DNA-AND BSA-BINDING STUDIES OF DINUCLEAR PALLADIUM(II) COMPLEXES WITH 1,5-NAPHTIRIDINE BRIDGING LIGANDS

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INTERAKCIJE DINUKLEARNIH 1,5-NAFTIRIDINSKIH KOMPLEKSA PALADIJUMA(II) SA DNK I BOVIN SERUM ALBUMINOM

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Received/Primljen: 22.01.2021.

Accepted/Prihvaćen: 24.02.2021.

ABSTRACT

The interactions of metal complexes with important biomolecules such as deoxyribonucleic acid (DNA) or bovine serum albumin (BSA) are responsible for their antitumor activity due to different modes of interaction with DNA and their transport through the blood system to cells and tissues via serum albumin. Therefore, the dinuclear palladium(II) complexes, $[\{Pd(en)Cl\}_2(\mu-1,5-nphe)](NO_3)_2$ (**Pd1**) and $[\{Pd(1,3-pd)Cl\}_2(\mu-1,5-nphe)](NO_3)_2$ (**Pd2**) (*en* is ethylenediamine, 1,3-*pd* is 1,3-propylenediamine and 1,5-*nphe* is the bridging 1,5-naphthyridine ligand) were synthesized and characterized by different spectroscopic methods. The UV-Vis and fluorescence emission spectroscopy were applied for evaluation of binding modes of **Pd1** and **Pd2** complexes to DNA as well as their interaction with BSA. The emission spectra indicate that the investigated **Pd1** and **Pd2** complexes can displace the ethidium bromide intercalator from DNA/EtBr molecules and act as intercalators showing strong interactions with DNA. The fluorescence intensity shows that **Pd1** and **Pd2** complexes can bind to BSA and then be transported to the cell.

Keywords: Dinuclear palladium(II) complexes; 1,5-naphthyridine; DNA; bovine serum albumin (BSA)

SAŽETAK

Interakcije kompleksa jona metala sa biološki važnim molekulima kao što su dezoksiribonukleinska kiselina (DNK) i bovin serum albumin (BSA) su od velikog značaja kako za objašnjenje njihove antitumorske aktivnosti preko različitih načina međusobne interakcije, tako i njihovog transporta putem krvi do ćelija i tkiva pomoću proteina serum albumina. Iz tog razloga, u ovom radu su sintetisana dva dinuklearna kompleksa paladijuma(II) sa 1,5-naftiridinom kao mostnim ligandom, $[\{Pd(en)Cl\}_2(\mu-1,5-nphe)](NO_3)_2$ (**Pd1**) i $[\{Pd(1,3-pd)Cl\}_2(\mu-1,5-nphe)](NO_3)_2$ (**Pd2**), a zatim je izvršena njihova karakterizacija na osnovu rezultata elementarne mikroanalize, kao i primenom IR, UV-Vis i NMR (¹H i ¹³C) spektroskopije. Primenom UV-Vis spektrofotometrije i fluorescentne spektroskopije ispitivani su načini interakcije sintetizovanih dinuklearnih kompleksa paladijuma(II) (**Pd1** i **Pd2**) sa DNK, dok je njihova interakcija sa BSA ispitivana pomoću fluorescentne spektroskopije. Emisioni spektri su pokazali da ispitivani kompleksi paladijuma(II) mogu da istisnu interkalator etidijum-bromid iz DNK molekula i da sa njim ostvaruju jake interakcije, odnosno da se ponašaju kao interkalatori. Na osnovu intenziteta fluorescencije može se zaključiti da se **Pd1** i **Pd2** kompleksi mogu vezati za BSA i na taj način transportovati do ćelije.

Ključne reči: Dinuklearni paladijum(II) kompleksi; 1,5-naftiridin; DNK; bovin serum albumin (BSA)

ABBREVIATIONS

D₂O - deuterium oxide
K₂[PdCl₄] - potassium-tetrachloridopalladium(II)
EtBr - ethidium bromide
HCl - hydrochloric acid
KBr - potassium bromide

AgNO₃ - silver nitrate
AgCl - silver chloride
NMR - nuclear magnetic resonance
IR - infrared
UV-Vis - ultraviolet-visible

INTRODUCTION

Palladium(II) complexes are interesting due to their similarity with the corresponding platinum(II) complexes, so they can be used as model molecules for investigation of the antitumor action mechanism of cisplatin and other platinum chemotherapeutics. Reactivity of palladium(II) complexes is 10^4 - 10^5 times higher in relation to analogous with platinum(II) (1-4).

Although the first results did not show significant antitumor activity of the palladium(II) complexes, they were nevertheless studied more widely. It is considered that the lower activity of these complexes is a consequence of very fast ligands exchange and the inability of the complex to reach the biological target without changes in structure, which increases the risk of adverse effects on biochemical processes in the cell. Since the main reason for the poor biological activity of these palladium complexes lies in its thermodynamic and kinetic lability, it was necessary to make a good selection of ligands, and thus the leaving groups, in the synthesis. In order to overcome such problems, a large number of palladium(II) complexes containing chelating ligands, have been synthesized in order to reduce the reactivity of palladium(II) ion, and increase the stability of the obtained compounds (5). The reactivity and lipophilicity, as well as the antitumor activity of the complex itself, depend on the choice of appropriate ligands. With increasing of compound lipophilicity, its cytotoxicity also increases (6,7). The leaving group must not be extremely labile because it is necessary for the complex to sustain its structural integrity *in vivo* long enough. For example, 1,10-phenanthroline mononuclear and dinuclear palladium(II) complexes show very good antitumor activity (8). It is assumed that one of the main reasons for good cytotoxicity, in addition to lipophilicity, is the presence of the N-H group, more precisely the hydrogen atom that is suitable for the formation of the hydrogen bonds. This allows more efficient coordination of nucleic acids, containing the electron donor nitrogen atoms, for the palladium(II) ion. So far, only in medicine, the radioactive isotope ^{103}Pd has been used for treatment of fast-growing prostate cancer (9,10).

