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The study of complexation of copper(II) ions with ampicillin: II. Dynamics of reactions

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Summary

In the presence of ampicillin copper(II) ions form different coordination compounds. Their formation and the properties of particular species were examined by means of UV, Vis and IR spectrometry. The dynamics of complex formation was studied. Firstly, an unstable blue-violet coordination compound with an absorption maximum at 590 nm was formed, later being transformed into a more stable compound. Hydrolysis of the β-lactam group is promoted by copper(II) and/or hydroxide ions and as a result the ampicillin ligand (L) is transformed into ampicilloic acid (L*). Consequently, the coordination compounds $[CuL]^+$, CuL_2, $[CuL_3]^-$ are changed to $[CuL^*]^+$, CuL_2^* and $[CuL_3^*]^-$, respectively.

Introduction

Antibiotics can form coordinative compounds with metal ions. The formation of these usually slightly soluble compounds can exert an influence on the antibacterial activity of the parent antibiotic and can contribute to some of the side effects observed with antibiotic therapy, e.g., neurological symptoms, gall bladder colics, etc. (Silber and D'Angelo, 1985; Kucers and McBennet, 1988). Additionally, because antibiotics are synthesized in metal ion-containing microbiological systems the uncontrolled presence of metal ions can have not only an inhibitor effect but also a beneficial influence on biosynthesis (Helmut, 1985).

The interactions in the copper(II) ions-ampicillin system at equilibrium conditions were studied by electrochemical and spectroscopic method in previous work (Sher et al., 1993). It was reported that two aqueous soluble coordination compounds and one precipitate had been formed depending on the molar ratio of reactants in the solution. It is known that penicillins undergo hydrolysis in alkaline aqueous solutions resulting in the formation of the corresponding penicilloic acids (Rapson and Bird, 1963), the rate of hydrolysis depending on the pH of the solution. Also,

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the presence of some metal ions in the solution, e.g., copper(II) ions, promotes hydrolysis (Cressman et al., 1966; Niebergal et al., 1966; Davies and Abraham, 1974; Dideberg et al., 1980; Tomida and Schwartz, 1983; Page, 1984). The purpose of this investigation was to study the dynamics of reactions between copper(II) ions and ampicillin as a function of pH, metal ions and ligand concentration as well as their molar ratio composition.

Experimental

Materials

Ampicillin preparations in sodium salt and acidic forms were used in the investigation. Ampicilloic acid was prepared by hydrolysis of ampicillin at pH 12 for 30 min at room temperature in the manner described by Rapson and Bird (1963). The solutions were prepared just before performing the experiment. All reagents were of analytical grade purity. The solutions with molar metal-to-ligand ratios of 10:1 to 1:10 were prepared by pouring together aqueous solutions of ampicillin or ampicilloic acid and Cu(II) ion solution.

Apparatus and procedures

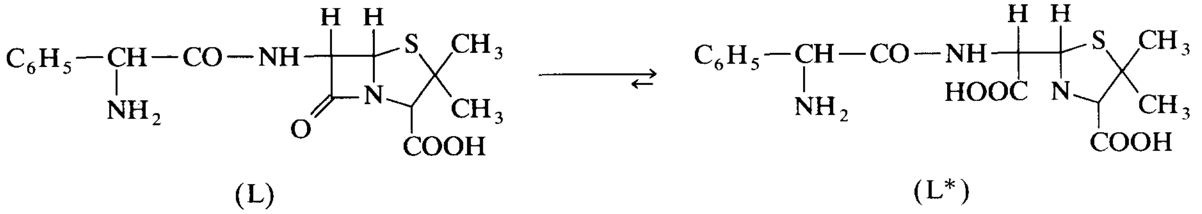
The copper content was determined using a 2280 Perkin Elmer AAS spectrometer, whereas UV and Vis spectra were recorded on an M40 Specord, Carl Zeiss spectrophotometer and IR spectra on a 727B Perkin Elmer infrared spectrophotometer. KBr pellets were prepared from solid precipitates or sample residues obtained after rapid evaporation and drying (air stream, room temperature) of sample solutions.

Results and Discussion

The reports of the hydrolysis of the β-lactam group of penicillins reported in the literature were confirmed by our results. Moreover, it was established that a knowledge of the dynamics of the processes taking in the presence of metal ions is necessary if the interactions involving the formation of coordination compounds with penicillins are to be elucidated.

