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Note

Copper(II) binding to tobramycin: potentiometric and spectroscopic studies

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Abstract

Protonation and Cu(II) binding by tobramycin, an aminoglycosidic antibiotic, was studied by potentiometry and UV–vis, CD and EPR spectroscopies. A range of mononuclear complexes of a general formula CuH_nL was found, with n between 3 and -2. Tobramycin anchors Cu(II) with an $\{NH_2, O^-\}$ chelate of the C-ring of its molecule. The amino and hydroxyl groups of the A-ring of tobramycin also participate in the binding at pH 7 and higher. The resulting structure involves both terminal aminosugar rings but eliminates the donors of the central streptamine unit from the coordination. A comparison between tobramycin and its close analog, kanamycin B [M. Jeżowska-Bojczuk, W. Bal and H. Kozłowski, *Inorg. Chim. Acta*, 275–276 (1998) 541–545] reveals the importance of the A3-OH group for the binding properties of these aminoglycosides.

Keywords: Aminoglycoside antibiotic; Tobramycin; Copper(II) complexes

1. Introduction

Aminoglycoside antibiotics interfere with protein synthesis by binding to the ribosome and perturbing several steps of translation. Although these molecular mechanisms are poorly understood, many aspects of the antibiotic actions in the cell are well described [1–4].

The role of transition metal ions in the biological activity of aminoglycoside antibiotics is not known, although our previous studies have shown that Cu(II) complexes with 1-deoxynojirimycin [5], kanamycin B [6] and amikacin [7] mediate very effectively the oxidative reactions of dioxygen and hydrogen peroxide like the oxidation of 2′-deoxyguanosine to its 8-oxo derivative. This oxidative property of antibiotics bound to metal ions may contribute to biological mechanisms of their antibacterial activity. For instance, copper tetracycline interactions have been implicated in the degradation of nucleic acids through free radical production [8].

Simple aminohexoses bind Cu(II) ions quite effectively, anchoring the metal ion to the amino nitrogen. When a hydroxyl group is properly located, and five- or six-membered chelate rings can be completed by a hydroxyl oxygen, then relatively strong complexes are formed with the metal ion bound to the amino nitrogen and protonated or deprotonated

Abbreviations: UV–vis, electronic absorption in the ultraviolet and visible range; CD, circular dichroism; EPR, electron paramagnetic resonance; CT, charge transfer; L, deprotonated tobramycin molecule.

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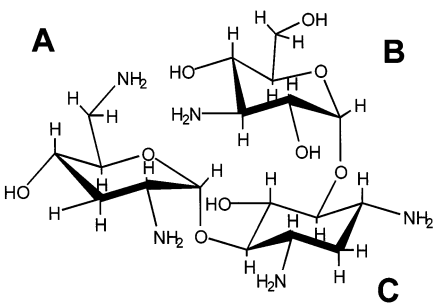
sugar oxygen(s) [9–12]. Aminoglycosidic antibiotics like kanamycin B [6] or amikacin [7] are much more sophisticated ligands by having several distinct potential binding sites. For example, kanamycin B binds Cu(II) ions through nitrogen and oxygen donor systems of two terminal aminosugar rings, while the central deoxystreptamine moiety does not participate in the metal ion coordination. Tobramycin (Scheme 1) is a close analogue of kanamycin B, differing only by the lack of the hydroxyl group at C-3 of the A ring. This difference may have a distinct impact on the binding ability of this antibiotic.

In this work, we present potentiometric and spectroscopic studies on Cu(II) coordination to tobramycin and the results are compared to those obtained for the other Cu(II)-aminoglycosidic antibiotic systems.

2. Experimental

Materials.—Tobramycin was used as obtained from Fluka, $CuCl_2$ and KNO_3 were obtained from Aldrich.

