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Calcium, Magnesium and Zinc Ion Coordination Equilibria of Vincristine

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Abstract

The zinc ion coordination of vincristine was studied by polarography; the analogous calcium ion coordination process was studied potentiometrically by a calcium ion selective electrode. In both cases, complexes of 1:1 composition were formed. The formation constant of the calcium complex was found to be $\lg K = 3.27 \pm 0.1$. On the basis of the substitution of zinc in its vincristine complex by calcium and magnesium ions respectively, the ratio of the corresponding stability constants could be estimated as $K_{Zn}:K_{Ca}$ (and $K_{Zn}:K_{Mg}$) $\sim 10^5–3 \times 10^4$. The complex formation processes proved to be pH-independent in the pH range 3.4–5.5, indicating that the metal ions are coordinated by the unprotonated oxygen donor atoms of vincristine.

Introduction

Natural bis-indole alcaloids and their derivatives show anti-tumor activity and are used in medical treatment of cancer [1–3]. They are administered as intravenous injections or as additives in infusions *i.e.*, in aqueous solutions. Unfortunately, the stability of these compounds in aqueous media is low; therefore, the aqueous pharmaceutical preparations have to be prepared by dissolving the solid substance (eventually made by freeze-drying its solution) in sterilized water just before their medical use.

Bis-indole alcaloids contain nitrogen and oxygen donor atoms [4]. They are expected to form metal complexes by coordinating metal ions in solution. Since metal complexes of organic ligands generally show a higher stability against hydrolytic decomposition than the free organic ligand [5], the preparation of water-soluble metal complexes of bis-indole alcaloids seems to be a promising way to produce stable aqueous injections of these drugs [6]. Naturally, only metal ions which have no undesirable physiological effects can be used for this purpose.

Our aim was, therefore, the equilibrium study of the coordination of zinc, calcium and magnesium ions, respectively, by bis-indole alcaloids in aqueous solution. We chose vincristine as our first model ligand because of its high biological activity. The zinc ion coordination of this molecule was investigated by direct polarographic measurements; the calcium and magnesium coordination was reflected by the effect of the latter ions on the zinc ion binding equilibria. The use of a special calcium ion-selective electrode made the direct determination of the formation equilibrium constant of the calcium complex possible. The results are presented in this report.

Experimental

Vincristine sulfate was obtained from Gedeon Richter Ltd., Budapest, and was stored in dark bottles in a refrigerator at −18 °C. For the equilibrium studies the sulfate ion of the compound was quantitatively exchanged by chloride using an ion exchange procedure.

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

The polarograms were recorded on a Radiometer PO4 Polarograph. Characteristic data of the capillary ($m = 2.22$ mg s^{-1}; $t = 3.06$ s; $m^{2/3}t^{1/6} = 2.05$ mg$^{2/3}$s$^{-1/2}$) were determined with an open circuit.

For the polarographic measurements, sodium acetate was used as the base electrolyte in 0.15 mol dm^{-3} solutions of pH = 5.5. For removal of the oxygen content, pure nitrogen was bubbled through the solutions.

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In each set of measurements the zinc ion concentration was kept at constant value ($\sim 10^{-4}$ mol dm^{-3}) and the vincristine concentration was increased from 10^{-5} to 10^{-3} mol dm^{-3}. The pH dependence of the zinc vincristine interaction was studied in the pH range 3.4–5.5. The effect of the ionic strength on the polarographic behaviour of the system was investigated by repeating a series of measurements in solutions of high (1–10 mol dm^{-3}) sodium chloride concentrations. For comparison in each set of measurements, the polarogram of a vincristine-free zinc solution was also recorded.

For the study of the effect of calcium and magnesium ions on the polarographic behaviour of the zinc–vincristine system, increasing amounts (from 10^{-1} to 5 mol dm^{-3}) of $CaCl_2$ and $MgCl_2$, respectively, were added to solutions of constant and equal zinc ion and vincristine concentrations (each 10^{-4} mol dm^{-3}).