At first the dynamics of ampicillin hydrolysis in solution lacking metal ions was studied. The UV spectrum of ampicillin changes rapidly at pH 12 while in the pH 6 acidic solution the shape of the curve remained unaltered even after 7 days. The same behavior was observed for ampicillin in acid as well as in sodium salt form at pH 12. The band at 1780 cm^{-1} corresponding to the β-lactam group was present in the IR spectrum of the residue after evaporation of the ampicillin solution at pH 6, but was absent in the sample obtained from the solution of pH 12 after 60 min. Accordingly, we can conclude that the hydrolysis of ampicillin to ampicilloic acid via the β-lactam group took place in alkaline media. The corresponding reaction could be represented as shown in Scheme 1.

The dynamics of interactions of copper(II) ions with ampicillin was studied at molar ratios of 1:1, 1:2 and 1:3 in order to obtain evidence on the behavior of compounds which are present in these concentration ranges (Sher et al., 1993). First, an unstable blue-violet coordination compound with



Scheme 1.

an absorption maximum at 590 nm is formed due to bonding of donor electrons and the amino group of ampicillin with the acceptor orbitals of copper(II) ions. The evidence for this conclusion is based on the similarity of the spectra of the compound discussed and of $[Cu(NH_3)_4]^{2+}$, the maxima of which occur at 590 and 600 nm, respectively. Then, rearrangement to a more stable coordination compound occurs in accordance with the metal-to-ligand molar ratio. Thus, in the region of molar ratio 1:1, the peak at 590 nm disappears with time and that at 755 nm increases. The band at 1780 cm^{-1} corresponding to the β-lactam group was also observed in the IR spectrum of the above residue. This suggests that the coordination compound $[CuL]^+$ was present in the solution. However, the band at 1780 cm^{-1} was absent in the spectrum of the solution after 48 h. Consequently, ampicillin ligand in the compound was transformed to ampicilloic acid as a result of hydrolysis of the β-lactam group and the coordination compound $[CuL^*]^+$ was formed in the system.

As in the previous case, the violet color also appears when pouring together Cu(II) and ampicillin solutions in a molar ratio of 1:2. However, when the color fades, the solution becomes turbid due to the formation of a precipitate. The IR spectra of ampicillin and its insoluble compounds were recorded. Between 1800 and 1300 cm^{-1}, C=O vibrations of the β-lactam, amide and carboxylic acid groups are expected. The bands at 1780 and 1680 cm^{-1} can be assigned to the C=O and C-O vibrations, respectively. These assignments were made on the basis of literature data (Nakamoto, 1978; Ogorevc et al., 1985). In the spectrum of ampicillin bands at 1780 cm^{-1} (β-lactam), 1680 cm^{-1} (amide), 1605 and 1400 cm^{-1} (carboxylic acid) were observed, while in that of the precipitate obtained at equilibrium, the bands of the amide and two carboxylic groups were shifted to 1660, 1610 and 1390 cm^{-1}, respectively, the band of β-lactam at 1780 cm^{-1} being absent. Thus, it can be supposed that the precipitate with ligand lacking the β-lactam group was present under these experimental conditions. However, the peak at 1780 cm^{-1} was observed in the spectrum of the precipitate investigated immediately after pouring together copper(II) ion and ligand solutions. Therefore, it can be assumed that the compound contained the β-lactam group. This compound can subsequently be transformed to the compound without the β-lactam group, i.e., the first compound contained ampicillin as a ligand and the second one ampicilloic acid.

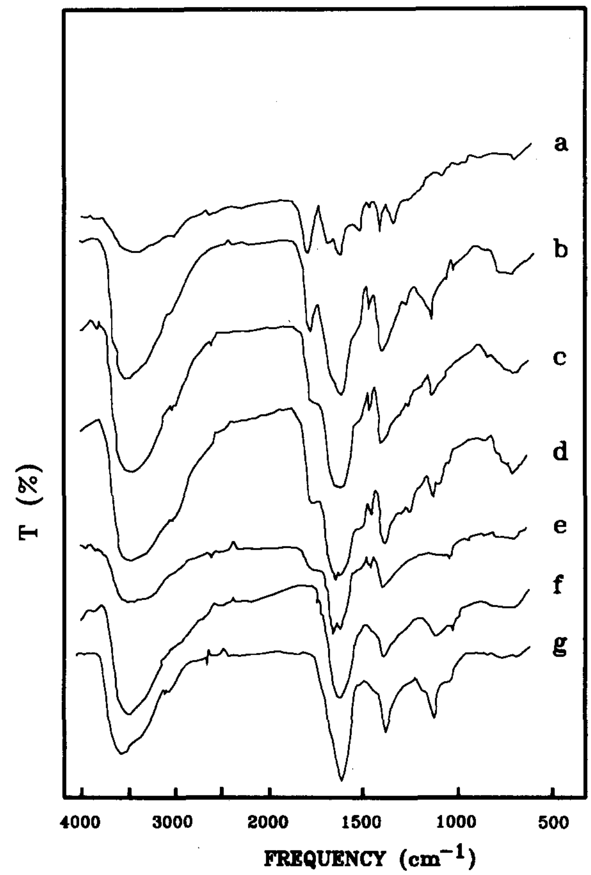


Fig. 1. The IR spectra of ampicillin (a) and precipitates with copper(II) ions (b–f): immediately (b), after 24 h (c), 48 h (d), 72 h (e), 150 h (f); and ampicilloic acid with copper(II) ions (g).

