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Surface-enhanced Raman studies of bradykinin using colloidal gold☆

Dominika Święch[a], Younkyoo Kim[b], Edyta Proniewicz[a,*]

[a] Faculty of Foundry Engineering, AGH University of Science and Technology, ul. Reymonta 23, 30-059 Kraków, Poland
[b] Department of Chemistry, Hankuk University of Foreign Studies, Yongin, Kyunggi-Do 449-791, Republic of Korea

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ABSTRACT

In this paper, bradykinin (BK), an endogenous peptide hormone, which is involved in a number of physiological and pathophysiological processes was deposited onto the colloidal Au nanoparticles. The surface-enhanced Raman spectroscopy (SERS) was used to determine the adsorption mode of BK under different environmental conditions, including: excitation wavelengths (514.5 nm and 785.0 nm), pH of aqueous sol solutions (from pH = 3 to pH = 11), and size of the colloidal nanoparticles (10, 20, and 50 nm). The metal surface plasmon of the colloidal suspended Au nanoparticles was examined by ultraviolet-visible (UV–vis) spectroscopy. The results showed that the *C*-terminal part of BK plays a crucial role in the adsorption process onto the colloidal suspended Au particles. The $Phe^{5/8}$ and Arg^{9} residues of BK mainly participate in the interactions with the colloidal Au nanoparticles. At acidic pH of the solution (pH = 3), the BK $—COO^-$ terminal group through the both oxygen atoms strongly binds to the Au nanoparticles. The Phe^{5}/Phe^{8} rings adopt tilted orientation with respect to the colloidal Au nanoparticles with diameters of 10 and 20 nm. As the particle size increases to 50 nm, the flat orientation of the Phe ring(s) with respect to the Au nanoparticles is observed.

1. Introduction

The kinins are a family of hormones, which are implicated in many physiological and pathophysiological processes [1,2]. They are generated in plasma and tissues due to infection, inflammatory changes, etc. [3]. Bradykinin (BK; Arg^{1}-Pro^{2}-Pro^{3}-Gly^{4}-Phe^{5}-Ser^{6}-Pro^{7}-Phe^{8}-Arg^{9}COOH, see Fig. 1), one of the most important kinin, produces different kinds of effects, including: induction of smooth muscles contraction; stimulation of the secretion of chloride ions by epithelial cells and endothelium prostaglandin secretion; stimulation of nitric oxide (NO) and prostacyclin release from endothelial cells and isolated arterial preparations; and induction of sodium/-water homeostasis [3,5–10]. This local hormone is also known as a factor of many diseases, such as: asthma, sepsis, rheumatoid arthritis, pancreatitis, and in the central nervous system: epilepsy or Alzheimer's disease [11–13]. It has been also suggested that BK is an important factor for cancer growth, such as small-cell lung carcinoma (SCLC), non-SCLC, and prostate cancer [14–16]. The above-mentioned biological effects of BK are mediated by the activation of two subtypes of the heptahelical transmembrane G-protein-coupled receptors (GPCRs), specifically, the B_1 and B_2 receptors [17]. The B_1 receptors are poorly detectable under the physiological conditions, whereas the B_2 receptors are continuously produced and trigger most of the BK biological effects [18].

Many research groups have provided the comprehensive theoretical and experimental studies on this peptide [4,19–27]. A number of investigations, involving: nuclear magnetic resonance (NMR), circular dichroism (CD), electron spin resonance spectroscopy (EPR), and molecular dynamics have been used to propose the model of binding of BK to the natural and model membranes [20–24]. Manna et al. have reported that positively charged BK stays on the anionic bilayer surface because of the electrostatic attraction of the two terminal *L*-arginines (Arg^{1} and Arg^{9}). These Arg residues are associated with the lipid bilayer surface via the lipid head groups [24]. In addition, the formation of the *C*-terminus β–turn structure, which is stabilized by several "inter-residue hydrogen bonds" between the amide NH of Arg^{9} and O=C of Pro^{7}, the amide NH of Phe^{8} and —O=C of Ser^{6}, and NH of Arg^{9} and OH of Ser^{6} has been suggested [24]. In the presence of the dodecyl sulfate micelles and phospholipids, the β-turn conformation of the Ser^{6}-Arg^{9} fragment, stabilized by a hydrogen bond between Ser^{6} and Arg^{9}, has been also proposed [25]. Kyle et al. by homology modeling and docking simulations have suggested a twisted S-shaped model of BK bound to the rat B_2 receptor, where the internal Phe^{5}, Phe^{8}, and Pro^{7} of BK are bound in the hydrophobic cavities [22]. Another

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* Corresponding author.
E-mail address: proniewi@agh.edu.pl (E. Proniewicz).

