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Synthesis and characterization of ruthenium(II)–oligopyridine–peptide conjugates. Interactions of the diasteromeres Δ- and Λ-[Ru(bpy)$_2$(4-COY-4′-Mebpy)]Cl$_2$ (Y = Gly-Lys^1-Lys^{2}CONH$_2$, Lys^1-Gly-Lys^{2}CONH$_2$, Lys^1-Lys^2-GlyCONH$_2$) with the oligonucleotide d(5′-CGCGAATTCGCG-3′)$_2$

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ABSTRACT

Diastereomeric complexes of the general formulae Λ- and Δ-[Ru(bpy)$_2$(4-COY-4′-Mebpy)]Cl$_2$ where bpy = 2,2′-bipyridine and Y = Gly-Lys^1-Lys^{2}CONH$_2$, Lys^1-Gly-Lys^{2}CONH$_2$, Lys^1-Lys^2-GlyCONH$_2$, were synthesized and characterized. The ability of these compounds to bind to the oligonucleotide duplex d(5′-CGCGAATTCGCG-3′) was studied with NMR techniques. Complex Λ-2, Λ-[Ru(bpy)$_2$(4-COLys^1-Gly-Lys^{2}CONH$_2$),4′-Mebpy)]Cl$_2$ (Mebpy = methyl-2,2′-bipyridine), interacts non-specifically causing changes for both complex and oligonucleotide ^{1}H NMR signals. Both Λ-1, Λ-[Ru(bpy)$_2$(4-COGly-Lys^1-Lys^{2}CONH$_2$),4′-Mebpy)]Cl$_2$ and Λ-3, Λ-[Ru(bpy)$_2$(4-COLys^1-Lys^2-GlyCONH$_2$),4′-Mebpy)]Cl$_2$, were bound to the oligonucleotide through both lysine aliphatic chains, indicating that the side chains of the sequential lysines create a kind of "clamp" to connect the complex with the oligonucleotide. Complex Δ-1, Δ-[Ru(bpy)$_2$(4-COGly-Lys^1-Lys^{2}CONH$_2$),4′-Mebpy)]Cl$_2$, interacts with the oligonucleotide duplex with both lysine side chains in a manner similar to Λ-1. Δ-2, Δ-[Ru(bpy)$_2$(4-COLys^1-Gly-Lys^{2}CONH$_2$),4′-Mebpy)]Cl$_2$, interacts with the oligonucleotide with the bipyridine ligands. In addition, the formation of a hydrogen bond between the Gly-NH and the carbonyl groups of the oligonucleotide bases was detected. A completely different binding mode was observed for Δ-3 Δ-[Ru(bpy)$_2$(4-COLys^1-Lys^2-GlyCONH$_2$),4′-Mebpy)]Cl$_2$, which at a ratio of 1:1 ([Ru]/[nucleotide]) opens the oligonucleotide strands. In addition, participation of all three peptidic NH of Δ-3 in hydrogen bonds was observed.

1. Introduction

Modifications of oligopyridine Ru(II) complexes with amino acids, peptides or their derivatives is an intensive subject of research interest in studies of bioinorganic chemistry field, as models either to study the photoinduced electron transfer in proteins [1–8], or the active radicals in enzyme–superoxide dismutase (SOD) systems [9,10] and as specific DNA binders or photochemical nucleases [9,11–23]. On the other hand, the cytotoxicity of the majority of metal complexes, including some of the ruthenium compounds as well, has been found to be related to their DNA binding [24]. These complexes could bind to DNA coordinatively, which is a non-specific mode as regards the DNA base sequence, or non-coordinatively, reversibly, through ligand–DNA interactions. For the latter, there is an increasing research interest in controlling the binding selectivity by adjusting the ligands of the complexes. On this basis, ruthenium oligopyridine *tris*-chelates, which are able to act as photosensitizers, causing oxidative damages to DNA [25], were studied extensively for many variations of ligands [26]. However, the DNA sequence specific recognition remains a goal of research interest [27]. Alterations of oligopyridine ligands with conjugated peptides offer a variety of advantages in DNA binding such as (i) improvement of the aqueous solubility of the complex at physiological pH, (ii) tuning the binding mode through the peptide sequence, (iii) increase of the binding strength of the complex through additional interactions between the peptidic moiety of the complex and the DNA. In addition, conjugated peptides offer better cellular uptake of ruthenium-based drugs or a specificity, by tuning the peptide sequence to target tumor endothelial cells, which are on the surface of the blood vessels, over the healthy ones [28].