For the potentiometric equilibrium measurements, a computer-controlled online automatic titration device was constructed. A Radelkis OP-208/1 type mV- and pH-meter served for the measurement of the EMF data. (Accuracy ±0.1 mV and ±0.001 pH). The standard solution was added by a Radelkis OP-930/1 type automatic burette (in 0.002 cm^3 or greater portions). The two devices were connected by a home-made interface to a Sinclair ZX-81 type microcomputer of 16 K RAM capacity. A 8255 type programmable peripheral interface was used for the transfer of the BCD signals of the mV-meter to the computer and for the transfer of the command signal of the computer to the mV-meter and burette. Programs written in BASIC language were made for operating the automatic apparatus. The computer not only operated the device, insured the addition of the standard solution in varying quantities and varying time intervals, made sure that only the data characteristic for the equilibrium state were reported, but also performed the online evaluation of the experimental data and the printing and representation of the results on a display and on the SEIKOSHA 50-s type printer.

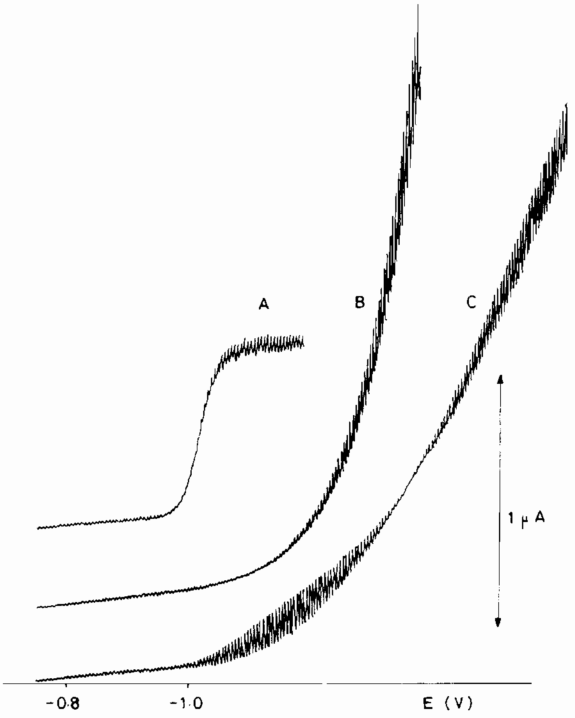

Fig. 1. Polarograms of zinc ions in the presence of vincristine. $[Zn^{2+}] = 10^{-4}$ mol dm^{-3}; pH = 5.5. [vincristine] = A, 0.0; B, 5×10^{-4} mol dm^{-3}; C, 10^{-4} mol dm^{-3}.

For the measurements of the change in the free calcium ion concentration due to the complex formation process, a calcium selective membrane electrode was prepared according to ref. 7. Calcium chloride of 0.01 mol dm^{-3} concentration served as internal standard solution. An Ag/AgCl electrode was used as reference. The 0.1 mol dm^{-3} calcium chloride standard solution was controlled by complexometric titration.

All the potentiometric measurements were performed in solutions of a total ionic strength of 1 mol dm^{-3}.

Results and Discussion

Polarographic Measurements

The study of the effect of vincristine on the polarogram of zinc ions in isotonic aqueous solution (ionic strength 0.15 mol dm^{-3}) shows that an equivalent amount of vincristine (molar ratio 1:1; total concentration 10^{-4} mol dm^{-3}) results in the disappearance of the zinc wave. The presence of zinc is reflected only by some oscillation on the corresponding part of the polarogram (Fig. 1, curve C) which disappears with increasing vincristine concentration (curve B). In solutions of lower ligand concentration, the zinc wave appears but shows some distortion caused presumably by the adsorption of the organic ligand on the mercury surface. The polarographic behaviour of the system does not show any pH-dependence in the pH range 3.4–5.5.