Numerous palladium complexes with ligands such as putrescine, spermine, edta-type ligands and coumarin derivatives have been synthesized and showed cytotoxic activity approximately equal or even better than cisplatin (11,12). Studies based on the biological activity of mononuclear palladium(II) complexes with ethylenediamine ligand have shown enhanced cytotoxic activity against human leukemia cell lines, relative to cisplatin and other platinum(II) complexes (13). Ethylenediamine dinuclear palladium(II) complexes with pyridine derivatives and phenanthroline as ligands have also shown cytotoxic activity, as well as the ability to reduce the viability of cervical tumor cells (14). The synthesis of palladium(II) complexes with voluminous *N*-donor ligands leads to the formation of *trans*-complexes, due to the steric effect. *Trans*-isomers of palladium(II) complexes have shown better cytotoxicity compared to corresponding *cis*-isomers (15).

Deoxyribonucleic acid (DNA), important biopolymer in the human body, forms different conformations under physiological conditions, because of interactions that occur within the molecule itself (16-18). DNA has a role to store and transmit encoded genetic data, which includes transcription, replication, and translation into proteins. Investigating the mechanism of action of cisplatin as a highly represented cytostatic in the treatment of different cancers, it was found that reversible and irreversible binding to the DNA molecule occur. Reversible bonding refers to the formation of covalent bonds between platinum(II) ions and nitrogen atoms from purine and pyrimidine bases, namely N7-nitrogen atoms from guanine, N7 and N1-atoms from adenine and N3-atom from cytosine and thymine (19, 20). NMR spectroscopy and X-ray analysis have shown that interlinked bonds between DNA molecules and metal ions can be formed in different ways. Analogous to platinum(II), studies of palladium(II) complexes interactions with DNA have been performed (21,22).

Serum albumins are a class of blood proteins whose primary function is the transport of molecules and regulation of osmotic pressure in the blood plasma. They play an important role in the transport of metal ions and their complexes to cells and tissues. The concentration of serum albumin in the blood is about $7.0 \cdot 10^{-4}$ M (23,24). Human serum albumin (HSA) and bovine serum albumin (BSA) belong to the group of the most studied proteins (23,25). Bovine serum albumin possesses amino acid sequence that has 76% similarity to the human serum albumin (23,26). Investigations of the interactions of serum albumin proteins and transition metal ion complexes used as drugs, is of great importance, because it can lead to a decrease or increase in the biological activity of the drug, as well as to the occurrence of new modes of complex transport. Rapid degradation of many drugs in the body, reduces their therapeutic power. HSA and BSA, as drug transporters, can be used to increase the half-lives of peptides and small molecules that are rapidly subject to degradation. In this way, serum albumins regulate the bioavailability of drugs and bioactive molecules. Due to the extraordinary binding capacity of different ligands, they are also used as model systems for studying interactions with bioactive molecules (27,28).

In this paper, we report synthesis and spectroscopic characterization of two dinuclear palladium(II) complexes with 1,5-naphthyridine bridging ligand, $[\{\text{Pd}(\text{en})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})](\text{NO}_3)_2$ (**Pd1**) and $[\{\text{Pd}(1,3\text{-pd})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})](\text{NO}_3)_2$ (**Pd2**). UV-Vis spectrometry and fluorescent measurements are applied for investigation of the reactions of palladium(II) complexes (**Pd1** and **Pd2**) with DNA and bovine serum albumin (BSA), which results can provide mechanism of their antitumor activity and their possibility to interact with important biomolecules as well as mode of these interactions.

EXPERIMENTAL

Materials

Chemicals and reagents ethylenediamine (en), 1,3-propylenediamine (1,3-pd), 1,5-naphthyridine (1,5-nphe), deoxyribonucleic acid isolated from calf thymus (CT-DNA), bovine serum albumin (BSA), ethidium bromide (EtBr), 0.01 M phosphate buffer and $K_2[PdCl_4]$ were purchased from Sigma-Aldrich Chemical Co. Bidistilled water was used to prepare a solution of these reagents. Other chemicals used in this work were commercial products of analytical purity and were purchased from a domestic manufacturer.

Instrumental methodes

All pH measurements were performed at 25 °C. For this purpose, a pH meter Mettler Toledo Seven Copmact S220-U was used, which was calibrated in relation to Mettler Toledo buffer solutions for pH = 4.0 and pH = 7.0. The measured pH values were not corrected for the deuterium effect.

Elemental microanalysis for C, H, and N parameters were performed in the Microanalytical Department of the Institute of Chemistry, Faculty of Chemistry, University of Belgrade.

D₂O as the solvent and TSP (trimethyl-silylpropane-3-sulfonate) as the reference standard were used to record the ¹H and ¹³C NMR spectrum. The spectra were recorded on a Varian Gemini 200 MHz spectrometer. All reactions were performed in 5 mm diameter NMR cuvettes.

Electronic absorption spectra were recorded on a Shimadzu UV-Vis spectrophotometer. Concentrations of dinuclear palladium(II) complexes were $5 \cdot 10^{-5}$ mol/dm³. The electron absorption spectra were recorded in the wavelength range 200–500 nm at 25 °C and the experimental results were processed using the computer program Microsoft Office Excel 2003.

Infra-red (IR) spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer using the KBr technique, in the wavelength range of 450 - 4000 cm⁻¹.

Fluorescence measurements were performed on an RF-1501 PC spectrophotometer (Shimadzu, Japan).

