Vanadyl Binding to Bleomycin

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Introduction

The bleomycins are largely employed in chemotherapy as antineoplastic agents in the treatment of a wide variety of carcinomas and lymphomas [1–4]. Furthermore these substances are receiving considerable attention as potential tumor scanning agents when complexed with an appropriate metal radioisotope [5]. The biological and pharmacological activity of these compounds must be related to the presence in the molecule of both metal-binding and DNA-interactive sites [6, 7]. Several results suggest that bleomycins utilize the bithiazole moiety for interacting with DNA [2, 3, 8–11], whereas the pyrimidine–imidazole–sugar region is involved in binding metal ions [12, 13]. In view of the relevant importance of the latter point in determining the biological activity of bleomycins, as well as its application in cancer radiodiagnosis, the coordination chemistry of bleomycins is currently the object of active research. Most investigations are dealing with the binding properties of 3d metal ions from iron through zinc [12, 13], but studies on bleomycin complexes of gallium, calcium and lanthanoids have also been reported [14, 15]. The vanadyl(IV) ion has been largely used as a spectroscopic probe to investigate the structure of biomolecules [16]. We report here evidence relative to the coordination of bleomycin to the oxovanadium(IV) cation.

Results and Discussion

Commercial bleomycin (BLM) is a mixture of two derivatives: BLM A_2 (70%) and BLM B_2 (30%). The two congeners differ in the nature of the substituents to the bithiazole moiety (Fig. 1): BLM A_2 contains the 3-aminopropylsulfonium residue in place of the agmatine residue of BLM B_2. Since it has been shown that the two congeners experience the same binding properties towards metal ions [12], in the present

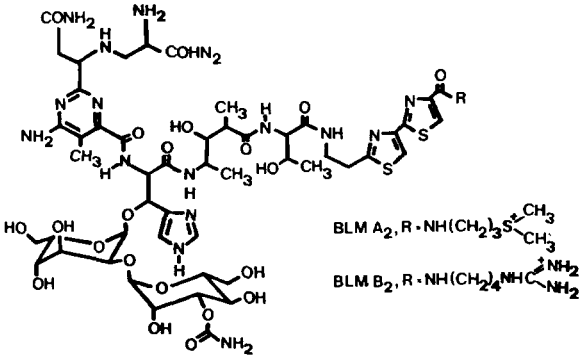

Fig. 1. The structures of the bleomycins A_2 and B_2.

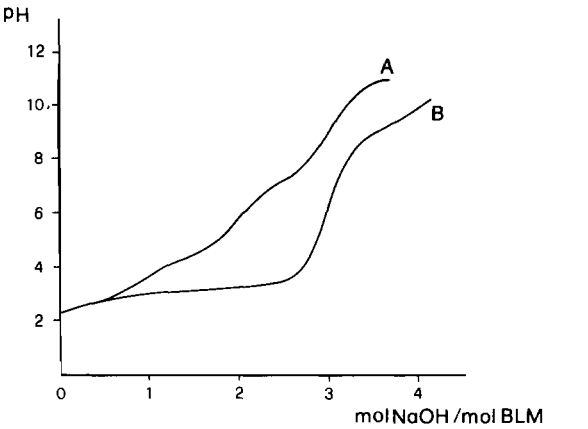

Fig. 2. Acid–base titration curves of aqueous solution containing BLM alone (A) and in the presence of VO^{2+} 1:1 (B).

investigation the clinical mixture was directly used, as is currently done when the coordination ability of this antibiotic is studied.

Potentiometric titration studies indicate that BLM behaves as triprotic acid in the pH range 2–11. Previous investigations [17] have attributed this result to deprotonation processes occurring at the diaminopropionamide residue and the imidazole group. When the titration is performed in the presence of vanadyl ions there is evidence of complex formation processes, as shown in Fig. 2. An additional equivalent of strong base is required in the titration because of the deprotonation process evidenced by the buffer zone in the pH range 9.5–10.5. Therefore a fourth acid–base equilibrium must be considered for the VO–BLM system,

The electronic spectra in the range 1000–500 nm show a pH dependent behaviour. The band at 745 nm, currently attributed to the $^2B_2 \rightarrow {}^2E$ transition in C_{4v} symmetry of the vanadyl ion [18], although not showing any significant shift in the pH range