Potentiometric measurements.—Potentiometric titrations of tobramycin and its complexes with Cu(II) in the presence of 0.1 mol dm^{-3} KNO_3 were performed at 25 °C over the pH range 3–11.0 with a MOLSPIN automatic titrator and NaOH as a titrant. Changes in pH were monitored with a combined glass–calomel electrode (Russell) calibrated daily in hydrogen concentration by HNO_3 titrations [13]. Samples of 1.5 mL, ligand concentration of 10^{-3} mol dm^{-3} and ligand-to-metal molar ratios between 4:1 and 1:1 were used. The potentiometric data were analyzed using the SUPERQUAD program [14]. Standard deviations computed by SUPERQUAD refer to statistical errors only. They give, however, a good measure of the importance of a given species in solution.



Scheme 1. The molecule of tobramycin, presented with all amino groups deprotonated (L).

CD spectra.—Spectra were recorded at 25 °C on a Jasco J-600 spectropolarimeter over the range 190–750 nm, using 1 and 0.1 cm cuvettes. Samples of 1:1 and 2:1 ligand-to-metal molar ratios were used with Cu(II) concentration of 10^{-3} mol dm^{-3}. Spectra are 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.

Absorption spectra (UV–vis).—Spectra were recorded on a Beckman DU-650 spectrophotometer over the range 250–900 nm in 1 cm cuvettes using the same samples as those in CD measurements.

EPR spectra.—Spectra were recorded at 120 K on a Bruker ESP 300E spectrometer at the X-band frequency (9.3 GHz) using 1:2 ethanediol–water as the solvent, for metal and ligand concentrations similar to those used in the other spectroscopic measurements.

3. Results and discussion

Protonation constants of tobramycin.—The protonation constants obtained from potentiometric titrations are collected in Table 1. Five protonation constants correspond to five amino groups present in the molecule of tobramycin. The highly positive charge of the antibiotic molecule in weakly acidic solutions

Table 1
Protonation constants (log β and pK values) of tobramycin at 25 °C and $I = 0.1$ M (KNO_3)[a]

Species	Tobramycin		Kanamycin B	
	log β	pK	log β	pK
HL	9.289(1)	9.289	9.121	9.121
H_2L	17.676(1)	8.387	17.378	8.257
H_3L	25.389(1)	7.713	24.979	7.601
H_4L	32.453(1)	7.064	31.893	6.914
H_5L	38.124(1)	5.671	37.637	5.744

[a] Statistical errors on the last digit are shown in parentheses. Constants for kanamycin B [6] are also included.

(all five amino groups protonated) leads to a significant lowering of the pK value of one of them to 5.67, while the four other pK values are in the range 7.0–9.3, typical for aminosugars possessing similarly located amino groups [9–12,15]. This effect was seen previously for kanamycin B [6]. In general, the differences between the corresponding pK values for these two aminoglycosides are negligible (Table 1), as should be expected.

Cu^{2+} complexes of tobramycin.—Combined results of spectroscopic and potentiometric studies indicate that in this system only monomeric equimolar metal complexes are formed. According to potentiometric results, six complex species are formed over the pH range 4–11. They all have CuH_nL stoichiometry, with n varying from $+3$ to -2. The stability constants and the spectroscopic data for these complexes are presented in Table 2. The species distribution diagram, illustrating the formation of the complexes as a function of pH, is shown in Fig. 1.

Part A of Table 2 compares stability constants of complexes of tobramycin and kanamycin B. One can see that the overall coordination process is similar in both aminoglycosides. Also the spectroscopic data for tobramycin (Table 2B) indicate the presence of just three coordination modes, as with kanamycin B [6]. They involve pairs of stoichiometric species: $CuH_3L + CuH_2L$ for type I, $CuHL + CuL$ for type II, and $CuH_{-1}L + CuH_{-2}L$ for type III.