In general ruthenium complexes with oligopyridine ligands tethered with peptides bind to oligonucleotide duplexes (realistic models for DNA) with a variety of modes depending on three major factors: (i) the chirality of the metal center, allowing Δ-type complexes to interact with the oligonucleotide duplex [15–18] by the oligopyridine ligands, (ii) the nature of the conjugated peptide, which has been found to affect the weaker binding of Λ-type complexes and (iii) the sequence of the oligonucleotide [15–18,20]. For the DNA polianionic

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strands, oligopyridines conjugated with peptides that have a side chain with protonated amino groups, such as lysine, have an additional affinity for the phosphate backbone [29]. In an attempt to investigate the role of lysine in the peptide sequence, tripeptides were chosen, consisting of two lysines and one glycine in all three possible combinations, as conjugates to complex $[Ru(bpy)_2(4\text{-}CO_2H\text{-}4'\text{-}Mebpy)]^{2+}$ (4-CO_2H-4′-Mebpy = 4′-methyl-2,2′-bipyridine-4-carboxylic acid, bpy = 2,2′-bipyridine). Thus, herein we report the synthesis and the spectroscopic characterization of the diastereomeric complexes Λ- and Δ-$[Ru(bpy)_2(4\text{-}COY\text{-}4'\text{-}Mebpy)]^{2+}$ where Y = Gly-Lys^1-Lys^2CONH_2, Lys^1-Gly-Lys^2CONH_2, Lys^1-Lys^2-$GlyCONH_2$, as well as their interactions with the oligonucleotide duplex d(5′-CGCGAATT CGCG-3′)$_2$ by means of NMR spectroscopy.

2. Experimental

2.1. Materials

2,2′-Bipyridine (bpy) and 4,4′-dimethyl-2,2′-bipyridine were purchased from Aldrich Chemical Company and used without further purification. Hydrated ruthenium trichloride, $RuCl_3 \cdot 3H_2O$, was purchased from Pressure Chemical Company (Pittsburgh, USA). The complexes *cis*-$[Ru(bpy)_2Cl_2]$ [30], Λ- and Δ-$[Ru(bpy)_2(py)_2]$A (where A = *O,O′*-dibenzoyltartaric acid) [31] and the ligand 4-methyl-2,2′-bipyridine-4′-carboxylic acid were synthesized according to literature methods [32]. All solvents were of analytical grade and were used without further purification. The deoxynucleotide d(5′-CGCGATC GCG-3′)$_2$ was purchased from Eurogentec and purified by standard purification option. Oligonucleotide concentrations were quantified by measuring the absorbance at 260 nm as previously reported. The resin (TentaGel S RAM) for the amino acid immobilization and the protected amino acids Fmoc-Lys-(Boc)-OH and Fmoc-Gly-OH (Fmoc = 9-fluorenylmethyloxycarbonyl group and Boc = tert-butyloxycarbonyl group), were purchased from Rapp Polymere Ltd and CBL Patras Ltd respectively.

2.2. Synthesis

2.2.1. Synthesis of ligands 4-COY-4′-Mebpy. (Y = Gly-Lys^1-Lys^2CONH_2, Lys^1-Gly-Lys^2CONH_2, Lys^1-Lys^2-$GlyCONH_2$)

The immobilization of the Fmoc-protected peptide on the resin was performed with the standard Fmoc protocol [33]. The conjugation of the ligand 4-CO_2H-4′-Mebpy (0.5 mmol) to the resin-bound peptide was achieved with the coupling agents benzotriazol-1-yl-oxytris(pyrrolidino)phosphanium hexafluorophosphate (PyBOP, 0.75 mmol) and diisopropylethylamine (DIPEA, 1 mmol). In a typical experiment 500 mg of resin bound peptide (substitution 0.22 mmol/g) was treated with 20% piperidine solution in NMP (5 mL), to deprotect the Fmoc group of the peptide. In the resulting resin bound deprotected peptide, a solution of 4-CO_2H-4′-Mebpy (0.5 mmol) in NMP (2 mL) containing DIPEA (1 mmol) and PyBOP (0.5 mmol) was added and remained to react for 3 h at room temperature. Then, the resin was filtered, washed with NMP (5 × 5 mL) and CH_2Cl_2 (2 × 5 mL). A small amount of the resin, which was used to check the purity of the prepared ligand (4-COY-4′-Mebpy), was treated with TFA/H_2O (95/5, v/v) and the crude product was precipitated with an excess of diethyl ether. *4-CO-Gly-Lys^1-Lys^2CONH_2-4′-Mebpy*. ESI-MS (electrospray ionization mass spectrometry): m/z = 527.2 ($M + H^+$), 263.5 ($M + 2H^+$). *4-CO-Lys^1-Gly-Lys^2CONH_2-4′-Mebpy*. ESI-MS: m/z = 264 ($M + 2H^+$). *4-CO-Lys^1-Lys^2-$GlyCONH_2$-4′-Mebpy* ESI-MS: m/z = 264 ($M + 2H^+$).

