Journal of Inorganic Biochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Metal complexes of 6-pyrazolylpurine derivatives as models for metal-mediated base pairs☆

Indranil Sinha [a,b], Alexander Hepp [a], Jutta Kösters [a], Jens Müller [a,b,*]

[a] Westfälische Wilhelms-Universität Münster, Institut für Anorganische und Analytische Chemie, Corrensstr. 28/30, 48149 Münster, Germany
[b] Westfälische Wilhelms-Universität Münster, NRW Graduate School of Chemistry, 48149 Münster, Germany

ARTICLE INFO

Article history:
Received 20 February 2015
Received in revised form 10 June 2015
Accepted 9 July 2015
Available online xxxx

Keywords:
DNA
Metal-mediated base pairs
Nucleobases

ABSTRACT

6-(3,5-Dimethylpyrazol-1-yl)purine has recently been introduced as an artificial nucleobase for the specific recognition of canonical nucleobases via the formation of a metal-mediated base pair. We report here the synthesis and structural characterization by single crystal X-ray diffraction analysis of a series of metal complexes of the corresponding alkylated model nucleobases 9-methyl-6-(3,5-dimethylpyrazol-1-yl)purine **2** and 9-methyl-6-pyrazol-1-yl-purine **7**. The sterically more demanding ligand **2** forms the Cu^{2+} complexes $[Cu(\mathbf{2})(NO_3)_2]$ and $[Cu(\mathbf{2})Cl_2]$ with a 1:1 stoichiometry of ligand and metal ion. In contrast, ligand **7** forms complexes $[Cu(\mathbf{7})_2(NO_3)](NO_3)$ and $[Ag(\mathbf{7})_2](ClO_4)$ with a 2:1 stoichiometry. The molecular structures of $[Cu(\mathbf{2})(NO_3)_2]$ and $[Cu(\mathbf{2})Cl_2]$ confirm the previously suggested coordination pattern, i.e. Cu^{2+} is coordinated via the pyrazole nitrogen atom and the purine N7 position. The fact that different relative orientations of the two ligands in $[Cu(\mathbf{7})_2(NO_3)](NO_3)$ and $[Ag(\mathbf{7})_2](ClO_4)$ are observed allows the prediction that the corresponding metal-mediated homo base pairs should be stable both in regular antiparallel-stranded and in the rare parallel-stranded double helices.

1. Introduction

Metal ions are involved in almost every aspect of nucleic acid chemistry [1]. In addition to the biological relevance of the interaction of natural nucleic acids with metal ions, the application of artificial nucleic acids with an increased metal-ion affinity has gained significant interest in the past years. The most prominent method for the site-specific introduction of metal-based functionality to a nucleic acid is the use of so-called metal-mediated base pairs. In these non-natural base pairs, the hydrogen bonds between complementary nucleobases are formally replaced by coordinate bonds to a central (transition) metal ion [2–4]. As a result, the metal ions are located along the helical axis inside the double helix [5–8]. While metal-mediated base pairs can be formed from the canonical pyrimidine nucleobases cytosine or thymine [9–12], the majority of examples has been reported with artificial nucleobases, e.g. imidazole [6,13,14], hydroxypyridone [15,16], salen [17], or 6-substituted purines [18–21]. Metal-mediated base pairs may even contain two metal ions per base pair [22–25]. Recently, a novel strategy towards an easy access to a family of nucleosides with bi- and tridentate ligands as aglycones has also been introduced [26–28]. Nucleic acids with metal-mediated base pairs have found applications in various fields [29], including the sensing of transition metal ions and small molecules, in charge transfer though DNA [30], and in expanding the genetic code [31].

Most recently, Lönnberg et al. have introduced a family of artificial purine-derived nucleosides that were designed with the aim of discriminating a natural nucleobase located opposite the artificial nucleoside [32–35]. Oligonucleotides capable of specifically recognizing short nucleic acid sequences are relevant in RNA interference and other therapeutic applications for the treatment of genetic disorders [36,37]. The artificial nucleosides used in those studies are based on purine, adenine, or hypoxanthine, and contain one or two dimethylpyrazolyl substituents (Fig. 1). All of them had been introduced as ribonucleosides into 2′-*O*-methyl oligonucleotides. Particularly the incorporation of Cu^{2+} into the resulting base pairs led to a significant increase in thermal stability of the respective double helix. The metal-binding behavior of 6-substituted purines such as 6-(3,5-dimethylpyrazol-1-yl)purine is of particular interest, as two different bidentate binding modes are feasible. Depending on the orientation of the dimethylpyrazolyl substituent, N1 or N7 binding of the purine ring is possible (Fig. 2). Metal binding via N1 results in the formation of a metal-mediated Watson–Crick base pair, whereas binding to N7 leads to a metal-mediated Hoogsteen base pair. This ambident behavior of 6-substituted purine derivatives has recently been demonstrated using 6-furylpurine [18,19]. As no molecular structures had been available for metal complexes of 6-(3,5-dimethylpyrazol-1-yl)purine, Cu^{2+}-binding to the N7 position of this purine derivative had been suggested based on a comparison

☆ Dedicated to Professor Giovanni Natile on the occasion of his 70th birthday.

* Corresponding author at: Westfälische Wilhelms-Universität Münster, Institut für Anorganische und Analytische Chemie, Corrensstr. 28/30, 48149 Münster, Germany.
E-mail address: mueller.j@uni-muenster.de (J. Müller).

http://dx.doi.org/10.1016/j.jinorgbio.2015.07.002
0162-0134/

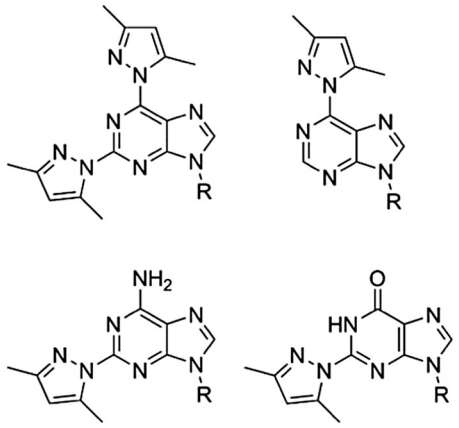

Fig. 1. Chemical structures of 3,5-dimethylpyrazolyl-substituted derivatives of purine, adenine, and hypoxanthine (R = nucleic acid backbone) as previously reported by Lönnberg et al. [32,33,35].

with the complexation behavior of the closely related adenine moiety [32]. We report here a systematic structural study of the metal-binding behavior of 9-methyl-6-(3,5-dimethylpyrazol-1-yl)purine **2**, acting as a model nucleobase. The use of alkylated ligands as model nucleobases is well-known from nucleobase chemistry [38] and has successfully been introduced to the study of metal-mediated base pairs, too [19,27,39–41].

2. Experimental

2.1. General

All solvents were dried prior to their use. 6-Hydrazinylpurine and 6-pyrazol-1-yl-purine **6** were synthesized according to literature procedures [42,43]. ESI-TOF measurements of compounds **1**, **2**, **3** and **7** were performed using the oa-TOF mass spectrometer MicrOTOF (Bruker Daltonik GmbH, Bremen, Germany) equipped with a standard ESI source. All mass spectra are quasi-internally mass calibrated by the measurement of an infused calibrant (ammonium formate) prior to the compound of interest. ESI-MS spectra of compounds **4**, **5**, **8** and **9** were measured on an LTQ Orbitrap XL (Thermo Scientific, Bremen, Germany), equipped with the static nanospray probe (slightly modified to use self-drawn glass nanospray capillaries). The elemental analyses were performed on a Vario EL III CHNS analyzer. NMR spectra were recorded at 300 K on Bruker Avance (I) 400 and Avance (III) 400 spectrometers with an internal standard relative to tetramethylsilane (δ = 0 ppm, $CDCl_3$), trimethylsilyl propionate (δ = 0 ppm, D_2O) or the residual solvent peak (δ = 2.50, DMSO-d_6). For the atom-numbering scheme used in the following lists of NMR data, please see Fig. 3. Single-crystal X-ray diffraction data were collected with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) on a Bruker D8 Venture diffractometer. The structures were solved by direct methods and were refined by full-matrix, least squares on F^2 by using the SHELXTL and SHELXL-97 programs [44]. Crystallographic data are listed in Table 1.

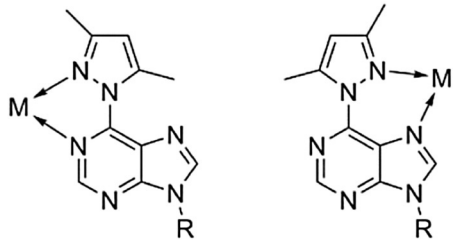

Fig. 2. Ambident metal-binding behavior of 6-(3,5-dimethylpyrazol-1-yl)purine, resulting from an unhindered rotation around the C–N bond (R = nucleic acid backbone).