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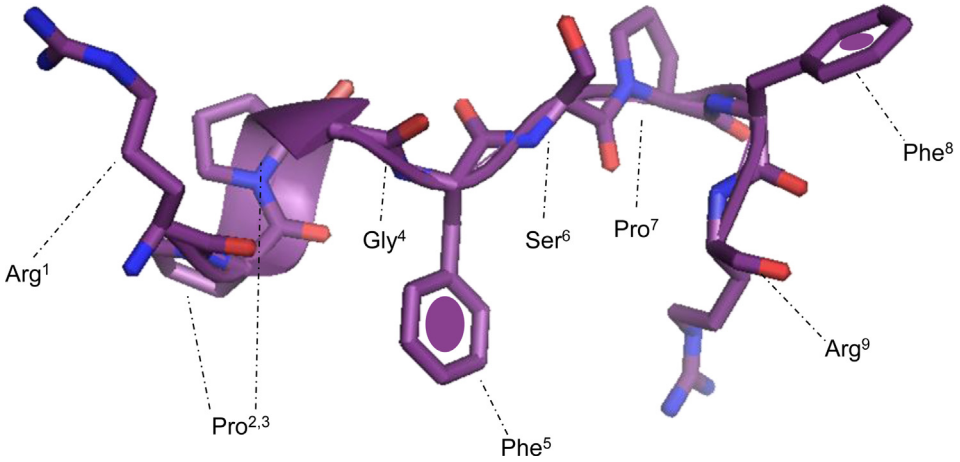

Fig. 1. The molecular structure of BK [4].

study has shown that the kinin receptor subtype selectivity depends on the presence of the charged terminal residues [26]. The B_2 receptor affinity is drastically reduced by the removal of Arg^9 what indicates that the B_2 receptor requires full-length BK for recognition [26,27]. In 2008, Lopez et al. based on the solid-state NMR data has proposed that the Ser^6-Arg^9 *C*-terminal fragment of BK is crucial for the BK interaction with the human B_2 receptor and BK adopts "distinct double-S-shaped structure" [4].

The biological significance of BK and the biological tests, showing the way of BK interactions with the transmembrane B_2 receptors and different membranes motivated our research group to undertake investigations on the BK behavior at solid/liquid interface, where a colloidal Au nanoparticles act as a solid. The reason for choosing the colloidal Au nanoparticles as a solid were our earlier interesting results on bombesin family peptides – neurotransmitters that also serve as the ligands of the transmembrane GPCR receptors [28–32]. The results of these studies have demonstrated some analogy in the information coming from the biological activity and SERS studies. For bombesin analogues, the relative potency for inhibition of binding of ^{125}I-[Tyr^4]BN to rat pancreas acini cells was correlated with the behavior of the amide bond on the colloidal Ag nanoparticles, while the contribution of the structural components to the ability to interact with the GRPR receptors was correlated with the SERS patterns. It means that the same molecular fragments of bombesin analogues interact with the transmembrane bombesin-prefered GPCR receptors and with the given metallic surface; and these molecular fragments, which do not interact with the GPCR receptors are not observed in the bombesin SERS spectra. In addition, our earlier experiments have shown that it is possible to control the orientation of the adsorbed molecule by changing the type and roughness size of the metallic surface.

Previously, we have characterized the orientation of BK deposited onto the roughened in the oxidation-reduction cycles Ag and Au electrode surfaces and in the Ag sol [33–36]. Thus, to provide missing information about the adsorption process of this biomolecule we decided to describe the behavior of BK adsorbed onto the colloidal Au nanoparticles.

The implication of this work is that an enhanced understanding of (1) the adsorption mode of bradykinin onto the colloidal Au nanoparticles and (2) the influence of the different environmental conditions, including: excitation wavelengths (514.5 nm and 785.0 nm), pH of aqueous sol solutions (from pH = 3 to pH = 11), and size of the colloidal nanoparticles (10, 20, and 50 nm) on this process that allow for future predictions regarding the interaction between a peptide of a known amino acid sequence and a given Au surface. These studies are important because the rheology of the adsorbed species in monolayer and submonolayer coverage of 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 [37]. Therefore, the SERS effect must be investigated for molecules immobilized on different defined metallic surfaces (different types of metals and different roughness size). Note that 90% of SERS studies have used a variety of Ag surfaces because they generally result in the strongest SERS signal [38,39]. Au has also been used as a SERS-active substrate because it is stable and provides surface plasmon resonance conditions in the visible and near-infrared spectral regions; however, it does not produce an enhancement as strong as that of Ag [40]. Compared with Ag surfaces, Au surfaces display several desirable properties: (1) they prevent surface oxidant formation; (2) Au has a higher oxidizing potential than Ag, allowing Au to be used in various redox studies on electrodes; (3) Au is suitable for chemical modification by deposition on metallic and nonmetallic materials, and (4) biomolecules bound to colloidal Au particles are known to retain their biological activity [41–43].

2. Experimental methods

2.1. Bradykinin

BK (powder, ≥98% (HPLC)) was purchased from Sigma-Aldrich (Poland).

2.2. UV–vis measurements

The UV–vis spectra of the aqueous Au sol and BK/Au sol system (measured after 15 min of mixing) (see Fig. 2) were recorded on a Shimadzu UV-3100 spectrophotometer.

2.3. Raman measurements

Raman measurements were performed for liquid samples (10^{-2} M peptide concentration) placed onto a glass plate. The Raman spectra were recorded on a Renishaw spectrometer (model inVia) operating in confocal mode combined with a Peltier cooled CCD detector and a Leica microscope (50× long-distance objective). Excitation line at 785 nm was used from a continuum-wave diode laser. The lasers power at the laser outputs was set at about 10 mW. The typical exposure time for the Raman measurement in this study was 160 s.