Monatshefte für Chemie 122, 131–138 (1991)

Monatshefte für Chemie
Chemical Monthly

A Study of Polyamine Complex Formation with H^+, Cu(II), Zn(II), Pb(II), and Mg(II) in Aqueous Solution

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Summary. The composition and stability of the following biogenic amine complexes have been investigated: 1,4-diaminobutane *(Put)*, 4-azaoctane-1,8-diamine *(Spd)*, 4,9-diazadodecan-1, 12-diamine *(Spm)* as well as homologues such as 1,3-diaminopropane *(Put3)*, 4-azaheptane-1, 7-diamine *(Spd3,3)* and 4,8-diazaundecan-1,11-diamine *(Spm3,3,3)* with H^+, Cu(II), Zn(II), Pb(II) and Mg(II). A potentiometric method was used. The VIS technique enabled the determination of coordination mode in copper/amine systems. It was found that Mg(II) does not form coordination compounds with any of the studied polyamines in solution. An increase in the concentration of ligand and metal was found to result in a stronger tendency towards the formation of protonated compounds accompanied by a decrease in the concentration of hydroxocomplexes. At physiological *pH* (7.4) an increase in the concentration of protonated compounds by approximately 15% was observed within the ligand concentration range from 0.001 mol dm^{-3} to 0.0001 mol dm^{-3} at a Cu(II) concentration of 0.000177 mol dm^{-3}.

Keywords. Biogenic amines; Complexes; Stability constants.

Untersuchungen zur Komplexbildung von Polyaminen mit H^+, Cu(II), Zn(II), Pb(II) und Mg(II) in wäßriger Lösung

Zusammenfassung. Anhand einer Analyse von potentiometrischen Daten wurden Zusammensetzung und Beständigkeit folgender biogener Aminkomplexe untersucht: 1,4-Diaminobutan *(Put)*, 4-Azaoktan-1,8-diamin *(Spd)*, 4,9-Diazadodekan-1,12-diamin *(Spm)*, sowie auch deren Homologen 1,3-Diaminopropan *(Put3)*, 4-Azaheptan-1,7-diamin *(Spd3,3)* und 4,8-Diazaundekan-1,11-diamin *(Spm3,3,3)* mit H^+, Cu(II), Zn(II), Pb(II) und Mg(II). Mit Hilfe der VIS-Technik wurde die Koordinationsweise in Kupfer/Amin-Systemen bestimmt. Es wurde festgestellt, daß Mg(II) keine Koordinationsverbindungen mit den untersuchten Polyaminen bildet. Eine höhere Konzentration von Ligand und Metall führte zu stärkerer Tendenz der Bildung protonierter Verbindungen, wobei die Konzentration von Hydroxokomplexen kleiner wurde. Bei physiologischem *pH* (7.4) wurde im Bereich der Ligand-Konzentration von 0.001 mol dm^{-3} bis 0.0001 mol dm^{-3} bei einer Cu(II)-Konzentration von 0.000177 mol dm^{-3} ein Anstieg der Konzentration protonierter Verbindungen um etwa 15% beobachtet.

Introduction

Biogenic amines, i.e. putrescine-*Put*, spermidyne-*Spd* and spermine-*Spm* [1–6], are believed to be among compounds that play a crucial role in the processes of living organisms. However, evaluations of the function of amines in biochemical processes have so far yielded information about the consequences of the presence (or absence) of amines in the cells, but have failed to explain the mechanism of interactions and structural changes resulting from the reaction amine/biomolecule [7–12]. The presence of such metal cations as for example Cu^{2+}, Zn^{2+} and Mg^{2+} in the cells makes it necessary to consider the existence of their complexes with amines as well as interactions of these compounds with biomolecules, e.g. DNA and RNA, in the investigations.