2.2.2. Synthesis of the complexes Λ- and Δ-$[Ru(bpy)_2(4\text{-}COY\text{-}4'\text{-}Mebpy)]Cl_4$, (Y = Gly-$Lys^1$-$Lys^2CONH_2$, Lys^1-Gly-Lys^2CONH_2, Lys^1-Lys^2-$GlyCONH_2$)

The diasteromeric complexes Λ- and Δ-$[Ru(bpy)_2(4\text{-}COY\text{-}4'\text{-}Mebpy)]Cl_4$, (Y = Gly-$Lys^1$-$Lys^2CONH_2$, Lys^1-Gly-Lys^2CONH_2, Lys^1-Lys^2-$GlyCONH_2$) were prepared in a similar way. An amount (0.09 mmol) of the resin bound ligand, 4-COY-4′-Mebpy, was refluxed under argon with Λ- or Δ-$[Ru(bpy)_2(py)_2]$A (0.1 mmol) (where A = *O,O′*-dibenzoyltartaric acid) and triethylamine (0.25 mL), in DMF/EtOH (3/1) until the solution was almost decolorized (about 24 h). The resin with the immobilized complex was carefully washed with DMF (N,N-dimethylformamide) (5 × 5 mL) and dried with CH_2Cl_2 (3 × 5 mL). The cleavage of the complex and the protecting groups from the resin was achieved using 5 mL of TFA/H_2O (95/5, v/v). The crude product was precipitated by the addition of 50 mL diethyl ether and cooling at 7 °C overnight. The red-brown precipitate was filtered, dissolved in 1 mL of methanol and added dropwise to 1 mL of an aqueous solution of HPF_6 (~0.1 M) and the complex was precipitated as $[PF_6]^-$ salt. The water-soluble chlorides of the complexes were achieved by conversion with LiCl in acetonic solutions [34]. Finally, all the prepared complexes were purified by reverse-phase HPLC on a Dionex chromatograph, with a Waters C18 column.

Λ-1: Λ-$[Ru(bpy)_2(4\text{-}CO\text{-}Gly\text{-}Lys^1\text{-}Lys^2CONH_2\text{-}4'\text{-}Mebpy)]Cl_4$ Yield: ~40%. *Anal.* Calcd: (%) for $C_{46}H_{56}N_{12}O_4Cl_4Ru$; C, 51.0; H, 5.2; N, 15.5. Found: (%) C, 51.1; H, 5.1; N, 15.5. High Resolution ESI-MS: $[M]^{2+}$ m/z = 470.1694; calcd. $[C_{46}H_{54}N_{12}O_4Ru]^{2+}$ m/z = 470.1712. UV-visible (UV–Vis) (50 mM phosphate buffer, pH = 7.0): λ_{max} (nm), ε (M^{-1} cm^{-1}); 245, (26 × 10^3); 284, (28 × 10^3); 459 (9 × 10^2). Circular dichroism (50 mM phosphate buffer, pH = 7.0): λ_{max} (nm), $\Delta\varepsilon$ (M^{-1} cm^{-1}): 278, (−35.2); 295, (+80.2); 459 (+6.3).

Δ-1: Δ-$[Ru(bpy)_2(4\text{-}CO\text{-}Gly\text{-}Lys^1\text{-}Lys^2CONH_2\text{-}4'\text{-}Mebpy)]Cl_4$ Yield: ~40%. *Anal.* Calcd: (%) for $C_{46}H_{56}N_{12}O_4Cl_4Ru$; C, 51.0; H, 5.2; N, 15.5. Found: (%) C, 51.1; H, 5.3; N, 15.6. High Resolution ESI-MS: $[M]^{2+}$ m/z = 470.1714; calcd. $[C_{46}H_{54}N_{12}O_4Ru]^{2+}$ m/z = 470.1712. UV–Vis (50 mM phosphate buffer, pH = 7.0): λ_{max} (nm), ε (M^{-1} cm^{-1}); 247, (25 × 10^3); 282, (28 × 10^3); 460 (9 × 10^2). Circular dichroism (50 mM phosphate buffer, pH = 7.0): λ_{max} (nm), $\Delta\varepsilon$ (M^{-1} cm^{-1}): 279, (+30.2); 295, (−81.3); 457 (−6.2).