The band at 1780 cm^{-1} was present in the spectrum of freshly prepared precipitate which was stable for a considerable period if dried. The band related to the β-lactam group was observed in the IR spectrum for a period of 3 months. However, the spectrum of the precipitate which was retained in the solution did not exhibit this peak, indicating that hydrolysis of the β-lactam group took place with in a few days (Fig. 1). The rate of hydrolysis increased sharply with rising pH of the solution. Additional proof for these suppositions was the observation that the spectrum of the precipitate after pouring together Cu(II) ion and penicilloic acid solutions was analogous to that of the hydrolyzed precipitate.

On the basis of all the observations mentioned above, it can be concluded that a precipitate, CuL_2 (I), is formed in the presence of the corresponding concentrations of copper(II) ion and ampicillin in aqueous solutions, which is subsequently transformed to CuL_2^* (I*) according to Scheme 2. It can also be supposed that the precipitate CuL_2^* is formed owing to the interactions of Cu(II) ions with the carboxyl group of hydrolized ampicillin. Additional evidence that the carboxyl group is involved in the bonding between copper(II) ions and penicillins is the absence of precipitate formation when the carboxyl group is blocked, e.g., in the case of bacampicilin.

Furthermore, when pouring together the Cu(II) ion and ampicillin solutions at a molar ratio of 1 : 3 or at higher concentrations of ligand, a blue-violet coordination compound with an absorption maximum at 590 nm was initially formed. As in the former cases, the compound was transformed to the more stable compound $[CuL]_3^-$. Thereafter, hydrolysis of the β-lactam group in this compound took place. The system under equilibrium conditions is thus characterized by the presence of only $[CuL_3^*]^-$. The same substance is also formed when excess of ligand solution is added to the precipitate CuL_2 or CuL_2^*. All the above can be confirmed by the similarity in the measured IR spectra with those of substances obtained by pouring together copper(II) ion and ampicilloic acid solutions at the same molar metal-to-ligand ratio.

On the basis of the measurements performed, the following reactions can be supposed to occur:

$$Cu^{2+} + L^- \rightleftharpoons [CuL]^+$$

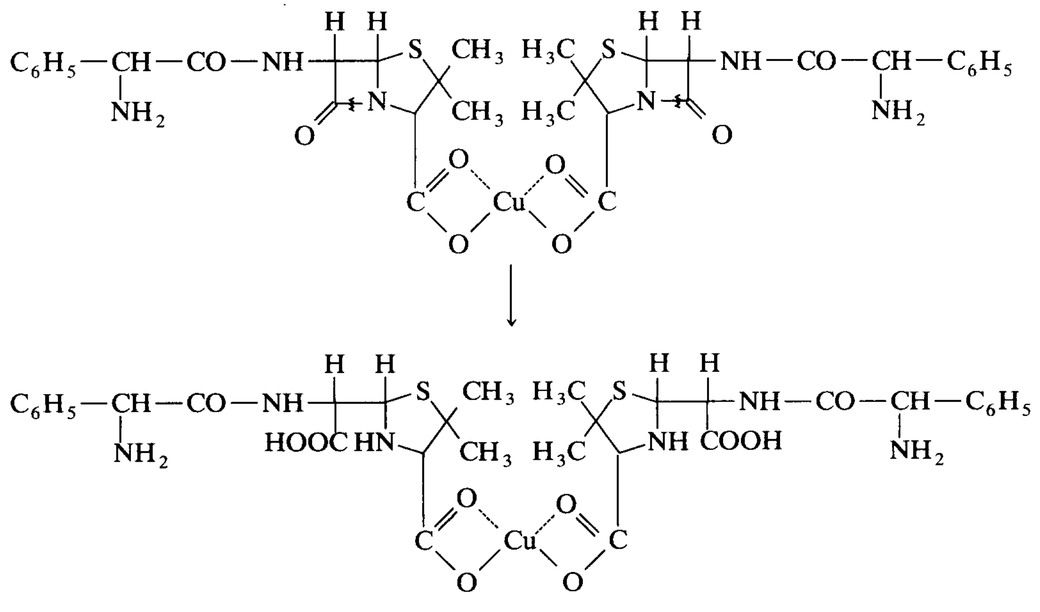
$$Cu^{2+} + 2L^- \rightleftharpoons CuL_2$$

$$[CuL]^+ + L^- \rightleftharpoons CuL_2$$

$$L^- \rightleftharpoons L^{*-}$$

$$[CuL]^+ \xrightleftharpoons{HOH,\ Cu^{2+}} [CuL^*]^+$$

$$Cu^{2+} + L^{*-} \rightleftharpoons [CuL^*]^+$$



(I)