When adding calcium or magnesium chloride to the previous solution (containing zinc and vincristine in a molar ratio of 1:1), an extremely great excess ($\sim$3 mol dm^{-3}) of the mentioned salts is needed for liberating the zinc ions from the vincristine complex (Figs. 2 and 3).

The polarographic study of zinc ion coordination of vincristine was repeated in a 10 mol dm^{-3} sodium chloride solution, which hindered the adsorption of the organic molecule on the electrode surface. The zinc wave appeared with a half-wave potential value that was 35–40 mV more negative than zinc ions show in a vincristine-free solution of otherwise

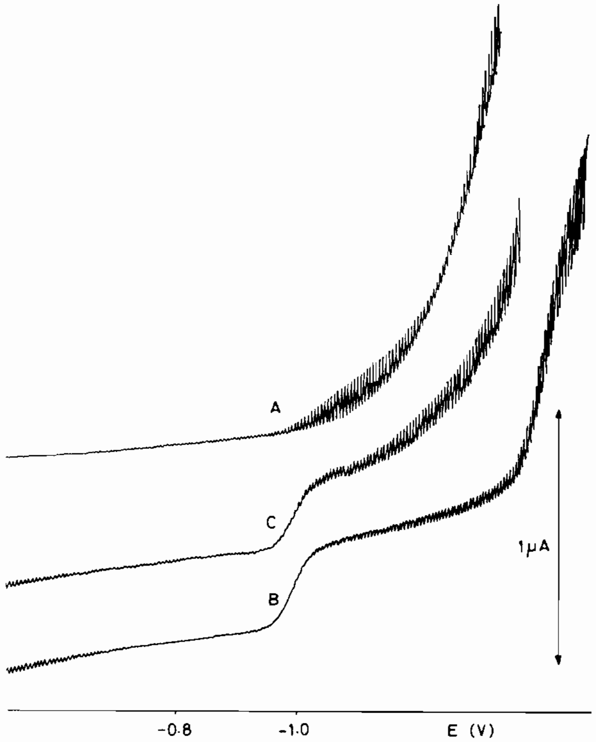

Fig. 2. Polarograms of zinc–vincristine in the presence of calcium chloride. A, $[Zn^{2+}]$ = [vincristine] = 6.25×10^{-5} mol dm^{-3}; B, A in the presence of 3.12 mol dm^{-3} $CaCl_2$; C, $[Zn^{2+}] = 6.25 \times 10^{-5}$ mol dm^{-3}, $[CaCl_2]$ = 3.12 mol dm^{-3}.

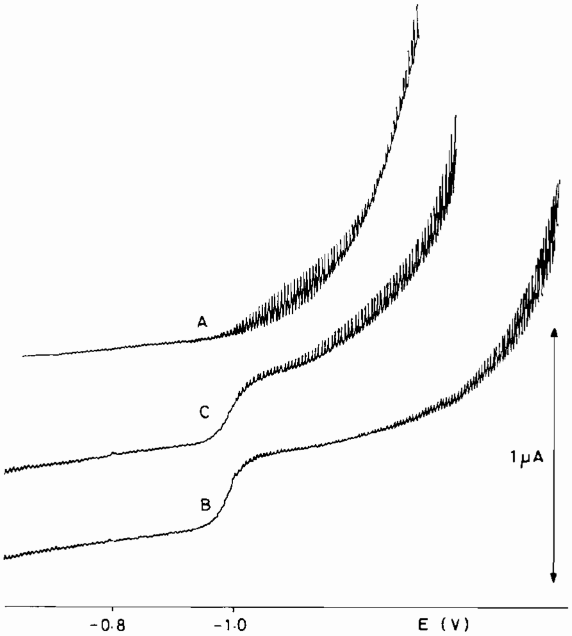

Fig. 3. Polarograms of zinc–vincristine in the presence of magnesium chloride. A, $[Zn^{2+}]$ = [vincristine] = 6.25×10^{-5} mol dm^{-3}; B, A in the presence of 3.12 mol dm^{-3} $MgCl_2$; C, $[Zn^{2+}] = 6.25 \times 10^{-5}$ mol dm^{-3}, $[MgCl_2]$ = 3.12 mol dm^{-3}.

