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Capreomycin — A polypeptide antitubercular antibiotic with unusual binding properties toward copper(II)

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ABSTRACT

Capreomycin is an important therapeutic agent having intriguing and diverse molecular features. Its polypeptidic structure rich in nitrogen donors makes the drug a promising chelating agent for a number of transition metal ions, especially for copper(II). The results of the model investigational studies suggest that capreomycin anchors Cu^{2+} ion with an amino function of the α,β-diaminopropionic acid residue at pH around 5. At physiological pH copper(II) ion is coordinated by two deprotonated amide nitrogen atoms of the α,β-diaminopropionic acid, the serine residue as well as the amino function deriving from the β-lysine. Above that pH value we observe a rearrangement within the coordination sphere leading to movement of Cu^{2+} to the center of the peptide ring with concurrent coordination of four nitrogen donors. Spin–lattice relaxation enhancements and potentiometric measurements clearly indicate that deprotonated amide nitrogen atom from the β-ureidodehydroalanine moiety is the fourth donor atom.

1. Introduction

Capreomycin (CPMY, Fig. 1), a polypeptide antibiotic, commonly grouped with aminoglycosides, isolated from *Streptomycetes caprelous*, is active against certain strains of tuberculosis, i.e. *Mycobacterium tuberculosis* and *Mycobacterium bovis*, as well as other strains, i.e. *Mycobacterium avium* [1–4]. Tuberculosis (TB) is perhaps the most persistent human disease of bacterial origin with 1.6–2 million fatalities annually. An increasing percentage of drug-resistant or multidrug-resistant strains has been isolated that severely impair the ability to treat the disease. Tuberactinomycins, the most effective antibiotics against multidrug-resistant TB so far, elicit their activity by interacting with both ribosomal subunits, thus inhibiting protein synthesis [5,6]. Viomycin was the first to be identified, but only capreomycin is nowadays commonly used clinically as a second-line antibiotic against infections by *Mycobacterium tuberculosis*.

The CPMY molecule is made of a cyclic pentapeptide containing two residues of α,β-diaminopropionic acid, β-ureidodehydroalanine, capreomycidine (a guanidine-containing amino acid) and β-lysine (Systematic IUPAC name = (3*S*)-3,6-diamino-*N*-[[(2*S*,5*S*,8*E*,11*S*,15*S*)-15-amino-11-[(4*R*)-2-amino-3,4,5,6-tetrahydropyrimidin-4-yl]-8-[(carbamoylamino)methylidene]-2-(hydroxymethyl)-3,6,9,12,16-pentaoxo-1,4,7,10,13-pentazacyclohexadec-5-yl]methyl]hexanamide). CPMY is a mixture of four isoforms, all active against mycobacteria: IA, IB, IIA and IIB. IA differs from IB by having an additional serine instead of an alanine, while IIA and IIB lack the β-lysine residue found in IA and IB respectively. The distribution of those components is as follows: 91% of IA and IB and 9% of IIA and IIB [7] (Supplementary Fig. S1).

The CPMY molecule contains 12 nitrogen donors potentially able to coordinate Cu^{2+}. Moreover, the large 16-member ring makes the molecule highly flexible, with the β-lysine and capreomycidine functions being able to rotate around the single bonds, such that accommodation of the metal ion in its center is made possible. Our previous studies with peptides-containing therapeutic agents, e.g. vancomycin [8], actinomycin D [9] and teicoplanin [10] showed that such compounds were able to bind Cu^{2+} ions strongly enough to interfere with the copper homeostasis. The increased copper level in plasma may enable complex formation during either severe inflammation, when vancomycin or teicoplanin are administered, or cancer, when actinomycin D may be used [11,12]. It has also been reported that the average serum copper level of tuberculosis patients may increase for ca. 21% (from 102 to 123.65 μg/dl) and that it comes down after antitubercular treatment [13,14]. Moreover, serum copper has been suggested as a useful biomarker for monitoring pulmonary tuberculosis [15]. The recent statement that several antitubercular drugs are able to chelate copper ions and thus affect its metabolism [16], became a starting point to the study of interaction between copper(II) and capreomycin.

In the present work protonation equilibria and Cu^{2+} binding by CPMY were studied by potentiometry, NMR, electronic absorption spectroscopy, circular dichroism, and EPR.

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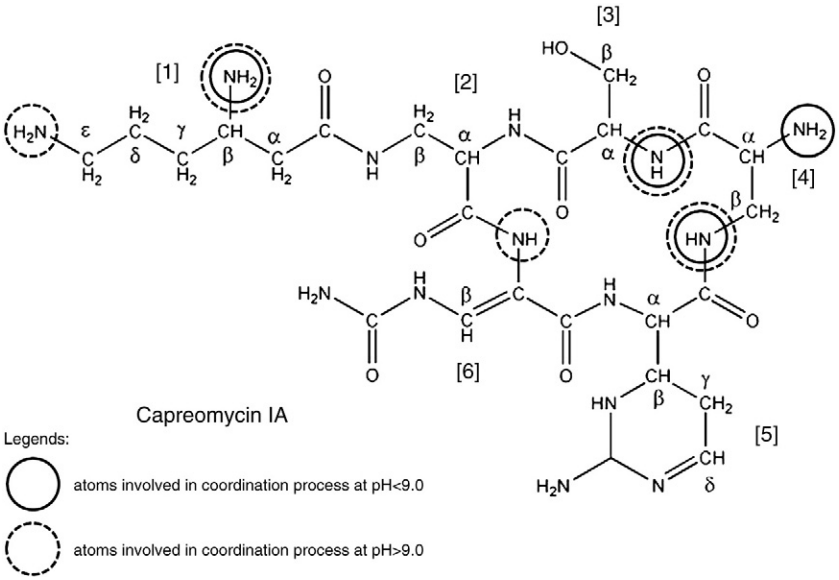

Fig. 1. The molecular formula of capreomycin with the Cu^{2+} binding sites highlighted.