We believe that the amount of protonation and stability constants of biogenic amine complexes and their homologues with metals may be an important factor to explain the mechanism of celluar regulation at the molecular level. Literature data concerning polyamine complexes with metals differ with respect to the values of stability constants as well as the composition of the occurring coordination compounds [3, 6, 13–18]. In the present study the potentiometric and the spectral method were used to determine unequivocally the composition and stability constants of biogenic amine complexes: 1,4-diaminobutane *(Put)*, 4-azaoctane-1,8-diamine *(Spd)*, 4,9-diazadodecan-1,12-diamine *(Spm)* as well as their homologues 1,3-diaminopropane *(Put3)*, 4-azaheptane-1,7-diamine *(Spd3,3)*, 4,8-diazaundecane-1,11-diamine *(Spm3,3,3)* with H^+, Cu(II), Pb(II) and Mg(II).

Experimental

The ligands were used in the form of hydrochlorides: *Put3* · 2HCl, *Put* · 2HCl, *Spd* · 3HCl, *Spd3,3 Spm* · 4HCl, *Spm3,3,3* · 4HCl. The hydrochlorides of *Put*, *Spd3,3*, *Spd*, *Spm3,3,3*, and *Spm* were obtained from the Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznań, the hydrochloride *Put3* was obtained from 1,3-diaminopropane (Sigma Chemical Company) after reaction with HCl in methanol. Elementary analyses of the hydrochlorides was carried out; for all compounds the results (%N, %C, %H) were in agreement with calculated values (±0.5%). Cu^{2+}, Mg^{2+}, Pb^{2+} and Zn^{2+} were used in the form of perchlorates, their preparation was described earlier [19]. The concentrations of Cu^{2+}, Zn^{2+}, Pb^{2+} and Mg^{2+} in the solutions were determined complexometrically [19]. Potentiometric measurements were carried out with a Radiometer pHM-26c *pH*-meter with ABU-1c autoburette. The GK-2401c electrode was used after previous calibration [20]. Concentrations of *Put3*, *Spd3,3*, *Spd*, *Spm3,3,3*, and *Spm* ranged from 0.0006–0.003 mol dm^{-3} (at metal to ligand ratio from 1 : 4.33 to 1 : 6.55), whereas the concentration of *Put* was 0.001 mol dm^{-3} (at a ratio of metal to Put from 1 : 6.0 to 1 : 8.5). Titration was carried out under argon, ionic strength $\mu = 0.1$ ($NaClO_4$), $T = 20^\circ C \pm 1$, the titrant was a CO_2-free solution of NaOH (0.0464 mol dm^{-3}). Stability constants and composition of the complexes formed in the system were calculated using the programs SCOGS [21] and MINIQUAD [22], the distribution of forms was determined with the program HALTAFALL [23]. Calculations were carried out on an EMC-RIAD-32 computer and an Amstrad 1512 microcomputer. VIS measurements of the investigated amines with Cu^{2+} were performed on an Shimadzu UV-160 spectrometer for concentrations of *Put* 0.003 mol dm^{-3}, Cu^{2+} 0.00052 mol dm^{-3}, and of *Put3*, *Spd*, *Spm3,3,3* 0.00225 mol dm^{-3}, and Cu^{2+} 0.00104 mol dm^{-3}.

Results and Discussion

The complex equilibria of *Put3*, *Put*, *Spd3,3*, *Spd*, *Spm3,3,3*, and *Spm* with Cu(II), Zn(II), Pb(II), and Mg(II) were studied using the potentiometric method. The protonation constants are listed in Table 1. For the investigated series of amines, $\triangle H$ of the protonation process is a result of changes in the interaction of charges ($-NH^+$) or dipoles ($-NH_2$), which become more negative with increasing length of

Table 1. Stability constants [log β] of polyamine complexes with H^+, Cu(II), Pb(II), Zn(II), and Mg(II). (In the calculations the forms $Cu(OH)^+$ and $Cu_2(OH)_2^{2+}$ [24] have been included. For the models where these compounds were present no convergence could be observed by means of the programs SCOGS and MINIQUAD)