Λ-2: Λ-$[Ru(bpy)_2(4\text{-}CO\text{-}Lys^1\text{-}Gly\text{-}Lys^2CONH_2\text{-}4'\text{-}Mebpy)]Cl_4$ Yield: ~35%. *Anal.* Calcd: (%) for $C_{46}H_{56}N_{12}O_4Cl_4Ru$; C, 51.0; H, 5.2; N, 15.5. Found: (%) C, 51.0; H, 5.1; N, 15.6. High Resolution ESI-MS: $[M]^{2+}$ m/z = 470.1718; calcd. $[C_{46}H_{54}N_{12}O_4Ru]^{2+}$ m/z = 470.1712. UV–Vis (50 mM phosphate buffer, pH = 7.0): λ_{max} (nm), ε (M^{-1} cm^{-1}); 247, (25.5 × 10^3); 279, (29.5 × 10^3); 459 (8.5 × 10^2). Circular dichroism (50 mM phosphate buffer, pH = 7.0): λ_{max} (nm), $\Delta\varepsilon$ (M^{-1} cm^{-1}): 279, (−34.8); 294, (+79.6); 460 (+6.6).

Δ-2: Δ-$[Ru(bpy)_2(4\text{-}CO\text{-}Lys^1\text{-}Gly\text{-}Lys^2CONH_2\text{-}4'\text{-}Mebpy)]Cl_4$ Yield: ~35%. *Anal.* Calcd: (%) for $C_{46}H_{56}N_{12}O_4Cl_4Ru$; C, 51.0; H, 5.2; N, 15.5. Found: (%) C, 51.2; H, 5.1; N, 15.6. High Resolution ESI-MS: $[M]^{2+}$ m/z = 470.1698; calcd. $[C_{46}H_{54}N_{12}O_4Ru]^{2+}$ m/z = 470.1712. UV–Vis (50 mM phosphate buffer, pH = 7.0): λ_{max} (nm), ε (M^{-1} cm^{-1}); 242, (23 × 10^3); 280, (27.5 × 10^3); 460 (8.5 × 10^2). Circular dichroism (50 mM phosphate buffer, pH = 7.0): λ_{max} (nm), $\Delta\varepsilon$ (M^{-1} cm^{-1}): 282, (+32.7); 296, (−83.6); 457 (−5.6).

Λ-3: Λ-$[Ru(bpy)_2(4\text{-}CO\text{-}Lys^1\text{-}Lys^2\text{-}Gly\text{-}CONH_2\text{-}4'\text{-}Mebpy)]Cl_4$ Yield: ~35%. *Anal.* Calcd: (%) for $C_{46}H_{56}N_{12}O_4Cl_4Ru$; C, 51.0; H, 5.2; N, 15.5. Found: (%) C, 51.1; H, 5.1; N, 15.4. High Resolution ESI-MS: $[M]^{2+}$ m/z = 470.1694; calcd. $[C_{46}H_{54}N_{12}O_4Ru]^{2+}$ m/z = 470.1712. UV–Vis (50 mM phosphate buffer, pH = 7.0): λ_{max} (nm), ε (M^{-1} cm^{-1}); 243, (26.5 × 10^3); 284, (29 × 10^3); 458 (7.5 × 10^2). Circular dichroism (50 mM phosphate buffer, pH = 7.0): λ_{max} (nm), $\Delta\varepsilon$ (M^{-1} cm^{-1}): 280, (−36.3); 290, (+77.2); 460 (+6.1).

Δ-3: Δ-$[Ru(bpy)_2(4\text{-}CO\text{-}Lys^1\text{-}Lys^2\text{-}GlyCONH_2\text{-}4'\text{-}Mebpy)]Cl_4$ Yield: ~35%. *Anal.* Calcd: (%) for $C_{46}H_{56}N_{12}O_4Cl_4Ru$; C, 51.0; H, 5.2; N, 15.5. Found: (%) C, 51.2; H, 5.1; N, 15.6. High Resolution ESI-MS: