Zinc, Calcium and Magnesium Ion Coordination of Vinblastine and Vindoline

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Abstract

Zinc ions were shown by polarography to coordinate strongly to vinblastine and to vindoline. A calcium or magnesium ion excess liberated these alkaloids from their zinc complexes. The calcium ion coordination of vinblastine was investigated with a calcium ion-selective electrode.

The complex formation processes proved to be pH-independent in the pH range 3.5–5.0. The experimental results, together with others on analogous vincristine-containing systems, indicate that the metal ions are coordinated to non-protonating oxygen donor atoms on the vindoline moiety of vincristine or vinblastine.

Introduction

Vincristine and vinblastine are bis-indole alkaloids [1] with antitumor activity [2–7]. They consist of a vindoline and a catharantine moiety. Changes in the vindoline moiety resulted in unpredicted changes in biological activity, while modifications in the catharantine moiety led to compounds with somewhat lower oncological activities, but also with lower toxicities [8].

Metal ions (mainly calcium, magnesium and zinc) have been shown [9–13] to influence the bioactivity of bis-indole alkaloids. The latter compounds decompose readily in aqueous solution, but the decomposition is prevented by metal ion coordination [14]. These results drew the attention of coordination chemists to the role of the metal–ligand interactions in these systems. Earlier studies led to the development of stable aqueous injections of these drugs [14] and ongoing investigations may result in a better understanding of the complicated biochemical processes.

In a previous paper [15] we reported results from a study of the calcium, magnesium and zinc ion coordination of vincristine. The present paper details results from a similar investigation of vinblastine and vindoline.

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Experimental

Vinblastine sulfate and vindoline were obtained from Gedeon Richter Ltd., Budapest, and were stored in dark bottles in a refrigerator at −18 °C. For the potentiometric equilibrium studies, the sulfate ion in vinblastine was exchanged by chloride.

All the chemicals used were of analytical purity. The solutions were prepared by using twice distilled water. The measurements were performed at 25 ± 0.1 °C.

The polarograms were recorded on a Radiometer PO4 polarograph. The characteristic data of the capillary (m = 2.22 mg s^{-1}, t = 3.6 s) were determined with an open circuit. The potentiometric measurements were carried out by using the computer-controlled automatic titration device described previously [15]. For the measurement of calcium ion concentration, a calcium-selective membrane electrode was prepared according to ref. 16, and it was equipped with a Diaflo UM 05 dialysis membrane to prevent the strong adsorption of vinblastine at the electrode surface. A Ag/AgCl electrode served as reference.

Results and Discussion

Polarographic Measurements

Polarograms of zinc ions in the presence of different concentrations of vinblastine are shown in Fig. 1. It can be seen that, in solutions containing the ligand in excess, the zinc wave does not appear in the measurable potential range. (The cathodic limit of this range is the catalytic hydrogen wave of the ligand.) On decrease of the ligand concentration until the metal:ligand ratio becomes 1:1, the zinc wave appears in the polarogram, but in a distorted form due to the adsorption of vinblastine on the electrode surface. Under these circumstances, polarography proved the formation of the zinc complex in the system, but it could not be used to determine the stability constants in an equilibrium study. When calcium or magnesium chloride was added in increasing concentrations to a solution containing zinc and vinblastine in a molar ratio of 1:1, a 5000-

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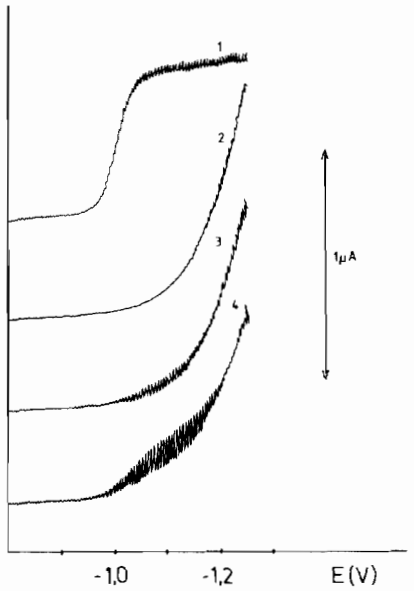

Fig. 1. Polarograms of zinc ions in the presence of vinblastine, $[Zn^{2+}] = 10^{-4}$ mol dm^{-3}, pH = 5.5, [vinblastine]: (1) 0.0 mol dm^{-3}; (2) 5×10^{-4} mol dm^{-3}; (3) 10^{-4} mol dm^{-3}; (4) 6×10^{-5} mol dm^{-3}.

fold excess of the former ions was found to liberate vinblastine from its zinc complex (Fig. 2), which is reflected by the appearance of the zinc wave in the polarogram.

Analogous results were obtained for the zinc–vindoline system. When vindoline was applied in excess, no polarographic zinc wave was obtained (Fig. 3). With a high concentration of calcium or magnesium ions, vindoline could be liberated from its zinc complex (Fig. 4). The polarographic behavior of both ligands proved to be pH-independent in the pH range 5.0–3.5.