Synthesis of [Pd(en)Cl₂] and [Pd(1,3-pd)Cl₂] complexes

Mononuclear palladium(II) complexes [Pd(en)Cl₂] and [Pd(1,3-pd)Cl₂] (en and 1,3-pd, are bidentate coordinated diamine ligands ethylenediamine and 1,3-propylenediamine, respectively) were synthesized according to a modified procedure previously described in the literature (29-31).

0.1632 g ($5.00 \cdot 10^{-4}$ mol) of $K_2[PdCl_4]$ was dissolved in 25 cm³ of water and the resulting brown solution was transferred to a 100 cm³ double-necked flask supplied with reflux condenser and dropper. The pH value of the solution was adjusted to about 2-3 by adding a solution of HCl with a

concentration of 0.1 M. Obtained solution was heated on a water bath, with slow instilment of an equimolar amount ($5.00 \cdot 10^{-4}$ mol) of ethylenediamine or 1,3-propylenediamine. Instilment is performed slowly for one hour with constant heating, stirring and occasional control of pH values (pH 2-3). During the reaction, the color of the solution changes from brown to light yellow. After refluxing for 5 h, the reaction mixture was left at room temperature overnight. The complexes crystallized from aqueous solution at room temperature. The obtained crystals were filtered off, washed with ethanol and air dried. The yield was about 90%.

Synthesis of dinuclear {[Pd(en)Cl]₂(μ-1,5-nphe)}(NO₃)₂ and {[Pd(1,3-pd)Cl]₂(μ-1,5-nphe)}(NO₃)₂ complexes

Dinuclear palladium(II) complexes, {[Pd(en)Cl]₂(μ-1,5-nphe)}(NO₃)₂ (**Pd1**) and {[Pd(1,3-pd)Cl]₂(μ-1,5-nphe)}(NO₃)₂ (**Pd2**), were obtained from the corresponding mononuclear complexes according to a modified procedure previously described in the literature (32-34).

In 5 cm³ of dimethylformamide 0.0572 g ($3.37 \cdot 10^{-4}$ mol) of AgNO₃ was dissolved and a suspension of the mononuclear [Pd(en)Cl₂] or [Pd(1,3-pd)Cl₂] complex ($3.43 \cdot 10^{-4}$ mol) in 10 cm³ of dimethylformamide was added. The reaction mixture was leaved overnight with stirring at room temperature and in the dark. Precipitated AgCl was removed by filtration and in the pale yellow solution of palladium(II) complex, [Pd(en)(dmf)Cl]⁺ or [Pd(1,3-pd)(dmf)Cl]⁺, solution of 1,5-naphthyridine (1,5-nphe) in dimethylformamide (molar ratio 2 : 1) was added dropwise. The reaction mixture was stirred at room temperature for about 4 h in the dark. The volume of the solution was reduced by evaporation of dimethylformamide on a rotary vacuum evaporator.

After the addition of about 20 cm³ of dichloromethane, a light yellow precipitate of the complex {[Pd(en)Cl]₂(μ-1,5-nphe)}(NO₃)₂ and {[Pd(1,3-pd)Cl]₂(μ-1,5-nphe)}(NO₃)₂ was obtained. The resulting precipitate of dinuclear palladium(II) complexes was separated by filtration, washed with methanol, then ether and air dried. The yield of dinuclear palladium(II) complexes was 65% for **Pd1** and 50% for **Pd2**.

Anal. Calcd. for **Pd1** (C₁₂H₂₂N₈Cl₂O₆Pd₂: FW = 658,11): C, 21.90%; H, 3.37%; N, 17.03%. Found: C, 22.11%; H, 3.26%; N, 17.29%. ¹H NMR (200 MHz, D₂O, δ, ppm): 8.14 (m, 2H, H3, H7), 9.58 (m, 2H, H4, H8), 10.21 (d, 2H, H2, H6). ¹³C NMR (50 MHz, D₂O, δ, ppm): 130 (C3, C7), 143 (C4a, C8a), 147 (C4, C8), 160 (C2, C6). IR (KBr, ν, cm⁻¹): ~3226, 3100 (N-H stretch); 1582, 1509 (C=C/C=N 1,5-naphthyridine group stretch); 1373, 1356, 1336 (ν_{as}(NO₃)). UV-vis (H₂O, λ_{max}, nm): 303 (ε = $7.8 \cdot 10^3$ M⁻¹cm⁻¹), 314 (ε = $8.0 \cdot 10^3$ M⁻¹cm⁻¹).

Anal. Calcd. for **Pd2** (C₁₄H₂₆N₈Cl₂O₆Pd₂: FW = 686,15): C, 24.51%; H, 3.82%; N, 16.33%. Found: C, 24.28%; H, 3.55%; N, 16.53%. ¹H NMR (200 MHz, D₂O, δ, ppm): 8.23 (m, 2H, H3, H7), 9.55 (d, 2H, H4, H8), 10.17 (d, 2H, H2,

H6). ^{13}C NMR (50 MHz, D_2O , δ , ppm): 131 (C3, C7), 143 (C4a, C8a), 146 (C4, C8), 160 (C2, C6). IR (KBr, ν , cm^{-1}): ~3241, 3135 (N-H stretch); 1659, 1600, 1589, 1510 ($\text{C}=\text{C}/\text{C}=\text{N}$ 1,5-naphthyridine group stretch); 1367, 1295 ($\nu_{\text{as}}(\text{NO}_3)$). UV-vis (H_2O , λ_{max} , nm): 303 ($\epsilon = 7.5 \cdot 10^3 \text{ M}^{-1}\text{cm}^{-1}$), 313 ($\epsilon = 7.2 \cdot 10^3 \text{ M}^{-1}\text{cm}^{-1}$).

