Complexes of Azithromycin with Some Divalent Metal Ions

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Received December 30, 1993; revised July 22, 1994; accepted July 25, 1994.

The formation of 2:1 complexes of azithromycin with copper(II), zinc(II), cobalt(II), nickel(II), magnesium(II) and calcium(II) ions was examined. These compounds (2–7) showed the same in vitro biological potency as azithromycin (1). The complexes (3–7) are isostructural and different when compared with azithromycin-Cu(II)-complex. Copper(II)-complex (2) was most stable and selected for structure determination by X-ray crystallography. Some physical characteristics and analyses of the complexes are also presented.

INTRODUCTION

Azithromycin (1),1–3 a semisynthetic 15-membered azalide antibiotic derived by chemical modification of erythromycin A, shows a broader antimicrobial spectrum, longer elimination half-life and higher tissue concentrations than erythromycin.4–8 It differs structurally from erythromycin A in the insertion of a 9a-methyl substituted nitrogen in the aglycone moiety, while the sugar moieties (desosamine and cladinose) stay unchanged.

The presence of metals and the formation of metal complexes might substantially influence the stability, distribution, biotransformation, elimination and other characteristics of the antibiotic. According to the early literature, the formation of a weak complex with erythromycin A was established in dilute solution only with Co(II) while with the other metal ions, like Cu(II), Ca(II), Mg(II), Ni(II) and Zn(II), such complexes were not observed.9 However, later on, the erythromycin complex with Zn(II) was prepared and structurally characterized in the solid state, but in this structure the zinc atom is coordinated only by one erythromycin molecule while the other ligands are one monodentately and one bidentately bonded acetate ions and one water molecule.10 In
this paper, we describe the formation of 2:1 complexes of azithromycin with some divalent ions (2–7). One of these complexes, the azithromycin-Cu(II) complex (2), was most stable and therefore selected for structural elucidation by X-ray crystallography.

EXPERIMENTAL

Azithromycin was produced by Pliva, Zagreb. All inorganic salts and other chemicals used were of analytical grade (Aldrich). Potentiometric (pH) measurements were carried out on a 672 Titroprocessor, Metrohm, and the UV spectra were recorded on a Pye Unicam SP8-100 instrument. The melting points were determined by a Fisher-Johns apparatus and are uncorrected. Atomic absorption spectrometry of metals was done using a Perkin-Elmer Model 5000 and polarographic measurements using an Amel Multipurpose Unit Model 563/564. X-ray diffractometer data were collected at room temperature on a Philips PW 1100 automatic diffractometer by using monochromatized Mo Kα radiation (λ = 0.71069 Å). Powder diffraction patterns were taken on a Philips diffractometer (monochromatized Cu Kα radiation).

Preparation of Complexes

*Azithromycin-Cu(II) complex (2).* To a stirred suspension of 1 (0.750 g, 1 mmol) in 48 ml of water, 1.7 ml of 0.1 M HCl was added to acidify the reaction mixture to pH 6.0. Then, 0.086 g (0.5 mmol) CuCl₂ × 2H₂O was added and the stirring was continued under a stepwise addition of 0.1 M NaOH until a pH value of 8.5 was achieved. Subsequently, the reaction mixture was stirred for 2 hours at constant pH value (pH stat), then the violet-coloured precipitate was filtered off, washed with water and dried, to obtain 0.64 g (81.8%) of 2. After crystallization from methanol, pale-violet prisms were obtained, m.p. 188–192 °C; UV(EtOH) λmax 292,485 nm.


Activity (in vitro biological potency): 834 U/mg against *Micrococcus luteus* ATCC 9341.

*Azithromycin-Zn(II) complex (3).* was prepared from 1 (0.750 g, 1 mmol) and ZnCl₂ (0.068 g, 0.5 mmol) at the pH value of 8.6, in a similar manner as described for 2, to give a colourless powder of 3, 0.61 g (77.9%); m.p. 120–123 °C.


Activity: 852 U/mg.

*Azithromycin-Co(II) complex (4).* was prepared from 1 (0.750 g, 1 mmol) and CoCl₂ × 6H₂O (0.118 g, 0.5 mmol) at the pH value of 8.6, as described in the preparation of 2, to give a light-green coloured product of 4, 0.63 g (80.7%); m.p. 128–131 °C; UV(EtOH) λmax 290,655 nm.


Activity: 849 U/mg.

*Azithromycin-Ni(II) complex (5).* was prepared from 1 (0.750 g, 1 mmol) and NiCl₂ × 6H₂O (0.119 g, 0.5 mmol) at the pH value of 8.6, as described in the preparation of 2, to give a light-green product of 5, 0.62 g (79.6%); m.p. 118–123 °C.


Activity: 853 U/mg.