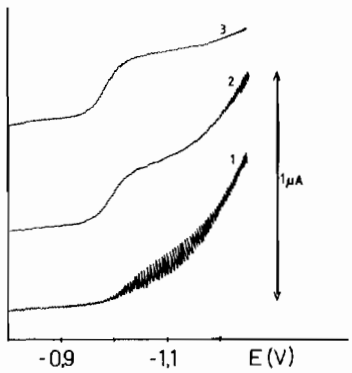

Fig. 2. Polarograms of the zinc–vinblastine complex in the presence of magnesium ions, (1) $[Zn^{2+}]$ = [vinblastine] = 5×10^{-5} mol dm^{-3}, $[Mg^{2+}] = 0.0$ mol dm^{-3}; (2) $[Zn^{2+}]$ = [vinblastine] = 5×10^{-5} mol dm^{-3}, $[Mg^{2+}] = 2.5$ mol dm^{-3}; (3) $[Zn^{2+}] = 5 \times 10^{-5}$ mol dm^{-3}, $[Mg^{2+}] = 2.5$ mol dm^{-3}, [vinblastine] = 0.0 mol dm^{-3}.

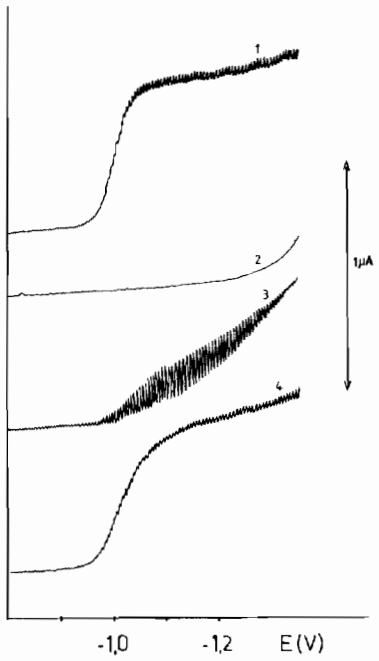

Fig. 3. Polarograms of zinc ions in the presence of vindoline, $[Zn^{2+}] = 10^{-4}$ mol dm^{-3}, pH = 5.0, [vindoline] = (1) 0.0 mol dm^{-3}, (2) 10^{-3} mol dm^{-3}, (3) 10^{-4} mol dm^{-3}, (4) 5×10^{-5} mol dm^{-3}.

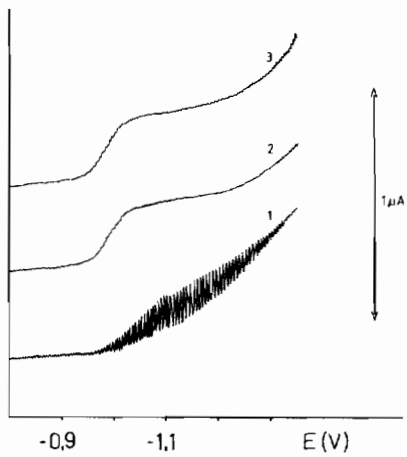

Fig. 4. Polarograms of the vindoline–zinc system in the presence of magnesium ions, (1) $[Zn^{2+}]$ = [vindoline] = 5×10^{-5} mol dm^{-3}, $[Mg^{2+}] = 0.0$ mol dm^{-3}; (2) system 1 in the presence of 2.5 mol dm^{-3} magnesium chloride; (3) polarogram of 5×10^{-5} mol dm^{-3} zinc in the presence of 2.5 mol dm^{-3} magnesium chloride.

All these measurements showed that zinc ions form stable complexes with vindoline and vinblastine, and that the stabilities of the complexes of calcium and magnesium ions with these ligands are several orders of magnitude lower than those of the

zinc complexes. The very similar polarographic behavior of vincristine [15], vinblastine and vindoline in the presence of zinc, calcium and magnesium ions suggests that the vindoline moiety of the bis-indole alkaloids is involved in complex formation.

Potentiometric Measurements

Calvin-type deprotonation titrations carried out in zinc-free and zinc ion-containing solutions of vinblastine and vindoline showed that the metal ions did not cause deprotonation of the alkaloids in the pH range 3–5.5 (below the pH of the hydrolysis of zinc). As the polarographic measurements indicated complex formation in this pH range, this process must be pH-independent, *i.e.* zinc ions coordinate to donor atoms which do not participate in protonation–deprotonation equilibria in the studied pH range. Analogous results were obtained with calcium ions, while with magnesium ions a small difference could be observed between the titration curves of the metal ion-free and magnesium-containing solutions of the ligands in the pH range 5.0–6.0 (Fig. 5). This slight difference indicates that, in the presence of such a high (about 150-fold) excess of magnesium ions, the predominant, pH-independent pathway is accompanied by some deprotonation of the ligand.