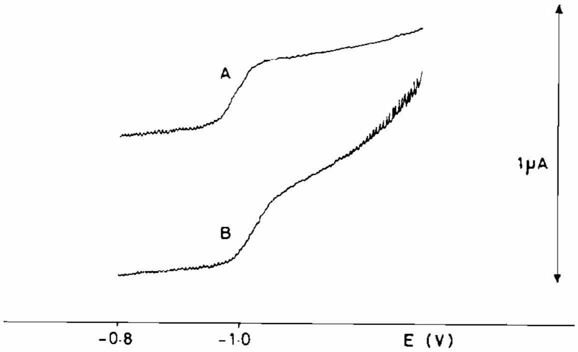

Fig. 4. Polarograms of zinc ions and zinc–vincristine in 10 mol dm^{-3} sodium chloride solution. A, $[Zn^{2+}] = 6.25 \times 10^{-5}$ mol dm^{-3}; B, $[Zn^{2+}]$ = [vincristine] = 6.25×10^{-5} mol dm^{-3}.

identical composition (Fig. 4). The log-plot analysis indicated that the reduction of zinc bound in the complex is an irreversible electrode process.

All these measurements showed that zinc ions form a complex of high stability with vincristine in aqueous solutions. The stability constant of the complex could not be determined from the polarographic data because of the distorted form of the zinc wave. On the basis of the excess needed for the substitution of zinc with calcium or magnesium ions in the vincristine complex, the ratio of the corresponding stability constants could be estimated as $K_{Zn}:K_{Ca}$ (and $K_{Zn}:K_{Mg}$) $\sim 10^5$–3×10^4 (supposing complexes of 1:1 metal:vincristine ratio).

Potentiometric Measurements

First, Calvin-type deprotonation titrations were performed for the potentiometric study of the interaction between zinc ions and vincristine. Comparing the titration curves of zinc-ion-free and zinc-ion-containing vincristine solutions, no deprotonation was observed in the pH range 3.0–5.6 (below the pH of the hydrolysis of zinc). The polarographic study of the system proved unambiguously the formation of zinc complexes; the potentiometric results indicate that the process is pH-independent. The zinc ions are coordinated to donor atoms unprotonated in the studied pH range. The analogous investigation of calcium and magnesium vincristine systems led to analogous results. The coordination of metal ions did not cause the deprotonation of vincristine in solutions where the formation of the complexes was well reflected by polarographic measurements.

The basicity of the donor atoms of vincristine was characterized by determining their protonation constants from pH-metric titration curves. The results showed the presence of two basic groups with pK values 5.50 ± 0.1 and 8.3 ± 0.2, which can be assigned to the nitrogen donor atoms of the molecule. Both are protonated in the studied pH-range. Vincristine contains, besides the nitrogens, the following functional groups which contain oxygen donor atoms: two hydroxy, one methoxy, one acetoxy, two methyl ester and one formyl group. Since these are not protonated in the studied pH-range, we assume that the metal ions are coordinated in a pH-independent process by some of these oxygens.

Because of the pH-independence of these complex formation reactions, the Calvin-type deprotonation studies naturally could not give information on the processes and on the corresponding stability constants. The polarographic studies led only to the ratios of $K_{Zn}:K_{Ca}$ and $K_{Zn}:K_{Mg}$, respectively. Therefore, for the characterization of the systems, the formation constant of at least one of the mentioned complexes had to be determined directly using a metal ion selective electrode.

Our preliminary investigations showed that the commercial ion-selective electrodes used for the measurement of zinc, calcium and magnesium ions, respectively, are poisoned by vincristine. The calcium ion selective electrode prepared according to the prescription in ref. 7 and used in our previous investigation of the calcium ion coordination of heparin [8] proved to be suitable for the measurement of the calcium ion activity in the presence of vincristine. Therefore, this electrode was used in the equilibrium studies reported here.