		Put3	*Put*	*Spd3,3*	*Spd*	*Spm3,3,3*	*Spm*
H^+	HL	10.70 (2)	10.83 (1)	10.48 (2)	10.97 (1)	10.36 (3)	10.91 (2)
	H_2L	19.64 (2)	20.51 (2)	20.22 (2)	21.03 (1)	20.38 (3)	21.28 (2)
	H_3L	–	–	28.03 (3)	29.61 (2)	29.01 (3)	30.39 (3)
	H_4L	–	–	–	–	36.39 (4)	38.67 (3)
Cu^{2+}	ML	9.68 (12)	–	13.71 (3)	11.70 (6)	16.36 (3)	14.66 (3)
	ML_2	16.79 (26)	–	18.48 (24)	17.13 (12)	–	–
	ML_3	21.66 (32)	–	–	–	–	–
	MHL	15.78 (14)	–	18.87 (18)	18.91 (7)	–	–
	$ML(HL)$	–	–	–	–	–	29.32 (14)
	MH_2L	–	–	–	–	27.49 (7)	27.63 (26)
	$ML(H_2L)$	–	–	–	–	42.03 (7)	–
	MLOH	2.04 (11)	–	3.14 ((5)	2.90 (11)	–	–
	ML_2OH	–	0.065 (6)	–	6.72 (12)	–	–
Pb^{2+}	ML	6.29 (11)	?	–	–	–	–
	MHL	14.61 (16)		15.82 (14)	15.98 (12)	–	–
	MH_2L	–		–	–	25.90 (12)	25.98 (7)
Zn^{2+}	ML	–		–	–	8.20 (7)	
	MHL	14.96 (12)	?	–	14.89 (11)	–	?
	$ML(HL)$	20.22 (11)		22.83 (8)	22.29 (12)	–	
Mg^{2+}		–		–	–	–	–

the chain between individual donor atoms. The general basicity $\Sigma_i k_i$ (in comparison of compounds with an equal number of nitrogen atoms) was *Put3* > *Put*, *Spd* > *Spd3,3* and *Spm* > *Spm3,3,3*. The largest difference in the $\Sigma_i k_i$ value was determined for the last pair of compounds; this is due to the fact that in *Spm* the two -NH groups are separated by four methylene groups; consequently they should be treated as two independent diamine systems.

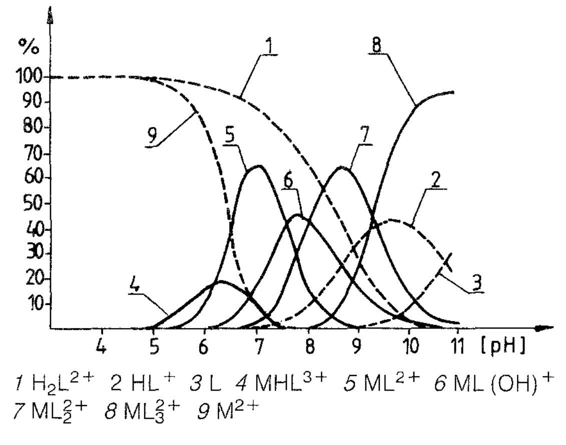

Fig. 1. Distribution diagram for the system *Put3*/Cu(II); $C_{Put3} = 0.001$ mol dm^{-3}, $C_{Cu(II)} = 0.00017$ mol dm^{-3}

