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Comparative studies of coordination properties of puromycin and puromycin aminonucleoside towards copper(II) ions

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Abstract

Protonation equilibria of puromycin (PM) and puromycin aminonucleoside (PAN) and their coordination by copper(II) ion were studied in solution by potentiometry, electronic absorption spectroscopy (UV–Vis), circular dichroism (CD), electron paramagnetic resonance (EPR) and mass spectrometry. For puromycin four mononuclear complexes were found, with stoichiometries $Cu(PM)^{2+}$, $CuH_{-1}(PM)^{+}$, $CuH_{-2}(PM)$ and $CuH_{-3}(PM)^{-}$. In each of them the Cu(II) ion was bound in the peptidic-like manner, the differences of stoichiometries are a consequence of subsequent deprotonations of the sugar C2'-OH group and the coordinated water molecule. The coordination mode for puromycin aminonucleoside was aminosugar-like. Two dimeric complexes, $Cu_2H_{-1}(PAN)_2^{2+}$ and $Cu_2H_{-2}(PAN)_2^{+}$, and one monomeric $CuH_{-2}(PAN)_2$ were found. The N^6,N^6-dimethyladenine moiety of PAN was not involved in the coordination process due to steric hindrance.

Keywords: Puromycin; Puromycin aminonucleoside; Nucleoside antibiotic; Copper(II) complexes

1. Introduction

Puromycin (6-dimethylamino-9-[3′-deoxy-3′-(*p*-methoxy-L-phenylalanylamino)-β-D-ribofuranosyl]-purine, PM, Fig. 1a), produced by *Streptomyces alboniger*, is a structural analogue of the 3′-end aminoacyl-tRNA [1]. As a result it is an antibiotic and antibacterial agent, inhibiting the peptidyl transfer in both prokaryotic and eukaryotic ribosomes, by being linked non-specifically to growing polypeptide chains [2–4]. The inhibitory effects of PM on amino acid transport, and collagen and DNA biosynthesis were also established [5,6]. At low concentrations PM is able to bind specifically to the C-termini of full-length proteins [7]. This compound is not applied in therapy due to a lack of selectivity towards prokaryotic cells [8–10], rapid occurrence of bacterial resistance [11,12], and inconvenient side effects including lipid peroxidation and lesions in the inner ear and kidney [13].

Puromycin aminonucleoside (6-dimethylamino-3′-deoxy-3′-β-D-ribofuranosyl]-purine, PAN, Fig. 1b) is one of several substrates in puromycin biosynthesis. It is also an RNA synthesis inhibitor [14]. Similarly to PM, PAN is responsible for nephrotoxic effects defined as nephrotic syndrome, a common manifestation of renal disease [15]. By this reason PAN is being used for *in vivo* rodent models of human nephrotic diseases [16,17].

The goal of this work was to establish the binding abilities of PM and PAN for cupric ion. This comparative study allowed us to solve an issue of the steric effect, caused by the presence of the aglycon chain at the C-3′ position in PM molecule.

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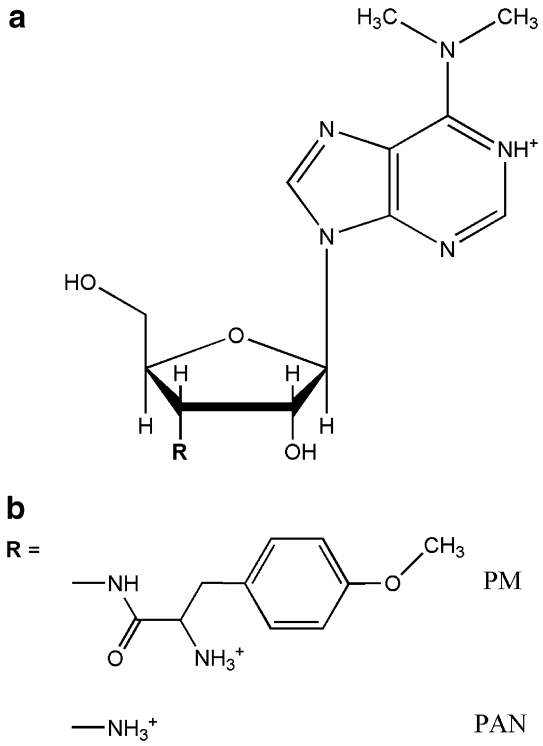

Fig. 1. The structure of the fully protonated: (a) puromycin molecule: $H_2(PM)^{2+}$, (b) puromycin aminonucleoside molecule: $H_2(PAN)^{2+}$.