2.2. Synthesis of 6-(3,5-dimethylpyrazol-1-yl)purine *1*

6-Hydrazinylpurine (1.77 g, 11.8 mmol) was suspended in acetylacetone (8.4 mL, 83 mmol), followed by the addition of CF_3COOH (8.8 μL, 0.12 mmol). The mixture was stirred overnight. The resulting solid was filtered, washed with cold water and diethyl ether. It was purified by silica gel column chromatography to produce the title compound **1** as a white solid in 42% yield (1.07 g, 4.99 mmol). ESI-TOF m/z: $[M + H]^+$ 215.1040 (calcd. 215.1045), $[M + Na]^+$ 237.0859 (calcd. 237.0865). Elemental analysis (%): found: C 56.3, H 4.7, N 39.5; calcd. for $C_{10}H_{10}N_6$: C 56.1, H 4.7, N 39.2. ^{1}H NMR (400 MHz, DMSO-d_6), δ/ppm: 12.8 (s, 1H, NH), 8.76 (s, 1H, H2p), 8.61 (s, 1H, H8p), 6.25 (s, 1H, H4*), 2.75 (s, 3H, H6*), 2.33 (s, 3H, H7*). ^{13}C NMR (101 MHz, DMSO-d_6), δ/ppm: 151.8 (C3*), 151.5 (C2p), 150.7 (C8p), 147.4 (C6p), 146.2 (C4p), 145.0 (C5p), 142.6 (C5*), 110.4 (C4*), 14.7 (C6*), 13.4 (C7*).

2.3. Synthesis of 9-methyl-6-(3,5-dimethylpyrazol-1-yl)purine *2* and 7-methyl-6-(3,5-dimethylpyrazol-1-yl)purine *3*

Compound **1** (0.627 g, 2.93 mmol) was suspended in DMF (20 mL). After the addition of NaH (0.141 g, 3.51 mmol) and stirring for 15 min at 50 °C, methyl iodide (0.219 mL, 0.495 g, 3.51 mmol) was added and stirring continued for 6 h. The reaction mixture was allowed to reach room temperature, diluted with water, and extracted with ethyl acetate thrice. The combined organic phase was dried ($MgSO_4$), and the crude product mixture was purified by silica gel column chromatography to produce a white solid in 26% yield (0.174 g, 0.762 mmol), containing both N9 and N7 alkylated isomers **2** and **3** in a ratio of about 55:45. The isomers could be unambiguously identified by NMR spectroscopy. The mixture was used for further reactions without the necessity for separation. ESI-TOF m/z: $[M + H]^+$ 229.1196 (calcd. 229.1202), $[M + Na]^+$ 251.1016 (calcd. 251.1021). Elemental analysis (%): found: C 55.2, H 5.5, N 34.7; calcd. for **2**/**3** · 0.67 H_2O: C 55.0, H 5.6, N 35.0. N9-alkylated isomer **2**: ^{1}H NMR (400 MHz, $CDCl_3$), δ/ppm: 8.77 (s, 1H, H2p), 8.14 (s, 1H, H8p), 6.07 (s, 1H, H4*), 3.92 (s, 3H, H9p), 2.69 (s, 3H, H7*), 2.38 (s, 3H, H6*). ^{13}C NMR (101 MHz, $CDCl_3$), δ/ppm: 154.1 (C4p), 152.8 (C3*), 151.3 (C2p), 149.8 (C6p), 145.1 (C8p), 143.0 (C5*), 123.8 (C5p), 110.2 (C4*), 30.0 (C9p), 14.6 (C7*), 14.1 (C6*). N7-alkylated isomer **3**: ^{1}H NMR (400 MHz, $CDCl_3$), δ/ppm: 8.91 (s, 1H, H2p), 8.17 (s, 1H, H8p), 6.09 (s, 1H, H4*), 3.96 (s, 3H, H9p), 2.57 (s, 3H, H7*), 2.29 (s, 3H, H6*). ^{13}C NMR (101 MHz, $CDCl_3$), δ/ppm: 164.0 (C4p), 151.4 (C2p), 150.9 (C3*), 149.8 (C8p), 145.0 (C6p), 143.0 (C5*), 118.3 (C5p), 109.3 (C4*), 36.3 (C7p), 13.5 (C7*), 13.0 (C6*).

2.4. Synthesis of [Cu(*2*)$(NO_3)_2$] *4*

$Cu(NO_3)_2 \cdot 3\,H_2O$ (0.158 g, 0.657 mmol) was added to a methanolic solution of **2** and **3** (0.150 g, 0.657 mmol). The mixture was heated at 60 °C for 18 h. After one week at 4 °C, a blue crystalline compound could be isolated. The crystals proved to be [Cu(**2**)$(NO_3)_2$] **4** according to single crystal X-ray diffraction analysis. ESI-MS m/z: $[Cu(\mathbf{2})(NO_3)]^+$ 353.0301 (calcd. 353.0298), $[Cu(\mathbf{2})_2(NO_3)]^+$ 581.1432 (calcd. 581.1421).

2.5. Synthesis of [Cu(*2*)Cl_2] *5*

$CuCl_2 \cdot 2\,H_2O$ (75 mg, 0.44 mmol) was added to a methanolic solution of **2** and **3** (0.100 g, 0.438 mmol). The mixture was heated at 60 °C for 18 h. The solvent was evaporated, affording a green solid