*Azithromycin-Mg(II) complex (6).* was prepared from 1 (0.750 g, 1 mmol) and MgCl₂ × 6H₂O (0.102 g, 0.5 mmol) at the pH value of 8.6, as described in the preparation of 2, to give a colourless product of 6, 0.55 g (75.0%); m.p. 121–124 °C.

*Anal.* Calcd. C₇₆H₁₄₂O₂₄N₄Mg: C 60.05, H 9.43, N 3.69, Mg 1.60; found: C 59.85, H 9.42, N 3.60, Mg 1.54.

Activity: 850 U/mg.
COMPLEX OF AZITHROMYCIN

Azithromycin-Ca(II) complex (7), was prepared from 1 (0.750 g, 1 mmol) and CaCl₂ x 2H₂O
(0.074 g, 0.5 mmol) at the pH value of 8.6, as described in the preparation of 2, to give a col-
ourless product of 7, 0.60 g (77.9%); m.p. 120-123 °C.

Anal. Calcd. for C₇₆H₁₄₂O₂₄N₄Ca: C 59.43, H 9.32, N 3.65, Ca 2.61; found: C 59.15, H 9.40,
N 3.52, Ca 2.30.

Activity: 856 U/mg.

Structure Determination of Azithromycin-Cu(II) Complex by X-ray analysis

The pale-violet crystals obtained from methanolic solution of the title compound were of
irregular shape and poor quality and, above all, very sensitive. Left in the air, they started
immediately to lose solvent, became opaque and white and clove into very thin layers. For pro-
tection they were sealed in Lindemann glass capillaries together with a droplet of methanol.
However, in spite of that, they decomposed significantly during the data collection.

Accurate cell dimensions were determined from the least-squares refinement based on 18
reflections within the θ range from 4.4 to 7.2°.

Crystal data: Cu(C₃₀H₇₁N₄O₁₂)₂, monoclinic, space group P₂₁, a = 19.143(5), b = 23.491(5),
c = 12.486(4), Å, β = 95.71(3)°, V = 5586.9 Å³, Z = 2, Dₜ = 1.182 Mg m⁻³ (pycnometrically).

An irregular crystal of approximate dimensions 0.2 x 0.3 x 0.4 mm was used for intensity
data collection by the ω-2θ scan technique (scan width 1.2°, scan speed 0.04° s⁻¹) in the range
of 2 ≤ θ ≤ 25°. Three check reflections monitored every two hours indicated crystal decay of
ca 20% and the intensities were corrected accordingly. A total of 2396 unique reflections were
collected, of which 2336 were greater than Io(F) and used in the structure solution and refine-
ment. The data were corrected for the Lorentz and polarization factors but not for absorption.

The copper atom position was obtained from a three-dimensional Patterson synthesis, all
other non-hydrogen atoms by using direct methods and subsequent Fourier map calculations
Due to the limited number of diffraction data, the least-squares refinement was performed as-
suming anisotropic temperature factors for the copper atom and isotropic temperature factors for
all other atoms. Hydrogen atoms attached to the azithromycin ligands were placed on geometrical
grounds and their contributions included in the structure factor calculations. At R = 0.154, ten
water-oxygen atoms were located in the cavities formed between the complex molecules. The
refinement converged at R = 0.112. The final atomic parameters, with temperature factors and
lists of the interatomic distances and angles, and of the observed and calculated structure fac-
tors are obtainable from the authors on request. All calculations were performed using the
SIR,¹¹ SHEXL76¹² and SHELEX93¹³ programmes, the distances and angles were calculated by
means of the CSU¹⁴ and the diagrams drawn by the EUCLID programme.¹⁵

RESULTS AND DISCUSSION

Chemical Preparation

The process of complexing azithromycin with particular metal ions was carried
out by adjusting the pH of the solution to values convenient for precipitation of the
product (see Experimental). The following metal complexes were prepared and ana-
yzed: Cu(II), Zn(II), Co(II), Ni(II), Mg(II) and Ca(II). Some physical characteristics
and analyses of complexes are also presented in the Experimental section. It is ob-
vious that these compounds showed similar in vitro biological potency as azithromy-
cin (867 U/mg against Micrococcus luteus ATCC 8341). It appears that an introd-
uction of metal atoms and the formation of the corresponding complexes with the
azithromycin molecule do not change its antibacterial activity.

Complexes of EPR and polarographic active metals were chosen for recording
the EPR spectra and for polarographic measurements. The EPR spectra of the az-
ithromycin-copper(II) (2) and the azithromycin-cobalt(II) complexes (4) (Figure 1)
confirmed the presence of new species (azithromycin itself showed no EPR ability). Formation of the azithromycin-Zn(II) complex (3) was detected by shifting the polarographic wave of Zn(II) by about 0.2 V (Figure 2).