The coordination of a $\{NH_2, O^-\}$ donor set (type I) is well supported by the d–d

Table 2
A. Stability constants ($\log\beta$ and pK values) of Cu(II) complexes of tobramycin and kanamycin B [6] at 25 °C and $I = 0.1$ M (KNO_3)

Species	Tobramycin		Kanamycin B		ΔpK
	$\log\beta$[a]	pK	$\log\beta$[a]	pK	
CuH_3L	29.80(1)		28.85		
CuH_2L	23.45(1)	6.35	23.06	5.79	+0.56
$CuHL$	16.66(1)	6.79	16.45	6.61	+0.18
CuL	9.22(1)	7.44	8.81	7.66	−0.22
$CuH_{-1}L$	0.63(1)	8.59	0.70	8.11	+0.48
$CuH_{-2}L$	−9.31(1)	9.94	−8.56	9.26	+0.68

B. Spectroscopic characterization (CD, UV–vis, EPR) of Cu(II) complexes of tobramycin

Species	UV–vis[b]	CD[b]	EPR[c]	
	λ (ε)	λ ($\Delta\varepsilon$)	$g_{\|\|}$	$A_{\|\|}$
Complex I (CuH_3L and CuH_2L)	680 (43)[d]	650 (+0.15)[d] 280 (−0.50)[e,f]	2.30	163
Complex II ($CuHL$ and CuL)	608 (71)[d]	665 (+0.05)[d] 573 (−0.07)[d] 323 (−0.15)[e] 253 (+1.20)[f]	2.26	178
Complex III ($CuH_{-1}L$ and $CuH_{-2}L$)	612 (66)[d]	588 (−0.16)[d] 348 (−0.06)[e] 268 (−2.53)[f]	2.24	188

[a] $\beta(MH_nL) = [MH_nL]/[M][L][H^+]^n$, standard deviations on the last digit are given in parentheses.
[b] λ units are nm; ε and $\Delta\varepsilon$ units are $dm^3\ mol^{-1}\ cm^{-1}$.
[c] $A_{\|}$ units are Gauss.
[d] d–d electronic transitions of Cu(II) in a tetragonal complex.
[e] $O^- \rightarrow$ Cu(II) charge transfer band.
[f] $NH_2 \rightarrow$ Cu(II) charge transfer band.

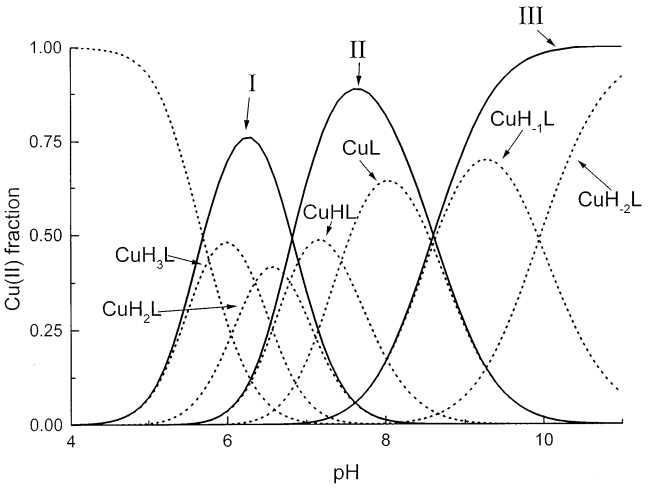


Fig. 1. A species distribution diagram of tobramycin (L) complexes of Cu(II) for concentrations used in spectroscopic studies (2×10^{-3} M (L), 10^{-3} M (Cu(II)). (---) stoichiometric species; (—) spectroscopic species.