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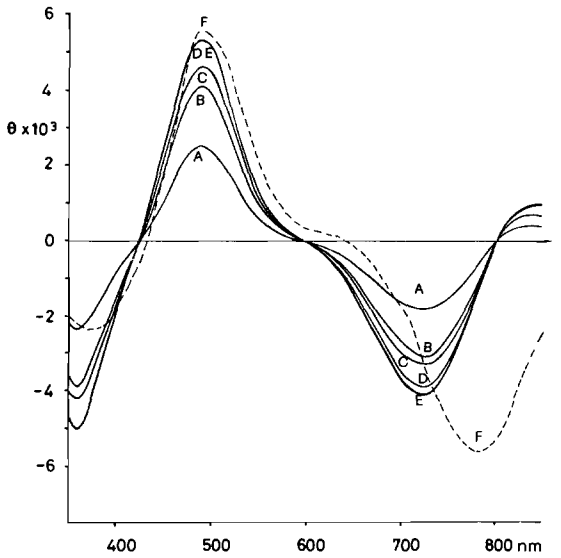

Fig. 3. CD spectra of aqueous solution containing VO(BLM) at pH = 2.6 (A), pH = 3.1 (B), pH = 3.5 (C), pH = 4.5 (D), pH = 5.5–7.7 (E) and pH = 11.0 (F).

2–7, exhibits a progressive increase in intensity on increasing pH. Typical values are ϵ = 26 at pH = 2.4 and ϵ = 51 at pH = 6.0. In alkaline medium a slight blue shift to 740 nm is observed, the molar absorption coefficient decreasing to 40. A shoulder at 485 nm can also be detected for acidic pH values, but at higher pH intense charge transfer bands cover this band.

Circular dichroism spectra in the range 1000–350 nm are in this respect a more rich source of information. Bands at 850, 725, 487 and 363 nm with a shoulder at 385 nm are detected in the pH range 2.5–7, thus supporting the existence of a direct interaction between the vanadyl ion and the optically active BLM. Whereas the positions of the absorption maxima do not show any appreciable change in this pH range, the band intensities exhibit the pH dependent behaviour shown in Fig. 3. Apparently this result indicates the existence of an equilibrium involving the formation of an optically active chromophore, the process being virtually complete at pH = 5.5. In alkaline medium a similar but not identical spectrum is obtained, thus indicating that a different chromophore exists as predominant species in this pH range.

Similar conclusions are reached when ESR spectra of glassy water solutions containing both the vanadyl ion and BLM are recorded. In the pH range 2.5–5.0 two patterns of resonance signals, whose intensities increase and decrease respectively depending on the actual pH values of the solution, are simultaneously detected. The spectral parameters of the resonating species, whose intensity increases with increasing

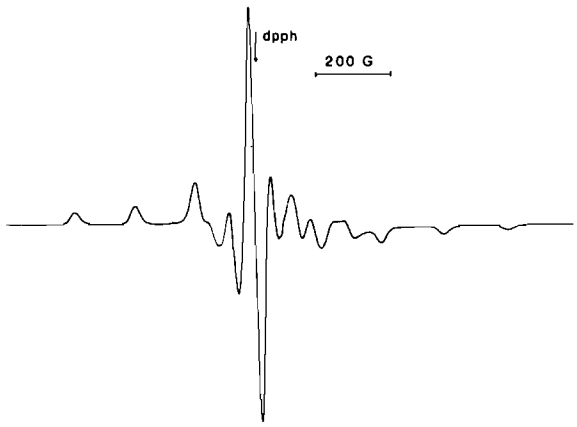

Fig. 4. ESR spectrum (X-band) at 90 K of an aqueous solution containing VO(BLM) at pH = 5.8.

pH, are $g_{\parallel}$ = 1.955, $g_{\perp}$ = 1.985, $A_{\parallel}$ = 165 × 10^{-4} cm^{-1}, $A_{\perp}$ = 60 × 10^{-4} cm^{-1}, whereas the second species, whose concentration decreases on increasing pH, is characterized by the spectral parameters typical for the vanadyl aquo-cation. Again, therefore, it is suggested that a complex formation between the vanadyl ion and BLM occurs. A typical spectrum at pH = 6 is reported in Fig. 4.

In agreement with the optical and CD spectra this VO–BLM derivative exists as the predominant species in solutions up to pH = 8. At higher pH values the spectra are slightly different, as shown by the comparison of the high field features. In any case the spectral parameters are very close to those at lower pH, showing eventually a small increase in $A_{\parallel}$, whose best fit value seems to be 167 × 10^{-4} cm^{-1}.

All the above spectral results show that BLM binds to oxovanadium(IV) in a pH dependent fashion The relative equilibria can be formulated as

$$VO^{2+} + BLM \rightleftharpoons VO(BLM)^{2+} \quad (1)$$

$$VO(BLM)_2 + OH^- \rightleftharpoons VO(H)_{-1}(BLM) \quad (2)$$

The titration data can be interpreted suggesting that both primary and secondary amino groups of the diaminopropionamide residue as well as the imidazole group are implicated in metal binding. The EPR data can be compared with those previously reported for vanadyl complexes, relating the $g_{\parallel}$ and $A_{\parallel}$ values. Following the correlation suggested by Holyk [16, 19], our present data are seen to be intermediate between those of $VO(N_2O_2)$ and $VO(N_4)$ chromophores, thus showing that at least two nitrogen donors are involved in coordination to the vanadyl ion. We suggest that actually there are 3 bound nitrogen atoms, *i.e.* the two amino groups of the diaminopropionamide residue and the imidazole group. The coordination of the last group is support-

ed by ^{1}H NMR data in which the imidazole proton signals are washed out by the interaction with the paramagnetic ion, whereas the bithiazole proton signals are only broadened by the same interaction. Since the ligand exchange in the vanadyl coordination sphere is slow on the ^{1}H NMR time scale it was not possible to perform selective broadening experiments.

A support to the hypothesis of a $VO(N_3O)$ chromophore comes from the additivity calculation of $A_\parallel$ [16, 19] which yields 166 X 10^{-4} cm^{-1}, in fair agreement with the experimental value. For N_2O_2 and N_4 chromophores the expected values are 171 and 161 X 10^{-4} cm^{-1} respectively.

The oxygen donor atom in the suggested chromophore is presumably provided by a water molecule. At higher pH this can be substituted by an hydroxo group in the coordination sphere. The pK_a value of *ca.* 10 associated with the loss of a proton in equilibrium (2) would then be reasonable for the deprotonation of the coordinated water molecule and it would compare well with the acid–base behaviour commonly observed for vanadyl complexes [20]. Also the minor changes in the ESR, CD, and electronic spectra are in agreement with this model.

The coordination of these VO(BLM) derivatives is different from that suggested for the iron, cobalt, nickel, copper, and zinc derivatives [12, 13], since for these ions MN_5 or MN_5O chromophores are believed to be formed. The 4-amino-pyrimidine and the amido group of the β-hydroxy-histidine, which are postulated to bind to the heavier metal ions, should not coordinate to the vanadyl ion.

Experimental

Bleomycin sulfate (Blenoxane) was a generous gift of Bristol Laboratories (Syracuse, New York). It consists of approximately 70% BLM A_2 and 30% BLM B_2. A molecular weight of 1550 was assumed. Solutions containing the oxovanadium(IV)–BLM derivatives were obtained by adding appropriate amounts of vanadyl sulfate solutions to *ca.* 10^{-2} *M* solutions of BLM. Since the VO(BLM) derivatives tend to be air oxidized in alkaline medium, all operations were carried out under oxygen free atmosphere.

Potentiometric titrations were carried out by the addition of 20 μl steps of NaOH *ca.* 0.03 *M* to 2 ml of a BLM solution *ca.* 10^{-2} M. The pH values were determined by using a potentiometer Orion Model 801 equipped with a glass/calomel microelectrode. The electronic spectra were recorded using a Cary 17D spectrophotometer. The CD spectra were carried out using a Jasco J-500 C spectropolarimeter. The EPR spectra were obtained with a Varian E-9 spectrometer, operating at 9 GHz, using DPPH as external standard. The sepctra were analyzed taking into account second order effects [21]. ^{1}H NMR spectra were recorded on a Varian CFT 20 spectrometer operating at 80 MHz using D_2O as solvent.

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