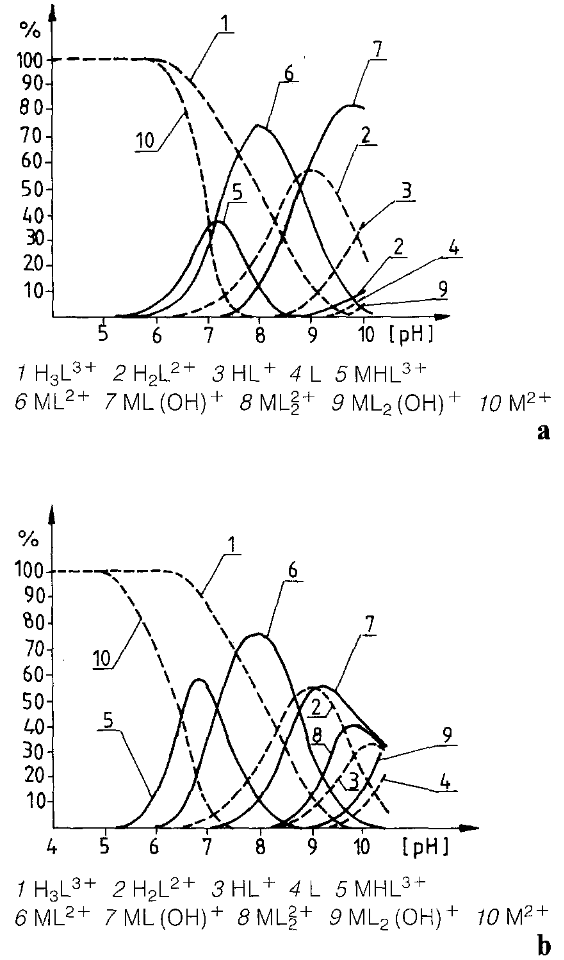

Fig. 2. Distribution diagrams for the systems **a** *Spd*/Cu(II) $C_{Spd} = 0.00075 \text{ mol dm}^{-3}$, $C_{Cu(II)} = 0.00017 \text{ mol dm}^{-3}$; **b** *Spd*/Cu(II)$C_{Spd} = 0.0075 \text{ mol dm}^{-3}$, $C_{Cu(II)} = 0.0017 \text{ mol dm}^{-3}$

The most stable coordination compounds were determined for Cu(II) (Tab. 1). The complexing process of Cu(II) in the system Put3/Cu(II) begins at a *pH* of approximately 5.0 (lowest value), where *M*H*L* is formed (Fig. 1). Its formation begins at a lower pH than that of the protonated compound *M*H*L* *Spd* with Cu(II) (beginning at pH of ca. 5.5 Fig. 2 a), despite the fact that in the latter system β_{MHL} is higher (Tab. 1). However, taking into consideration the competitive participation of the proton the equilibrium constants of the reaction should be compared:

$$M + H_2L \rightleftharpoons MHL + H^+ \ (\log \beta^{M}_{MHL} = 0.80) \ ,$$

$$M + H_3L \rightleftharpoons MHL + 2H^+ \ (\log \beta^{M}_{MHL} = 0.65) \ .$$

The equilibrium constant K^{M}_{MHL} is higher for CuH*Put3* and this explains the earlier formation of the protonated compound with the participation of *Put3*. The *M*H*L* complex is also formed by *Spd3,3*. In order to determine the mode of coordination in the complexes *M*H*L* *Spd* and *Spd3,3* their equilibrium constants K^{M}_{MHL} should be compared. This constant expresses the stability of the bonding of the ligand by the metal and is 8.34 for Spd/Cu(II) and 8.39 for Spd3,3/Cu(II). The two values are similar, which shows that the same mode of coordination is present in both

Table 2. Spectral data of the Cu(II)/amine systems

	Put3		*Put*		*Spd*		*Spd3,3*		*Spm*		*Spm3,3,3*	
Species	*pH*	λ [nm]	*pH*	λ [nm]	*pH*	λ [nm]	*pH*	λ [nm]	*pH*	λ [nm]	*pH*	λ [nm]
$M\mathrm{H}L$			7.00	705	6.25	646	5.70	630.5				
ML	7.02	646			8.01	617	8.00	616	8.00	561	7.00	595[a]
$ML(\mathrm{OH})$					9.75	606						
$ML(\mathrm{H}L)$									10.2	557		
$ML(\mathrm{H}_2L)$											10.10	409
ML_2	8.70	575					10.60	585				
$ML_2\mathrm{OH}$			10.40	630.5	10.5	577						
			11.60	601.0								
ML_3	10.5	559										

[a] This form occurs in the mixture with other complex compounds

complexes. In the complex $M\mathrm{H}L$ *Spd3,3* there is only the possibility of the formation of a 6-membered ring and since for both *Spd3,3* and *Spd* the same mode of coordination has been determined, a 6-membered chelate ring must be present in both complexes. The stabilities of the CuL complexes of the investigated polyamines show the order Cu*Put3* < Cu*Spd* < Cu*Spd3,3* < Cu*Spm* < Cu*Spm3,3,3* depending on the number of 6-membered and 7-membered rings formed [14, 16, 25].