2. Materials and methods

2.1. Materials

Puromycin (PM) and nitric acid were obtained from Sigma-Aldrich Co. (St. Louis, MO). Puromycin aminonucleoside (PAN), $NaClO_4$ and $Cu(ClO_4)_2$ were purchased from Fluka Chemie GmbH (Buchs, Switzerland). KNO_3 and NaOH were obtained from Merck AG (Darmstadt, Germany). Ethanediol was purchased from POCH S.A. (Gliwice, Poland).

2.2. Potentiometry

Potentiometric titrations of PM and PAN and their complexes with copper(II) ions were performed at 25 ± 0.5 °C in the presence of 0.1 M KNO_3, within the pH range of 2.8–10.5 (Molspin automatic titrator, Molspin Ltd, Newcastle upon Tyne, UK) with CO_2-free 0.1 M NaOH as a titrant. Changes in pH were monitored with a combined glass-Ag/AgCl electrode (InLab 422, Mettler-Toledo, Warsaw, Poland), calibrated daily in hydrogen ion concentrations by HNO_3 titrations [18]. Sample volumes of 2 ml were used. Ligands concentrations were 1 mM, and metal to ligand molar ratios of 1:1.5, 1:2 and 1:3 were used. The collected data were analyzed using SUPERQUAD program [19]. Standard deviations computed by SUPERQUAD refer to random errors only.

2.3. Electronic absorption spectroscopy

The electronic absorption spectra were recorded at 25 °C on a Cary 50 Bio spectrophotometer (Varian Inc., Palo Alto, CA) over the spectral range of 190–1100 nm, in 1 or 0.1 cm cells. The metal to ligand molar ratios were 1:2 for Cu(II) ion to PM and PAN, and the concentration of the former one was 1 mM. The measurements were done in the presence of 0.1 M $NaClO_4$, rather than KNO_3, due to the transparency of the former in far UV.

2.4. Circular dichroism

The CD spectra were recorded under the same conditions as UV–Vis measurements, on a Jasco J-715 spectropolarimeter (JASCO, Japan Spectroscopic Co., Hiroshima, Japan), over the range of 190–800 nm, using 0.1 and 1 cm cells. The spectra were expressed in terms of $\Delta\varepsilon = \varepsilon_l - \varepsilon_r$, where ε_l and ε_r are molar absorption coefficients for left and right circularly polarized light, respectively.

2.5. Electron paramagnetic resonance

The spectra of the Cu(II) complex with puromycin were recorded at 77 K on a Bruker ESP 300E spectrometer (Karlsruhe, Germany) at the X-band frequency (9.3 GHz). Ethanediol–water (1:2) was used as solvent to obtain homogeneity of frozen samples. Samples concentrations were the same as those applied in other spectroscopic measurements.

2.6. Mass spectrometry

ESI-MS experiments for the Cu(II)–PAN complex were performed on a MicroTOF-Q mass spectrometer (Bruker Daltonics, Germany) equipped with a standard electrospray ion source and operated at source and desolvation temperatures of 200 °C. Sample solutions were introduced into the source region of the instruments by conventional electrospray, performed in the positive mode of ionization, with a capillary voltage of 4.5 kV and a flow of 3 μl/min. The analyzed compound was dissolved in water with the same concentration as in spectroscopic experiments. The sample was investigated at pH 6.02, corresponding to the maximum abundance of the dimeric complex.

2.7. HyperChem calculations

The calculations for the dimeric $Cu_2H_{-4}(PAN)_2^{2-}$ complex were made using Hyperchem 7.51 (Hypercube, Inc., Gainesville, FL). The geometry optimization based on molecular mechanics (MM^+ force field was used) led to structures, which represent a potential energy minimum. The Polak–Ribiere routine was applied and root-mean-square (RMS) gradient of 0.1 was set as the termination condition. The *cis* and *trans* coordination conformers, with respect to positions of nitrogen and oxygen donors in the