![Figure 1. EPR Spectra of the azithromycin-copper(II) complex (—□—) and azithromycin-cobalt(II) complex (■)](image)

Figure 2. Polarographic curves of Zn(II) (curve a) and the azithromycin- zinc(II) complex (curve b); 10^{-3} M solutions in methanol -0.1 N KCl (1:1).

X-ray Structure Analysis of Azithromycin-Cu(II) Complex

The structure of azithromycin-Cu(II) complex (2) is shwon in Figure 3. The copper atom links two azithromycin molecules (A) and (B), being bonded to two tertiary nitrogen [N(3′A) and N(3′B)] and two hydroxyl oxygen [O(2′A) and O(2′B)] atoms from two desosamine moieties. Analogous bonding to the desosamine moiety was found in the above mentioned zinc acetate complex with erythromycin. The coordination is very irregular, with the angles at the copper atom ranging from 88(1) to 97(1)°. The bond lengths Cu-O of 1.88(2) and 1.85(2) Å and Cu-N of 2.00(3) and 2.02(3) Å are as expected. The bond lengths and angles in the 15-membered rings and
Figure 3. A perspective view of the azithromycin-Cu(II) complex molecule. The copper atom is coordinated by two azithromycin ligands, (A) and (B), and both have the analogous atom-numbering scheme. Hydrogen atoms are omitted for clarity. Bond lengths (Å) and angles (°) within the copper coordination sphere: Cu-O(2'A), 1.88(2); Cu-O(2'B), 1.85(2); Cu-N(3'A), 2.00(3); Cu-N(3'B), 2.02(3); O(2'A)-Cu-N(3'A), 88(1); O(2'B)-Cu-N(3'B), 88(1); O(2'B)-Cu-N(3'A), 96(1); N(3'B)-Cu-O(2'A), 97(1); O(2'A)-Cu-O(2'B), 162(1); N(3'A)-Cu-N(3'B), 152(1).

Figure 4. Packing of molecules in the unit-cell seen along the crystallographic c axis. A cavity formed between the molecules is extended along the b axis.
Sugar components are similar to those observed in azithromycin itself, as well as in the analogous but differently substituted 15-membered aglycone rings. In the C(2)-C(5) region, both aglycone rings exhibit the «folded in» conformation in contrast to the «folded out» conformation observed in the free azithromycin. Azithromycin adopts the same «folded in» conformation in solution, as proved by the NMR spectroscopy.

An outstanding feature of the structure is a significant cavity formed around the two-fold screw axis, i.e., between the columns built up from the complex molecules and extended along the crystallographic b axis (Figure 4). Remarkable distances between the complex molecules, such as C(7')..C(14B') (transformation of the asymmetric unit (x,y,z): (i) = 1 - x, 1/2 + y, 1 - z) and C(7')..C(14B) amounting to 3.81(6) and 11.69(6) Å, respectively, indicate that such a cavity can accommodate a considerable number of solvent (water and/or methanol) molecules (Figure 5). This is exactly what has been observed: if freshly prepared crystals were left in a desiccator over calcium chloride up to the constant weight, they lost about 22% of their original weight. This loss corresponds approximately to 24 water or 14 methanol molecules per one complex molecule. This is also in good agreement with the difference observed between the measured and calculated density, 1.18 versus 0.925 Mg m⁻³. However, only ten water molecules were located in the crystal structure. Therefore, the loss of solvent may be the reason for the observed crystal decomposition as well as for the low accuracy of the diffraction data. Unfortunately, all attempts to prepare better crystals from a number of different solvents were unsuccessful.

According to the powder diffraction data, the complexes (3–7) are isostructural and differ from those of the azithromycin-Cu(II) complex.

Acknowledgement. - The authors wish to thank Miss Z. Veksli, Ph.D. and Prof. S. Popović, the Ruder Bošković Institute, Zagreb, and Mr. A. Dürrigl, M.S., Pliva, Zagreb, for helpful instrumental analyses. In vitro activities were determined in the Pliva Control Department, Zagreb. The work was partially supported by the Ministry of Science and Technology of the Republic of Croatia.
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SAŽETAK

Kompleksi azitromicina s nekim dvovalentnim metalnim ionima

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Proučavano je nastajanje 2:1 kompleksa azitromicina s ionima bakra(II), cinka(II), kobalata(II), nikla(II), magnezija(II) i kalcija(II). Ti spojevi (2–7) pokazuju jednaku biološku aktivnost in vitro kao azitromicin (1). Kompleksi (3–7) su izostrukturni, ali različiti u usporedbi s kompleksom Cu(II). Kompleks (2) bakra(II) bio je najstabilniji i odabran je za određivanje strukture metodom rentgenske kristalografije. Također su navedena neka fizikalna svojstva i analize kompleksa.