Interactions of complexes with CT-DNA

UV-Vis spectrophotometric measurements

Interactions of $[\{\text{Pd}(\text{en})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})](\text{NO}_3)_2$ and $[\{\text{Pd}(1,3\text{-pd})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})](\text{NO}_3)_2$ complexes with deoxyribonucleic acid isolated from calf thymus (CT-DNA) were investigated using UV-Vis spectrophotometry. Based on these measurements, the internal binding constant (K_b) of CT-DNA molecules for dinuclear palladium(II) complexes was determined. A 0.01 M phosphate buffer solution (pH = 7.4) was used to prepare the solutions for UV-Vis measurements. All reactions were performed at 37 °C. The concentration of CT-DNA was determined from the ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}). An absorption ratio of 1.8 to 1.9 indicates that CT-DNA has been released from the protein portion, and such a solution was stored at 4 °C for no longer than 7 days. The concentration of CT-DNA was determined based on UV absorption at 260 nm and extinction coefficient $\epsilon = 6600 \text{ M}^{-1}\text{cm}^{-1}$ (35,36).

To determine the internal binding constant (K_b) of CT-DNA molecules and dinuclear palladium(II) complexes, UV-Vis spectra of the solution obtained by mixing dinuclear palladium(II) complexes with CT-DNA were recorded. In all solutions, the concentration of palladium(II) complex was constant ($4.51 \cdot 10^{-5} \text{ M}$), while the concentration of CT-DNA was in the interval $(0 - 9.02) \cdot 10^{-5} \text{ M}$ ($[\text{complex}] / [\text{CT-DNA}] = 0.0 - 2.0$). The internal binding constants (K_b) were determined according to the equation: $[\text{DNK}]/(\epsilon_a - \epsilon_f) = [\text{DNK}]/(\epsilon_b - \epsilon_f) + 1/K_b \cdot (\epsilon_b - \epsilon_f)$ (37), where [DNA] is the concentration of CT-DNA, ϵ_a and ϵ_b are the extinction coefficients of the free complex and the complex when the CT-DNA molecule is bound to it, respectively. The extinction coefficient ϵ_f was defined from the calibration curve obtained by determining the absorption of the free complex at different concentrations. The extinction coefficient ϵ_a was calculated based on Lambert-Beer's law, as the ratio of the absorption of the tested solution (A_{obs}) and the concentration of the complex in that solution ($A_{\text{obs-DNA}}/[\text{complex}]$). The obtained results are presented graphically as the dependence of $[\text{DNK}]/(\epsilon_a - \epsilon_f)$ versus [DNK]. The slope of the obtained line has the value $1/(\epsilon_b - \epsilon_f)$, while the segment on the y axis is $1/K_b \cdot (\epsilon_b - \epsilon_f)$. The internal binding constant (K_b) was determined from the ratio of the slope of the line and the segment on the y axis.

Fluorescent measurements

Interactions of dinuclear palladium(II) complexes, $[\{\text{Pd}(\text{en})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})](\text{NO}_3)_2$ and $[\{\text{Pd}(1,3\text{-pd})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})](\text{NO}_3)_2$, with CT-DNA in the presence of ethidium bromide (3,8-diamino-5-ethyl-6-phenylphenanthridium bromide, EtBr) were examined using emission fluorescence spectroscopy. A solution of palladium(II) complex was added in to the mixture of EtBr and CT-DNA ($1.28 \cdot 10^{-5} \text{ M}$) in a 1: 1 molar ratio, so that obtained solution have concentration ratio of Pd(II) complex and CT-DNA in range from 0.0 to 0.9. All reactions were carried out in 0.01 M phosphate buffer (pH = 7.4). In order to examine the competitive reactions of the interaction of dinuclear palladium(II) complexes and EtBr with CT-DNA, fluorescent spectra of EtBr/CT-DNA were recorded in the absence and presence of dinuclear Pd(II) complexes. The emission spectra were recorded in the wavelength range 550 - 750 nm, with extinction at 527 nm and fluorescent emission at 612 nm. The Stern-Volmer constant (K_{sv}) was determined based on the equation: $I_0/I = 1 + K_{\text{sv}}[Q]$ (38), where I_0 and I are the fluorescence intensities before and after the addition of the palladium(II) complex to the EtBr/CT-DNA solution, while $[Q]$ is the concentration of the Pd(II) complex. The obtained results are presented graphically as the dependence of I_0/I from $[Q]$. The Stern-Volmer constant (K_{sv}) was obtained from the slope of the acquired line. The stability constant (K_a) as well as the number of binding sites (n) was obtained based on the equation: $\log(I_0 - I)/I = \log K_a + n \cdot \log[Q]$ (39-41). The obtained results are presented graphically as the dependence of $\log(I_0 - I)/I$ from the $\log[Q]$. The value of K_a was obtained from the intersection of the line with the y axis, and the number of connecting points (n) from the slope of the line.

Interactions of Pd(II) complexes with albumin

Emission fluorescence spectroscopy were used to examine interactions of dinuclear palladium(II) complexes $[\{\text{Pd}(\text{en})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})](\text{NO}_3)_2$ and $[\{\text{Pd}(1,3\text{-pd})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})](\text{NO}_3)_2$ with bovine serum albumin (BSA). Emission spectra were recorded in the wavelength range 300–500 nm, with extinction at 295 nm (32). The binding effect of the tested albumin complexes was observed based on the reduction of the albumin emission intensity ($1.6 \cdot 10^{-6} \text{ M}$ in 0.01 M PBS) to 352 nm after the addition of palladium(II) complexes ($0\text{-}4.0 \cdot 10^{-5} \text{ M}$). All emission spectra were recorded under the same experimental conditions.