The composition of the calcium complex and the corresponding formation equilibrium constant were determined from the data of the potentiometric titrations of vincristine with a 0.1 mol dm^{-3} calcium chloride standard solution. (Ionic strengh 1.0 mol dm^{-3}, total vincristine concentration: 10^{-3} mol dm^{-3}). Since the complex formation process proved to be a time reaction, a computer-operated automatic potentiometric device was used for collecting the EMF data as a function of the total calcium ion concentration, which assured that only data corresponding to equilibrium conditions were recorded. The calibration of the calcium electrode was performed before and after each titration of vincristine. The agreement of the two independent calibration curves proved the reliable function of the electrode. A pair of typical calibration and vincristine titration curves is shown in Fig. 5. The Bjerrum formation curve calculated from the potentiometric data is presented in Fig. 6. It can be seen that in the concentration range studied, only one calcium vincristine complex of 1:1 composition is formed. The quantitative evaluation of the data led to the equilibrium constant:

$$\lg K = 3.27 \pm 0.1$$

Using this constant the computer simulated the $\bar{n} - \lg[Ca^{2+}]$ curve seen in Fig. 6. The agreement between the points calculated from the primary experimental data and the computer simulated curve prove the correctness of the results.

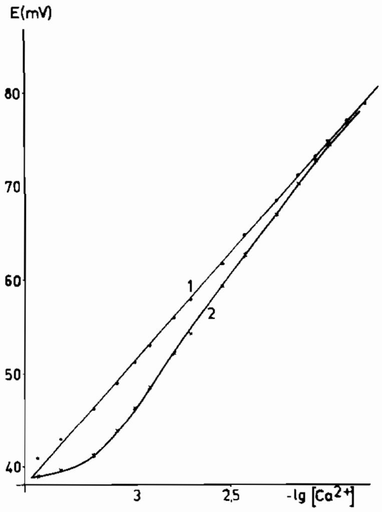

Fig. 5. Potentiometric titration of vincristine. (1) Calibration curve of the electrode; (2) Titration curve of 10^{-3} mol dm^{-3} vincristine solution with 0.1 mol dm^{-3} $CaCl_2$ standard solution.

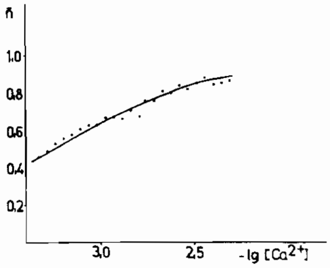

Fig. 6. The Bjerrum formation curve of the calcium–vincristine complex. The points are experimental values; the full line was calculated by using the stability constant of lg K = 3.27 value.

Similarly to the previous polarographic investigations and in agreement with the deprotonation studies, the potentiometric calcium ion coordination measurements reflected no pH-dependence in the pH-range 3.4–5.5.

Conclusion

All these investigations prove that in aqueous solutions vincristine coordinates calcium, magnesium and zinc ions, respectively. The equilibrium studies cannot give direct information on the binding sites of such ambidentate ligands. On the basis of the pH-independence of the process, however, one can conclude that the basic nitrogens do not participate in the coordination. From the structural model of vincristine (Fig. 7) one may suggest that acetoxy, formyl and methyl ester oxygens of the molecule are situated according to symmetry favourable for

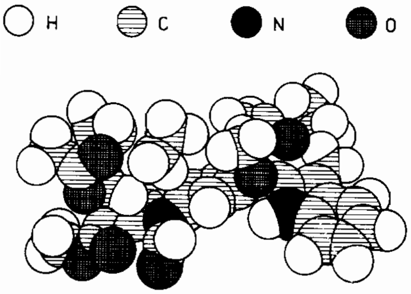

Fig. 7. The structural model of vincristine.

the coordination of the mentioned ions. For the unambiguous assignment of the coordination sites, ^{1}H NMR and ^{13}C NMR studies of the system are in progress. The results will be published elsewhere.

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