	–	–	–
$\rho_\omega(CH_2)$, δ(S-CH_3), δ(CCH_2C)	1337	–	–	–	–	–	1323
ν(C-NH_2)	1295						
His[δ(ring) + $\rho_{ipb}(C_2H)$]	–	1304	–	–	1301	1297	–
Amide III	1249	1264	1268	1269	1259	1272	–
W10 [$\boldsymbol{\nu}$(C_3-C_β + $\boldsymbol{\nu}$(C-*H)]*	–	–	–	–	–	1244	1249
$\rho_\tau(CH_2)_{Trp}$	–	–	–	–	–	–	1217
ν(C—N_A), $\rho_b(C_AN_AHC)$, $\delta(CC_AO_ANA)$	–	–	1194	–	1177	1197	–
W12 [$\delta(N_1H)$*]*	–	–	–	–	–	–	1165
ρ_w(C—NH_2)	1146	1158	–	–	–	1152	–
ν_{as}(CCN), δ(C—NH_2), δ(NCH_2C_A), ν(C—N_A)	–	1134	1135	1135	1135	1135	–
His [$\rho_{ipb}(C_2H)$], $\rho_\tau(NH_2)$	–	1083	–	–	1078	–	–
ν(C—C)	1027	–	1025	–	–	–	–
W16 [phenyl and pyrrole ring out-of-phase breathing]	–	–	–	–	–	–	1004
His [δ(ring)],ν(CN), $\rho_b(NH_2)$	–	–	–	999	1004	–	–
ν(C—C/N), δ(C—NH_2)	972	972	961	–	948	–	–
His [δ(ring) + $\delta(N_1H)$] or ν(C—C=O)	918	–	–	–	917	900	918
W1 [o.o.p. CH benzene def.]	–	–	–	–	–	–	887
ν_{as}(CSC) or *W11 [phenyl]*	817	–	–	–	833	818	–
W18 [sym phenyl/pyrrole ring breathing]	–	–	–	–	–	–	789
ν(C-S) P_C-T or *W19*	–	–	–	–	–	–	713
ν(C-S) P_C-G	685	–	–	–	–	–	–
His [ρ_{oopb}(CH)]	–	–	669	–	–	–	–
His [ρ_{oopb}(CH)]	–	646	654	–	–	–	–
W and/or ν(C-S) P_H-G/T	636	–	–	639	636	636	637
ν(C-S) P_H-G	610	–	–	–	–	612	–

Abbreviations: φ: benzene ring; ν: stretching; ν_{as}: antisymmetric stretching; ν_s: symmetric stretching; δ: deformation; ρ_{oop}: out-of-plane deformation; ρ_{ipb}: in- plane deformation; ρ_w: wagging; ρ_b: bending; ρ_τ: twisting vibrations; W: tryptophan; C_A: the carbon atom of the amide bond; N_A: the nitrogen atom of the amide bond; O_A: the oxygen atom of the amide bond.

a metallic surface and the strength of the competitive interaction of certain molecular fragments with this surface clearly depend on the nanostructure of the metallic surface and thus, on the controlled distribution of the metal surface plasmon [19–21]. Therefore, the SERS effect must be investigated for molecules immobilized on different defined metallic surfaces.

SERS is the most common *in situ* technique in the contexts involving the adsorption process at solid/liquid interface because of its simplicity and ability to rapidly recognize the structures of peptides, neurotransmitters, enzymes, antibodies, or DNA in an aqueous solution as well as its ability to probe different types of supramolecular architectures and study the peptide-level adsorption phenomena [22–25]. These interactions are believed to be of great significance for a number of fundamental and applied fields, including: bio-catalysis, bio-electrochemistry, biosensor construction, biomaterial biocompatibility analysis, and the understanding of the *in vivo* behavior of implants [26–28]. The determined properties of the nanostructural interactions between biological and physical systems can be used in the future to construct biological sensors, understand periprocedural adverse events, occurring when the implant is in contact with body fluid components (*e.g.* blood plasma), design optimal coating layers of bone implants (stents) and artificial hearts, and improve drug design and synthesis processes.

2. Experimental

2.1. Materials

Bombesin and its carboxyl terminal fragments: BN^{13-14}, BN^{12-14}, BN^{11-14}, BN^{10-14} and BN^{9-14} (X–14 fragments of the BN amino acid sequence) were purchased from Sigma-Aldrich, Japan, whereas BN^{8-14} was purchased from Bachem Co., Switzerland. All the samples were used without further purification.

Gold colloid solutions (~20 nm nanoparticles size; polydispersity index (PDI) <0.2; concentration: ~0.01% as $HAuCl_4$; pH 7; proprietary surfactant as stabilizer) were purchased from Sigma (Poland).

2.2. Preparation of peptide samples

All the investigated peptides were dissolved in deionized water (0,08 μS/cm) to give solutions with a peptide concentration of 10^{-4} Mol/dm^3 and pH of 7.