In order to characterize the basic donor atoms of vinblastine and vindoline, the protonation equilibrium constants ($\log K$) values were determined by potentiometric titration. The measurements reflected the presence of one basic donor atom for each ligand,

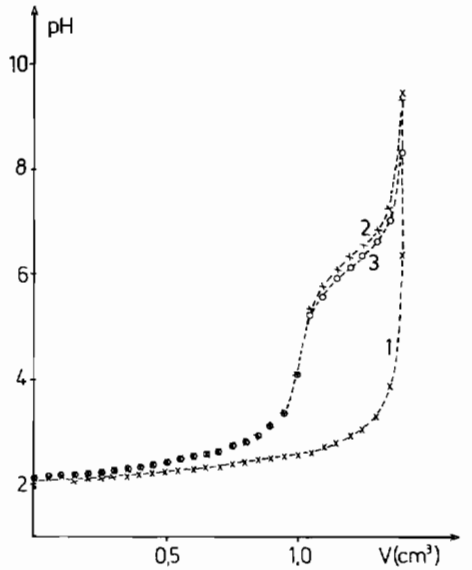

Fig. 5. Titration curve of vindoline in the presence of magnesium ions, (1) titration curve of 10 cm^3 0.0100 mol dm^{-3} HNO_3 with 0.100 mol dm^{-3} NaOH, I = 1 mol dm^{-3}; (2) 1 in the presence of 2.5×10^{-3} mol dm^{-3} vindoline; (3) 2 in the presence of 0.330 mol dm^{-3} magnesium nitrate.

with $\log K = 5.7$ for vinblastine and $\log K = 6.08$ for vindoline (at an ionic strength of 0.15 mol dm^{-3}). These values could be assigned to the nitrogen atoms of the molecules. From a comparison of these results with the analogous data for vincristine ($\log K_1 = 8.1$, $\log K_2 = 5.5$), it can be concluded that the more acidic nitrogen is situated in the vindoline moiety of the molecules. The deprotonation of the second nitrogen of vinblastine could not be investigated because the vinblastine precipitated from the solution after the loss of the first proton.

The pH-independence of the complex formation reactions meant that the Calvin-type deprotonation studies could not give any information concerning the stability and composition of the complexes formed. From the polarographic results, the stability ratios K_{Zn}:K_{Ca} and K_{Zn}:K_{Mg} could only be estimated. For a quantitative characterization of the systems, the formation constant of at least one of the complexes in question had to be determined directly. Since polarography could not be used for this purpose, metal ion-selective electrodes were selected for these studies.

Unfortunately, vinblastine and vindoline poisoned not only the commercially available metal ion-selective electrodes, but also the special PVC-based membrane which was successfully used in the calcium ion coordination study of vincristine. Therefore, the stability and composition of the calcium complex of vinblastine were determined by titrating the vinblastine solution (ionic strength 1.0 mol dm^{-3}, total vinblastine concentration 10^{-3} mol dm^{-3}) with a standard 0.1 mol dm^{-3} calcium chloride solution in the presence of a dialysis membrane-protected calcium-selective membrane electrode. A pair of typical calibration and titration curves is shown in Fig. 6. Quantitative evaluation of the data led to the equilibrium constant $\log K = 3.2 \pm 0.2$, essentially the same as was obtained for vincristine. (The large error in the constant is due to the above-mentioned experimental difficulties.) This means that the methyl substituent by which the two alkaloids differ does not change the stability or composition of the calcium complex. The analogous study on vindoline was unsuccessful because, due to its small molecular weight and size, vindoline passed through the pores of the dialysis membrane and poisoned the surface of the electrode.

Conclusions

All these investigations prove that, similarly to vincristine [15], vinblastine and vindoline form stable complexes with calcium, magnesium and zinc ions. The similar polarographic behavior of the three ligands in the presence of metal ions, the pH-independence of the complex formation reactions, the

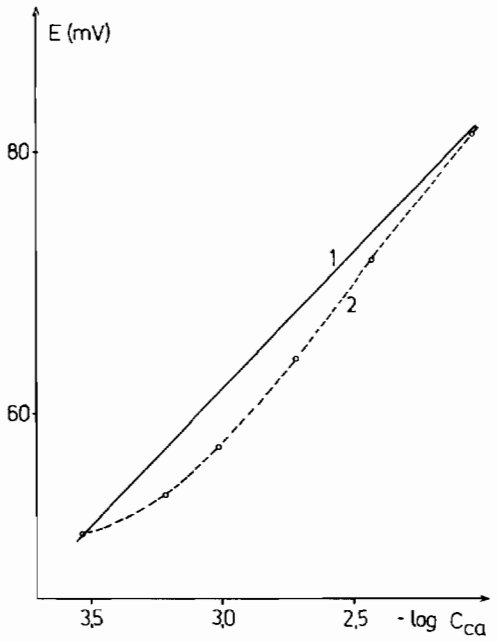

Fig. 6. Potentiometric titration of vinblastine, (1) calibration curve of the electrode; (2) titration curve of 10^{-3} mol dm^{-3} vinblastine with standard 0.1 mol dm^{-3} $CaCl_2$ solution.

identical formation constants of the calcium complexes of vincristine and vinblastine and structural considerations all indicate that non-protonating oxygen donor atoms in the vindoline moiety of the alkaloids act as coordination sites in the complex formation processes.

Acknowledgements

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