2. Results and discussion

CPMY behaves as a tetra-protic acid, H_4L, in water. The values of the constants that may be attributed to each of the deprotonation steps are collected in Table 1. The lowest pK_a value (~6.2) belongs to the primary amino function of the α,β-diaminopropionic acid residue. The next two pK_a values (7.9 and 10.0) refer to deprotonation of the β-NH_3^+ and ε-NH_3^+ of the β-lysine respectively. The constants calculated from the present potentiometric titrations agree with data reported in the literature for similar systems [17–19]. The highest pK_a value (~11.4) can be attributed to the capreomycidine moiety. The guanidine function within capreomycidine forms tautomeric species, that yield a pK_a value>11.0 [20].

In the presence of Cu^{2+}, seven Cu^{2+}-CPMY monomeric complexes are formed with the general formula CuH_nL, with *n* varying from +3 to −3. The SUPERQUAD calculations did not allow for the model, which would have contained CuL_2 or CuL_3 stoichiometry. On the other hand, titration done with an excess of the metal yielded precipitation of the copper hydroxide. The binding process starts at pH 4.5 with the CuH_3L species, as shown by the distribution diagram (Fig. 2). The minor d–d band at 696 nm in UV–visible (Table 1) is characteristic for copper(II) complexes with one nitrogen donor involved into the coordination sphere, as also ratified by EPR parameters (A_{II} = 146 G; g_{II} = 2.33). By just looking at the model structure of CPMY and considering its acid–base properties the amino group of the α,β-diaminopropionic acid residue can be suggested to act as the anchoring donor (α-nitrogen of residue no. 4 in Fig. 1), exactly as it was previously reported for hygromycin B [21].

With the rise of pH, the species distribution diagram reveals the occurrence of the new species CuH_2L (Fig. 2). The very low abundance did not allow to obtain spectroscopic parameters; however the pK_a value of its formation (~6.64) is consistent with deprotonation and coordination of a peptide bond amide function [22,23], as it may be expected by considering the usual behavior of peptides that bind Cu^{2+} *via* the N-terminal amino group and, depending upon the pH, of the neighboring ionized amide nitrogens [10,12]. Although CPMY does not possess an N-terminal amino group, the protruding amine from the α,β-diaminopropionic acid (α-nitrogen of residue no. 4, Fig. 1) together with the neighboring serine imino group (nitrogen of residue no. 3) matches the expected coordination model typical of peptides.

The pK_a value of formation of the CuHL species is 6.27, ca. 0.4 log units lower than that of the CuH_2L form. Such convergence in the potentiometric calculations is not usual but it is typical for simultaneous deprotonation processes. A difference lower than 0.6 log units between the consecutive constants suggests that both species form at a similar pH value [24]. The amide nitrogen is again the deprotonating function [22,25]. The UV–vis spectra yield evidence of an increase in intensity of the d–d band, while CD spectra display several Cotton effects not observed for CuH_2L (Table 1). The appearance of the band at 320 nm may reflect the involvement of the amide donor in the coordination [22]. The α,β-diaminopropionic acid amide (β-nitrogen of residue no. 4) most likely enters the coordination sphere leading to the {NH_2, N^-_{amide}, N^-_{amide}} binding model, in agreement with EPR parameters typical for Cu^{2+} complexes with three nitrogen donors (A_{II} = 199 G, g_{II} = 2.23, Table 1) [23,26].

The next CuL species is a major complex form that predominates in solution in the pH range 7.2–8.0 (Fig. 2). The spectral parameters obtained for this complex are not much diverse from those monitored for the previous species, such that invariance of the coordination sphere around Cu^{2+} might apparently be suggested. However, the pK_a value accompanying its formation (7.12) is likely to represent a mean value arising from a number of deprotonating nitrogen containing functions, whether spontaneous or Cu^{2+}-extorted. It is therefore troublesome to assess which particular function deprotonates upon formation of CuL.

NMR measurements were therefore carried out in order to gain a detailed information on CuL. NMR data were obtained in D_2O at pH 7.4 (pD 7.8); the assignment of 1D NMR spectra is reported in Table 2. The ^{1}H-NMR spectrum of CPMY IB was also assigned (Table S1 — Supplementary data), showing that, in most cases, the signals are not completely overlapped to those of CPMY IA. Moreover, even in cases of extensive overlap, the T1-TOCSY sequence (see Experimental section) allowed to measure the relaxation rates of CPMY IA. At pH 7.4, addition of 0.04 equivalents of Cu^{2+} yielded sizeable effects upon line widths and spin lattice relaxation rates, as summarized in Table 3. Deeper information on the Cu^{2+}-CPMY IA interaction was gained by ^{1}H–^{1}H TOCSY maps, revealing the greatest paramagnetic effects on α-CH_2 and β-CH of residue 1, β-CH_2 of residue 4 and α-CH of residue 3. At this pH value, it is evident that the metal is coordinated by deprotonated amide nitrogen donors of the α,β-diaminopropionic acid (β-nitrogen of residue no. 4) and of the neighboring serine (nitrogen of residue no. 3). However, NMR data are revealing for the amino group of the α,β-diaminopropionic acid (α-nitrogen of residue no. 4) being not involved in coordination, at variance with the previously discussed complexes. On the contrary,