transition below 700 nm (680 in UV–vis and 650 in CD, Fig. 2), its $\Delta\varepsilon$ magnitude above 0.1, and EPR parameters 'in between' the 1 N and 2 N coordination characteristics [9–12,16]. The spectroscopic data suggest that both CuH_3L and CuH_2L are 1 N complexes with the metal ion bound, most likely, to the amino group at the C-3 moiety of the C-ring, the same as with kanamycin B. Such binding is favored by the stabilizing interaction of the Cu(II) ion with a vicinal hydroxyl group [9,11,12]. However, a complex with this group protonated, typical for simple aminosugars, is not seen for tobramycin (and kanamycin B) because the hydroxyl deprotonation is facilitated by a high overall electrostatic charge of the complex molecule (4+ in CuH_3L). The proton dissociation from the CuH_3L complex occurs at the B-ring amino group. Despite their acidity, B-ring donors cannot participate in the binding together with C-ring donors, for sterical reasons [6,17]. The difference in pK of this event between tobramycin and kanamycin B (tobramycin higher by 0.56 log units, Table 2A) cannot be easily explained, and may indicate that the absence of the OH-3 group at the A-ring results in different conformational properties of tobramycin. This apparently small change strongly affects other properties of the complex, like solubility, which is very good for tobramycin, in contrast with kanamycin B [6]. Perhaps the A3-OH in kanamycin B is involved in association phenomena, which might explain the above mentioned difference of pK values.

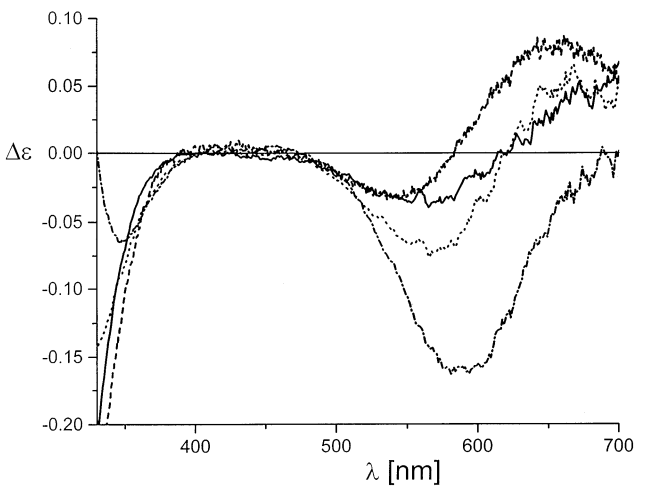


Fig. 2. CD spectra of Cu(II) complexes of tobramycin, (—) pH 6.5; (---) pH 7.3; (···) pH 8.1; (-·-·-) pH 9.4.

The formation of the type II of coordination of tobramycin correlates with the formation of CuHL. The spectra support the $\{2 \times NH_2, O^-\}$ binding mode. The pH of this event is very similar to that seen for kanamycin B, and supports the binding of the N-2 atom of ring-A. The formation of CuL occurs without the effect on Cu(II) binding, which is consistent with the deprotonation of the remaining B-ring amine. However, a characteristic charge transfer band at ca. 350 nm in the UV spectra, originating from the hydroxyl binding to Cu(II), which was prominent in the absorption spectra of kanamycin B [6], is absent from the spectra of tobramycin. This difference supports the notion of the participation of the protonated OH-3 of ring-A in the coordination of Cu(II) by kanamycin B. Tobramycin lacks this hydroxyl, and the second chelate ring has to be completed with the use of the OH-4 group (the A6 amine remains protonated). This group is less suitably located for coordination, forming a more relaxed and labile six-membered chelate ring. This flexibility effectively kills the CT band.

The same reason underlies the increase of the pK of formation of $CuH_{-1}L$ and the associated type III coordination by 0.48 log units. The spectra of type III are consistent with the $\{2 \times NH_2,\ 2 \times O^-\}$ coordination. The final deprotonation, to yield $CuH_{-2}L$,

does not affect the spectra, and thus pertains to the deprotonation of the A6 amine. This process is delayed by 0.68 log units compared to kanamycin B, indicating the importance of the conformation of the coordinated sugar ring for protonation phenomena.

4. Conclusion

Our study provides further evidence that the $\{NH_2, O^-\}$ chelate formation is preferred instead of a $\{NH_2, NH_2\}$ binding in streptamine-containing aminoglycosides. Both terminal aminosugar rings of tobramycin can participate in the binding, similarly to kanamycin B [6]. The comparison of these two aminoglycosides revealed the importance of the hydroxyl group in the position A3 to the complex formation.

Acknowledgements

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