The Stern-Volmer constant (K_{sv}) was determined based on the equation: $I_0/I = 1 + K_{\text{sv}}[Q] = 1 + k_q\tau_0[Q]$, where I_0 is the initial fluorescence intensity of tryptophan in BSA, and I is the fluorescence intensity of tryptophan in BSA after addition of palladium(II) complex in protein solution, k_q is the fluorescence quenching constant, τ_0 is the average time for fluorescence of albumin in the absence of the complex and $[Q]$ is the concentration of the complex. The binding constant (K_a) as well as the number of binding sites (n) were obtained on the basis of Scatchard's equation: $\log(I_0 - I)/I = \log K_a + n \cdot \log[Q]$. The results are presented graphically as the

dependence of $\log(I_0-I)/I$ from $\log [Q]$, the value of K_a is acquired from the intersection of the obtained line with the y

axis, and the number of connecting points (n) from the slope of the line.

RESULTS AND DISCUSSION

In this work, two dinuclear palladium(II) complexes, $[\{Pd(en)Cl\}_2(\mu-1,5-nphe)](NO_3)_2$ (**Pd1**) and $[\{Pd(1,3-pd)Cl\}_2(\mu-1,5-nphe)](NO_3)_2$ (**Pd2**), containing 1,5-naphthyridine (1,5-nphe) as bridging ligand, while ethylenediamine (en) and 1,3-propylenediamine (1,3-pd) are bidentate coordinated diamine ligands, were synthesized and characterized by elemental microanalysis, NMR (^1H and ^{13}C), IR spectroscopy

and UV-Vis spectrophotometry. **Figure 1** shows the structural formulas of ligands, and the synthesized dinuclear palladium(II) complexes. Interactions of dinuclear palladium(II) complexes with calf thymus deoxyribonucleic acid (CT-DNA) were investigated using UV-Vis spectrophotometry and fluorescence spectroscopy. In addition, fluorescence spectroscopy was used to study the interaction of **Pd1** and **Pd2** complexes with bovine serum albumin (BSA).

Figure 1. Structural formulas of 1,5-naphthyridine (1,5-nphe), ethylenediamine (en) and 1,3-propylenediamine (1,3-pd), and dinuclear palladium(II) complexes **Pd1** and **Pd2**.

Synthesis and characterization of dinuclear palladium(II) complexes

Dinuclear palladium(II) complexes, $[\{Pd(en)Cl\}_2(\mu-1,5-nphe)](NO_3)_2$ (**Pd1**) and $[\{Pd(1,3-pd)Cl\}_2(\mu-1,5-nphe)](NO_3)_2$ (**Pd2**), were obtained from the corresponding mononuclear complexes according to a modified procedure previously described in the literature (32-34).

As shown in **Figure 1**, the synthesized dinuclear palladium(II) complexes have the same bridging

1,5-naphthyridine ligand and different bidentate coordinated diamine ligands. ^1H and ^{13}C NMR spectra of 1,5-naphthyridine, ethylenediamine, 1,3-propylenediamine as well as **Pd1** and **Pd2** complexes were recorded in D_2O as solvent. NMR data for ligands and dinuclear palladium(II) complexes is given in Table 1, while ^1H and ^{13}C NMR spectra of **Pd1** and **Pd2** complexes in **Figure 2**.

Table 1. ^1H and ^{13}C NMR chemical shifts (δ , ppm) for 1,5-naphthyridine (1,5-nphe), ethylenediamine (en), 1,3-propylenediamine (1,3-pd) and the corresponding dinuclear palladium(II) complexes

ligand/ complex	NMR shifts								
	^1H				^{13}C				
	H2, H6	H4, H8	H3, H7	alifatic protons	C2, C6	C4, C8	C3, C7	C4a, C8a	L
1,5-nphe	8.57 <i>dd</i>	7.89 <i>d</i>	7.48 <i>dd</i>		154	139	128	144	
en				2.64 <i>s</i> (CH_2)					45 (CH_2)
Pd1	10.21 <i>d</i>	9.58 <i>d</i>	8.14 <i>m</i>	2.76 <i>m</i> (CH_2)	160	147	130	143	50 (CH_2)
1,3-pd				1.60 <i>m</i> (H2, CH_2) 2.47 <i>m</i> (H1, H3, CH_2)					29 (C2, CH_2) 39 (C1, C3, H_2)
Pd2	10.17 <i>d</i>	9.55 <i>d</i>	8.23 <i>m</i>	1.85 <i>m</i> (H2, CH_2) 2.76 <i>m</i> (H1, H3, CH_2)	160	146	131	143	30 (C2, CH_2) 43,44 (C1, C3, CH_2)

In the ^1H NMR spectrum of free 1,5-naphthyridine, two multiplets (doublet-doublets, *dd*) occur at 7.48 (H3, H7) and 8.57 (H2, H6) ppm, derive from the equivalent protons of the two condensed pyridine rings. The doublet at 7.89 ppm originates from the H4 and H8 protons of 1,5-nphe.

Figure 2. ^1H and ^{13}C NMR spectra of $[\{\text{Pd}(\text{en})\text{Cl}\}_2(\mu\text{-1,5-nphe})](\text{NO}_3)_2$ (**Pd1**) and $[\{\text{Pd}(1,3\text{-pd})\text{Cl}\}_2(\mu\text{-1,5-nphe})](\text{NO}_3)_2$ (**Pd2**) complexes.

After coordination of diamine ligand to the palladium(II) ion, the signal shifts downfield (**Fig. 2** and Table 1). The shifts of the signals, which correspond to the 1,5-nphe protons after coordination for the palladium(II) ion, are a consequence of the delocalization of the charge transmitted through the pyridine rings (20,32,42). The multiplet at 2.76 ppm in the ^1H NMR spectrum of the **Pd1** complex, correspond to methylene protons of bidentate coordinated ethylenediamine. This signal was shifted by $\Delta\delta = 0.12$ ppm downfield in regard to free ethylenediamine. In the aliphatic part of the ^1H NMR spectrum of the **Pd2** complex, two multiplets appear at 1.85 and 2.60 ppm due to the CH_2 protons at position 2 and the CH_2 protons at position 1 and 3 (**Fig. 1**, Table 1). Signals derived from these protons after coordination were shifted downfield ($\Delta\delta = 0.25$ ppm for methylene protons at position 2 and $\Delta\delta = 0.19$ ppm for same protons at positions 1 and 3) in regards to the uncoordinated 1,3-pd.