(I*)

Scheme 2.

$$\mathrm{CuL_2} \xrightleftharpoons{\text{HOH, Cu}^{2+}} \mathrm{CuL_2^*}$$

$$[\mathrm{CuL^*}]^+ + \mathrm{L^{*-}} \xrightleftharpoons{\quad} \mathrm{CuL_2^*}$$

$$\mathrm{Cu^{2+}} + 2\mathrm{L^{*-}} \xrightleftharpoons{\quad} \mathrm{CuL_2^*}$$

$$\mathrm{CuL_2} + \mathrm{L^-} \xrightleftharpoons{\quad} [\mathrm{CuL_3}]^-$$

$$[\mathrm{CuL}]^+ + 2\mathrm{L^-} \xrightleftharpoons{\quad} [\mathrm{CuL_3}]^-$$

$$\mathrm{Cu^{2+}} + 3\mathrm{L^-} \xrightleftharpoons{\quad} [\mathrm{CuL_3}]^-$$

$$[\mathrm{CuL_3}]^- \xrightleftharpoons{\text{HOH, Cu}^{2}} [\mathrm{CuL_3^*}]^-$$

$$\mathrm{CuL_2} + \mathrm{L^{*-}} \xrightleftharpoons{\text{HOH, Cu}^{2+}} [\mathrm{CuL_3^*}]^-$$

$$\mathrm{CuL_2^*} + \mathrm{L^{*-}} \xrightleftharpoons{\quad} [\mathrm{CuL_3^*}]^-$$

$$[\mathrm{CuL^*}]^+ + 2\mathrm{L^{*-}} \xrightleftharpoons{\quad} [\mathrm{CuL_3^*}]^-$$

$$\mathrm{Cu^{2+}} + 3\mathrm{L^{*-}} \xrightleftharpoons{\quad} [\mathrm{CuL_3^*}]^-$$

Acknowledgement

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References

Cressman, W.A., Sugita, E.T., Doluisio, J.T. and Niebergall, P.J., Complexation of penicillins and penicilloic acids by cupric ion. *J. Pharm. Pharmacol.,* 18 (1966) 801–815.

Davies, R.B. and Abraham, E.P., Metal cofactor requirements of β-lactamase II. *Biochem. J.,* 143 (1974) 129–135.

Dideberg, O., Joris, B. and Frere, J.M., The exocellular dd-carboxypeptidase of *Streptomyces albus* G: a metallo ($\mathrm{Zn^{2+}}$) enzyme. *FEBS Lett.,* 117 (1980) 215–218.

Helmut, S., *Metal Ions in Biological Systems,* Vol. 19, Academic Press, New York, 1985.

Kucers, A. and McBennet, N., *The Use of Antibiotics,* Medical Books, London, 1988.

Nakamoto, K., *The Infrared and Raman Spectra of Inorganic and Coordination Compounds,* Wiley, New York, 1978.

Niebergall, P.J., Hussar, D.A., Grosman, W.A., Sugita, E.T. and Doluisio, J.T., Metal binding tendencies of various antibiotics. *J. Pharm. Pharmacol.,* 18 (1966) 729–736.

Ogorevc, B., Hudnik, V., Gomiščcek, S., Smyth, M.R. and Vos, J.G., A spectroscopic and polarographic investigation of the complexation of cefazolin with copper(II) ions. *Inorg. Chim. Acta,* 108 (1985) 13–16.

Page, M.I., The mechanism of reactions of β-lactam antibiotics, *Acc. Chem. Res.,* 17 (1984) 144–150.

Rapson, H.D.C. and Bird, A.E., Ionization constants of some penicillins and of their alkaline and penicillinase hydrolysis products. *J. Pharm. Pharmacol.,* 15 (1963) 227–231.

Sher, A., Veber, M., Marolt-Gomiščcek, M. and Gomiščcek, S., Complexation of copper(II) ions with ampicillin: I. Spectroscopic and electrochemical investigation of interactions under equilibrium conditions. *Int. J. Pharm.,* 90 (1993) 181–186.

Silber, T.J. and D'Angelo L., Psychosis and leisures following the injection of penicillin G procaine, *AJDC,* 139 (1985) 335–341.

Tomida, H. and Schwartz, M.A., Further studies on the catalysis of hydrolysis and aminolysis of benzyl penicillin by metal ions. *J. Pharm. Sci.,* 72 (1983) 331–339.