The complexing behaviour of the system *Put*/metal has been controversial. Some authors even suggest that this ligand does not form complexes with metals [2, 6, 13, 16–18]. Our potentiometric investigations have shown a complexing of *Put* with Cu(II), resulting in the formation of the compound $ML_2\mathrm{OH}$ with $\log\beta = 0.065$ at $pH > 9$. This complex practically binds all the metals above a *pH* of 10.5 [26].

The analysis of VIS spectra yielded additional information about the mode of coordination of Cu(II) with the investigated ligands (Tab. 2). The spectra were recorded for *pH* ranges in which individual complex forms are dominant (the

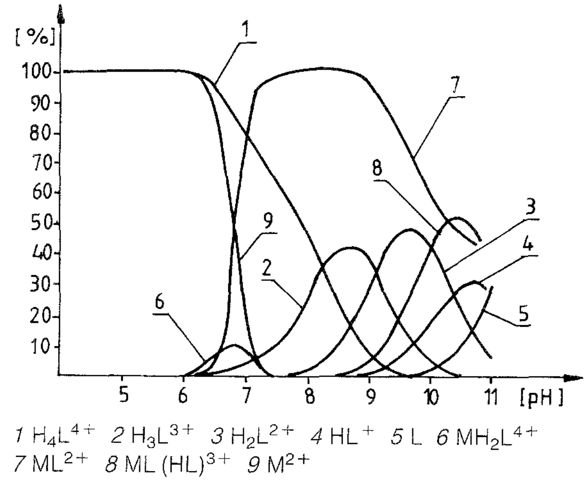

Fig. 3. Distribution diagram for the system *Spm*/Cu(II); $C_{Spm} = 0.00075$ mol dm^{-3}, $C_{\mathrm{Cu(II)}} = 0.00012$ mol dm^{-3}

adequate *pH* values were obtained from the analysis of distribution curves). For example in the system *Spm*/Cu(II) the *ML* complex is dominant in the *pH* range from approximately 7.0 to 9.0, and at $pH = 8.0$ it is the only compound of copper with the ligand (Fig. 3). The wavelength corresponding to this complex is $\lambda_{max} = 561$ nm and correlates with four coordinated nitrogen atoms [6, 14]. This structure has been confirmed in crystallographic studies of spermine – Cu(II) perchlorate which demonstrates a square planar coordination of Cu(II) by four nitrogen atoms of the same spermine molecule [27]. The maximum absorption wavelength for Cu*Spd3,3*, Cu*Spd* and Cu*Put3* shifts towards longer waves, thus reflecting the weakening of the ligand field due to the participation of an ever smaller number of nitrogen atoms in the coordination. Therefore it may be claimed that the ever weaker transition energy (reflected in λ_{max}) for the complexes Cu*Spm*, Cu*Spd3,3*, Cu*Spd* and Cu*Put3* corresponds to the coordination by 4, 3 and 2 nitrogens atoms, respectively. In the spectrum of *Put*/Cu(II) at $pH = 10.4$, where practically all the metal is bound in ML_2OH, λ_{max} is 630 nm; this wavelength corresponds to 3 coordinated nitrogens. Along with increasing *pH* λ_{max} shifts to shorter wavelength, reaching at $pH = 11.6$ the value $\lambda_{max} = 601$ nm which signifies the participation of 4 nitrogen atoms in the coordination. According to the results of potentiometric studies, at $pH \sim 9.0$ there is only one compound of putrescine with Cu(II) present CuL_2OH. Thus the difference in λ_{max} demonstrates a different coordination mode that is dependent on *pH*; i.e. at $pH \sim 10.0$ in CuL_2OH one ligand forms a 7-membered ring, the other is coordinated by one nitrogen atom. Along with the increase in *pH* a second 7-membered ring closes [20]. The above conclusions are in agreement with results of potentiometric studies which show that above *pH* 11.0 putrescine occurs mostly in the completely deprotonated form (Tab. 1) which facilitates the formation of two chelate rings with the participation of all donor atoms. In the VIS spectrum of *Put*/Cu(II) at *pH* 7.0 there is an absorption maximum at $\lambda_{max} = 705$ nm. This wavelength indicates the participation of one nitrogen atom in the coordination, hence a protonated *M*H*L* compound of *Put* with Cu(II) is present in the system. The presence of this complex was not confirmed in potentiometric studies of the binary system *Put*/Cu(II). The value of the stability constant of the protonated compound of *Put* with Cu(II) was determined on mixed complexes of putrescine and adenosine with Cu(II): it is $\log\beta = 15.83(17)$. An additional coordinating factor (adenosine) prevents here the premature precipitation of metal ions enabling measurements in a wider *pH* range. In contrary to Cu(II), Mg(II) does not form coordination compounds with any of the investigated amines. For Pb(II) and Zn(II) the complexes have been found to be weaker than their analogues with Cu(II) (Tab. 1). The complexing process of Pb(II) and Zn(II) with the investigated polyamines begins only in a basic environment. At lower *pH* values these metals do not compete with the proton. The equilibria constants $\log K^{M}_{MHK}$ for the complexes *M*H*L* *Put3* with Zn(II) are 4.26 and 4.32. The values of the constants are similar and since there is no chelate ring in the protonated compound *Put3* with Zn(II) (one nitrogen atom participates in the coordination, the other one is protonated) the conclusion may be drawn that also in the *M*H*L* complex of *Spd* with Zn(II) there is no chelate ring. It is, however, present in PbH*Spd*. The $\log K^{M}_{MHK}$ value for this compound is 5.32 which is higher in comparison with the analogous value 3.91 for PbH*Put3* (Tab. 1). The above conclusions are also confirmed by the comparison of the $\log\beta$ values of the discussed forms of Zn(II) and Pb(II) with