In the ^{13}C NMR spectra of the **Pd1** and **Pd2** complexes, in the aromatic region, four signals from the carbon atoms of the 1,5-naphthyridine ligand (C2 and C6, C4 and C8, C4a and C8a, C3 and C7) appears. The position of these signals differs significantly from those of the uncoordinated 1,5-nphe (Table 1). After coordination of 1,5-nphe for Pd(II) the signals for the carbon atoms in the heterocyclic aromatic ring move downfield. The signal at 50 ppm originating from from the carbon atoms of bidentate-coordinated ethylenediamine in **Pd1**, are shifted by $\Delta\delta = 5$ ppm in regarde to the uncoordinated en ligand. Also, in the spectrum of the **Pd2** complex, the signals corresponding to the C atoms of the coordinated 1,3-pd ligand are shifted by $\Delta\delta = 1\text{--}5$ ppm downfield relative to the signals of the uncoordinated ligand.

UV-Vis spectrophotometry

The UV-Vis spectra of the Pd1, Pd2 complexes and free 1,5-nphe ligand are given in Figure 3. The first absorption maximum is located in the wavelength range of 296 to 310 nm, while the second maximum is in the range of 310 to 320 nm. All absorption maxima of the synthesized complexes have a bathochromic shift after the coordination of 1,5-naphthyridine, due to $\pi \rightarrow \pi^*$ electron transitions in the heterocyclic 1,5-nphe ligand.

Figure 3. UV-Vis spectra of dinuclear $[\{\text{Pd}(\text{en})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})]^{2+}$ and $[\{\text{Pd}(1,3\text{-pd})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})]^{2+}$ complexes and free 1,5-nphe ligand.

IR spectroscopy

IR spectra of Pd1 and Pd2 complexes, recorded in the wavelength range 4000 - 450 cm^{-1} , show bands corresponding to coordinated 1,5-nphe ligand, as well as bands of bidentate coordinated ethylenediamine (en) and 1,3-propylenediamine (1,3-pd) (**Fig. 4**).

In the IR spectra of dinuclear palladium(II) complexes, two very strong bands in the range of 3280 - 3098 cm^{-1} were observed, which correspond to asymmetric and symmetric vibrations of the coordinated amino group of ethylenediamine and 1,3-propylenediamine. The medium-strength band in the region of 1500–1650 cm^{-1} correspond to the C = C and C = N vibrations of the aromatic 1,5-naphthyridine ring. A strong and wide band at $\sim 1364\text{--}1384$ cm^{-1} indicates the existence of NO_3^- ions in the outer coordination sphere of dinuclear palladium(II) complexes.

Figure 4. IR spectra of **Pd1** and **Pd2** complexes, KBr, 4000-450 cm^{-1} .

Interactions of dinuclear palladium(II) complexes with DNA

UV-Vis spectrophotometric measurements

UV-Vis spectrophotometry is a simple and efficient method, which is used to determine the mode of coordination or interaction of metal complexes with deoxyribonucleic acid (DNA). Transition metal complexes can interact with DNA covalently or noncovalently (43). Coordination of DNA molecules, most commonly via N7 atoms from guanine to a metal ion, creates a covalent bond, while noncovalent interactions include intercalation, hydrogen bond formation, and

electrostatic interactions. After the interaction of the metal complex with the DNA, the intensity of the absorbance may decrease (hypochromic effect) or increase (hyperchromic effect). Also, the absorption maximum may have a bathochromic or hypsochromic shift. Based on the obtained results of UV-Vis spectrophotometry, the internal binding constants (stability constants) of DNA to the metal ion, can be calculated. The UV-Vis spectra of $[\{\text{Pd}(\text{en})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})]^{2+}$ and $[\{\text{Pd}(1,3\text{-pd})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})]^{2+}$ complexes in the absence and presence of different concentrations of CT-DNA, $[\text{complex}] / [\text{CT-DNA}] = (0.0 - 2.00) \cdot 10^{-4} \text{ M}$ are shown in Figure 5.

After the addition of CT-DNA to the solution of dinuclear palladium(II) complexes, a hyperchromic effect (increase of the absorption maximum) in UV-Vis spectra can be observed, based on which it can be concluded that the complex interacts with CT-DNA. The hyperchromic effect indicates that our complexes with CT-DNA achieve interactions of electrostatic nature or $\pi \rightarrow \pi^*$ interactions which occurs as a consequence of the interactions of aromatic rings in complexes with bases in CT-DNA (44).

The internal binding constants (K_b) were calculated from the change in absorption at the appropriate wavelength after the addition of CT-DNA according to the equation given in the Experimental part (2.5.1). The obtained results are presented graphically as the dependence of $[\text{DNK}]/(\epsilon_a - \epsilon_f)$ from DNA concentration. The slope of the obtained line has the value $1/(\epsilon_b - \epsilon_f)$, while the segment on the y axis is $1/K_b \cdot (\epsilon_b - \epsilon_f)$. The internal binding constant (K_b) is determined from the ratio of the slope of the line and the segment on the y axis (Fig. 5). The obtained values for the internal binding constants (K_b) are shown in Table 2. Based on K_b , we can conclude that strong interactions occur between investigated Pd(II) complexes and CT-DNA, which are characteristic for intercalators.

Figure 5. Absorption spectra of $[\{\text{Pd}(\text{en})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})]^{2+}$ and $[\{\text{Pd}(1,3\text{-pd})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})]^{2+}$ complexes in 0.01 M phosphate buffer before and after addition of CT-DNA.