$\log\beta$ of analogous compounds with Cu(II), whereby CuH*Spd* is characterized by a chelate ring ($\log\beta_{\mathrm{CuH}Put3} < \log\beta_{\mathrm{CuH}Spd}$). A similar relationship is characteristic for protonated compounds of Pb(II) ($\log\beta_{\mathrm{PbH}Put3} < \log\beta_{\mathrm{PbH}Spd}$ and $\log\beta_{\mathrm{ZnH}Put3} = \log\beta_{\mathrm{ZnH}Spd}$, (Table 1). An unequivocal evaluation of complexing equilibria in the system *Put3*/Pb(II), *Put*/Zn(II) and *Spm*/Zn(II) is impossible due to the appearance of a precipitate immediately after the beginning of the reaction.

In the protonated compounds of Cu(II), Pb(II) and Zn(II), a weakening of the labile proton bond is observed. The protonation constant $\log K^{M}_{M\mathrm{H}K}$ corresponds to the equilibrium constant of the reaction $ML + \mathrm{H} \rightleftharpoons M\mathrm{H}L$; for complexes of $M\mathrm{H}L$ *Put3* with Cu(II) and Pb(II) it is 6.10 and 8.32, respectively (the $\log K^{\mathrm{H}}_{\mathrm{H}L}$ for the ligand alone is 8.94). This effect has been observed for all investigated systems.

In the interaction with nucleic acid molecules the presence of amine molecules as well as their complexes with metals should be taken into consideration. The nature of these interactions depends on *pH* conditions, amine concentration, metal concentration and the dielectric constant of the system. It was found that an increase of ligand and metal concentration results in a significant increase in the tendency towards the appearance of protonated forms at the expense of the decreasing number of hydroxo complexes (Fig. 2a, b). On the other hand, a decrease in the concentration of these components leads to a relative increase in the concentration of the hydroxo complexes with a simultaneous decrease in concentration of protonated forms. It should be noted that protonated complexes are less "rigid" systems in relation to forms with the participation of a greater number of chelate rings and this behaviour changes the interaction potential of a complex of this type towards other biomolecules.

Acknowledgement

This work was financially supported by the Polish Academy of Sciences, project RPBP 01.6.

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Received May 23, 1990. Accepted June 18, 1990