K_b values of the tested complexes are about 10 times lower than the same for the typical intercalator ethidium bromide (EtBr) to DNA, ($K_b = 1.23 \cdot 10^5 \text{ M}^{-1}$) (45), indicating that the interactions of the Pd(II) complexes are weaker compared to EtBr. The higher value of K_b for the **Pd1** complex compared to the **Pd2**, indicates that the **Pd1** complex has a higher affinity of interaction with CT-DNA, which can be attributed to the smaller steric effect of the bidentate coordinated five-membered ethylenediamine ring compared to the six-membered 1,3-propylenediamine ring in **Pd2**. The change in Gibbs energy (ΔG , Table 2) of the Pd(II)/CT-DNA adduct was calculated using the following equation: $\Delta G = -RT \ln K_b$. Negative values of Gibbs energy indicate spontaneous interaction of dinuclear palladium(II) complexes, **Pd1** and **Pd2**, with CT-DNA.

Table 2. Internal binding constants (K_b), changes in Gibbs energy (ΔG) and hypochromism (H) of the investigated dinuclear palladium(II) complexes, **Pd1** and **Pd2**, with CT-DNA

complex	$K_b \cdot 10^4$ (M^{-1})	ΔG (kJ/mol)	H
$[\{\text{Pd}(\text{en})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})]^{2+}$	(7.50 ± 0.05)	-28.93	22.42
$[\{\text{Pd}(1,3\text{-pd})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})]^{2+}$	(4.00 ± 0.03)	-27.31	25.77

Fluorescent measurements

Fluorescence emission spectroscopy was used for studying the interactions of dinuclear Pd1 and Pd2 complexes with the CT-DNA molecule and ethidium bromide (EtBr) as a typical intercalator. Changes in EtBr/CT-DNA emission spectra after addition of the complex solution, decrease or increase in fluorescent emission, indicates that the complex replaces

EtBr, and a new adduct of CT-DNA/Pd(II) complex is formed (46).

The emission spectra of EtBr/CT-DNA in the presence of Pd1 and Pd2 complexes are shown in Figure 6. Addition of palladium(II) complex (with increase of complex concentration) leads to a decrease of the emission intensity at 612 nm, indicating competition reaction between EtBr and the tested Pd(II) complexes in relation to CT-DNA. Extrusion of EtBr from the EtBr/CT-DNA with Pd(II) complex proves that intercalation of the Pd(II) complex occurred.

The intensity of the interaction of **Pd1** and **Pd2** complexes with CT-DNA was determined based on the Stern-Volmer constant (K_{sv}) using the Stern-Volmer equation: $I_0/I = 1 + K_{sv}[Q]$, where I_0 and I are the fluorescence intensities before and after the addition of palladium(II) complex in EtBr/CT-DNA solution, while $[Q]$ is the concentration of Pd(II) complex. The obtained results are graphically presented as the dependence of I_0/I from $[Q]$. The Stern-Volmer constant (K_{sv}) was determined from the slope of the obtained line.

Figure 6. EtBr/CT-DNA emission spectra in the presence of $[\{Pd(en)Cl\}_2(\mu-1,5-nphe)]^{2+}$ and $[\{Pd(1,3-pd)Cl\}_2(\mu-1,5-nphe)]^{2+}$ complex. $[EtBr] = 1.28 \cdot 10^{-5}$ M, $[DNA] = 1.28 \cdot 10^{-5}$ M, $[Pd(II) \text{ complex}] = (0 - 1.28) \cdot 10^{-5}$ M.

Inserted graph: Dependence of I_0/I from concentration $[Q]$.

From K_{sv} values (Table 3), it can be concluded that the investigated dinuclear palladium(II) complexes show high affinity for CT-DNA and can displace EtBr from the EtBr/CT-DNA adduct. The stability constant (K_a) was determined from the experimental results, as well as the number of binding sites (Table 3), showing that the K_a follows the K_{sv} values. The obtained values for the Stern-Volmer constant (K_{sv}), the stability constant (K_a) and the number of binding sites (n) confirm that the dinuclear Pd(II) complexes, **Pd1** and **Pd2**, react with CT-DNA by intercalating between two nucleotide strands of DNA and displacing EtBr. These results are in agreement with the K_b values obtained by UV-Vis spectrophotometry. The higher number of binding sites for the **Pd1** complex, once again indicates the stronger interaction of this complex with CT-DNA compared to the **Pd2** complex.

Table 3. Stern-Volmer constant (K_{sv}), stability constant (K_a) and number of binding sites (n) of the investigated dinuclear palladium(II) complexes with CT-DNA.

complex	$K_{sv} \cdot 10^5$ (M^{-1})	$K_a \cdot 10^5$ (M^{-1})	n
$[\{Pd(en)Cl\}_2(\mu-1,5-nphe)]^{2+}$	(4.50 ± 0.04)	(8.80 ± 0.02)	1.7
$[\{Pd(1,3-pd)Cl\}_2(\mu-1,5-nphe)]^{2+}$	(2.80 ± 0.06)	(2.50 ± 0.05)	1.1

Interactions of dinuclear Pd(II) complexes, Pd1 and Pd2, with BSA

Serum albumin, as the most common blood plasma protein, is one of the most studied proteins (23,25). It plays an important role in the transport of metal ions and their complexes through the blood system to cells and tissues. Bovine serum albumin (BSA) is the most studied serum albumin due to its structural similarity to human serum albumin (HSA). Namely, human serum albumin contains one tryptophan at the Trp-214 position, while BSA contains two tryptophan residues, Trp-134 and Trp-214. The bovine serum albumin (BSA) solution shows intense fluorescent emission at $\lambda_{em, max} = 352$ nm, and after excitation at 295 nm (47). After the addition of the **Pd1** or **Pd2** complex to the BSA solution, the fluorescence intensity at $\lambda = 352$ nm decreases (Fig. 7) based on it can be concluded that the tested complexes interact with this biomolecule. The decrease in fluorescence intensity can be attributed to changes in protein structure, which occur due to changes in the tryptophan environment in BSA, because of protein binding to complexes (48).

Figure 7. BSA emission spectra in the presence of $[\{\text{Pd}(\text{en})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})]^{2+}$ and $[\{\text{Pd}(1,3\text{-pd})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})]^{2+}$ complexes. $[\text{BSA}] = 1.60 \cdot 10^{-6} \text{ M}$, $[\text{Pd(II) complex}] = (0 - 4.00) \cdot 10^{-5} \text{ M}$; $\lambda_{\text{ex}} = 295 \text{ nm}$.

Inserted graph: Dependence of $\log(I_0-I)/I$ from concentration $[Q]$.

The values of the dynamic Stern-Volmer constant (K_{sv}) and the fluorescence quenching constant (k_q) for complex interactions with BSA were determined using the Stern-Volmer equation. The values of the constants K_{sv} and k_q for the interaction of the studied **Pd1** and **Pd2** complexes with BSA are given in Table 4. In addition, Table 4 shows the values of binding constants (K_a) as well as the number of albumin binding sites (n) for Pd(II) complexes, which were obtained on the basis of Scatchard's equation (Experimental part 2.6). The obtained results are graphically presented as the dependence of $\log(I_0-I)/I$ from $\log[Q]$, the value of K_a is obtained from the intersection of the line with the y axis, and the number of connecting points (n) from the slope of the line.

Based on the linearity in Stern-Volmer graphs, it can be concluded that both complexes lead to the quenching of tryptophan emission in the BSA molecule. K_{sv} values for **Pd1** and **Pd2** complex (Table 4) indicate a very similar binding affinity of BSA for the tested complexes. The values of n for both complexes are 1.8, which indicates that there are approximately two possible sites in the protein suitable for complex binding. The quenching constants (k_q) are greater than 10^{10}

and indicate a static emission quenching mechanism (49). K_a value leads to conclusion that the **Pd1** and **Pd2** complexes can bind to BSA and thus be transported to the cell and hydrolyze the bond with BSA at the appropriate site in the cell.

Table 4. Stern-Volmer constant (K_{sv}), quenching constant (k_q), binding constant (K_a) and number of binding sites (n) for interactions between BSA and dinuclear **Pd1** and **Pd2** complexes.

complex	$K_{\text{sv}} \cdot 10^5$ (M^{-1})	$k_q \cdot 10^{12}$ ($\text{M}^{-1}\text{s}^{-1}$)	$K_a \cdot 10^9$ (M^{-1})	n
$[\{\text{Pd}(\text{en})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})]^{2+}$	(2.80 ± 0.03)	(28 ± 2)	(1.20 ± 0.05)	1.8
$[\{\text{Pd}(1,3\text{-pd})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})]^{2+}$	(3.90 ± 0.04)	(39 ± 1)	(1.20 ± 0.02)	1.8

CONCLUSION

In this paper, two dinuclear complexes, $[\{\text{Pd}(\text{en})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})](\text{NO}_3)_2$ (**Pd1**) and $[\{\text{Pd}(1,3\text{-pd})\text{Cl}\}_2(\mu\text{-}1,5\text{-nphe})](\text{NO}_3)_2$ (**Pd2**), (ethylenediamine (en) and 1,3-propylenediamine (1,3-pd) are bidentate coordinated diamine ligands and 1,5-naphthyridine (5-nphe) bridging ligand) were synthesized. The structure of these complexes was confirmed based on the results of elemental microanalysis, UV-Vis, IR, NMR (^1H and ^{13}C) spectroscopy.

Interactions of synthesized dinuclear palladium(II) complexes with deoxyribonucleic acid were investigated using UV-Vis spectrophotometry and fluorescence spectroscopy. UV-Vis spectrophotometric data showed that after the addition of CT-DNA to the solution of dinuclear palladium(II) complexes, a hyperchromic effect occurs, based on which we can conclude that the complex interacts with CT-DNA without changing its structure. Higher value of K_b for complex **Pd1** indicates that this complex has greater binding affinity in comparison to **Pd2**. The emission spectra demonstrate that the investigated **Pd1** and **Pd2** complexes can displace the ethidium bromide intercalator from the CT-DNA/EtBr adduct. From the high Stern-Volmer constants (K_{sv}) values it can be concluded that these Pd(II) complexes act as intercalators showing strong interactions with DNA, and higher value of the stability constant (K_a) for **Pd1** confirms results from UV-Vis spectrophotometry. Stronger binding of **Pd1** complex to the DNA helix can be attributed to the smaller steric effect of the five-membered ethylenediamine ligand in relation to the six-membered 1,3-propylenediamine ring.

Fluorescence spectroscopy were used for investigation of interactions of **Pd1** and **Pd2** complexes with bovine serum albumin (BSA). The fluorescence intensity of tryptophan decreases proportionally to the increasement in the total concentration of the added complex. From calculated values of the constants (K_{sv} and K_a) it can be concluded that **Pd1** and **Pd2** complexes can bind to BSA and then be transported to the cell. Also, the number of binding sites (n) show that two

binding sites of BSA are accessible for interaction with these metal complexes.

The results obtained in this paper contribute to a better understanding of the interactions of dinuclear palladium(II) complexes containing 1,5-naphthyridine as a bridging ligand, with biologically important molecules, such as DNA and BSA.

ACKNOWLEDGMENT

This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement No. 451-03-68/2020-14/200122) and the Faculty of Medical Sciences, University of Kragujevac (JP02/20, JP11/16, JP10/16).

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