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Potentiometric Studies on the Formation Equilibria of Binary and Ternary Complexes of Cobalt(II) with Adenosine-5′-mono-, -di-, and -triphosphate and some Biologically Important Polybasic Oxygen Acids

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Summary. Potentiometric equilibrium measurements have been made for the interaction of adenosine-5′-mono-, -di-, and -triphosphate, and Co(II) with biologically important secondary ligands (malic, maleic, succinic, tartaric, citric, and oxalic acid). The formation of various 1:1:1 mixed ligand complexes inferred from the potentiometric titration curves. Initial estimates of the formation constants of the resulting species and the acid dissociation constants of *AMP*, *ADP*, *ATP* and the secondary ligand acids have been refined with a computer program. In some systems, the ternary complexes are found to be more stable than the corresponding binary ones. In some ternary systems studied, interligand interactions or some cooperativity between the coordinate ligands, possibly H bond formation, have been found to be most effective in deciding the stability of the complexes formed in solution. Stabilities of mixed ligand complexes increase in the order $AMP < ADP < ATP$. With respect to the secondary ligands, the formation constants of the mixed ligand complexes decrease in the following order: succinic > maleic > tartaric > malic > citric > oxalic acid.

Keywords. Formation equilibria; Cobalt(II); *AMP*; *ADP*; *ATP*; Polybasic acids; Potentiometry.

Potentiometrische Untersuchung der Bildungsgleichgewichte von binären und ternären Komplexen von Kobalt(II) mit Adenosin-5′-mono-, -di- und -triphosphat und einigen biologisch bedeutenden polybasischen Sauerstoffsäuren

Zusammenfassung. Die Wechselwirkung von *AMP*, *ADP* und *ATP* mit Co(II) und einigen biologisch interessanten Sekundärliganden (Bernsteinsäure, Hydroxybernsteinsäure, Maleinsäure, Weinsäure, Zitronensäure und Oxalsäure) wurde bei 25 °C und einer Ionenstärke von 0.1 *M* KNO_3 potentiometrisch untersucht. Die Titrationskurven zeigen das Vorliegen von Species der Zusammensetzung 1:1:1 an. Die geschätzten Bildungskonstanten der Komplexe und die Dissoziationskonstanten der Liganden wurden mittels eines Computerprogramms optimiert. In einigen der untersuchten Systeme sind die ternären Komplexe stabiler als die sekundären. Die Stabilität der Verbindungen hängt im wesentlichen von Interligandwechselwirkungen – möglicherweise der Ausbildung von Wasserstoffbrückenbindungen – ab. Die Stabilität der Komplexe mit gemischten Liganden steigt in der Reihenfolge $AMP < ADP < ATP$ und fällt entsprechend der Serie Bernsteinsäure > Maleinsäure > Weinsäure > Hydroxybernsteinsäure > Zitronensäure > Oxalsäure.

Introduction

Metal ion complex formations are among the prominent interactions found in nature [1–3]. Polybasic oxygen acid residues are important metabolic intermediates in biological systems; the ribonucleotides *AMP*, *ADP*, and *ATP* are equally important as substrates for many enzymic reactions [4–7]. Ternary complexes of divalent transition metal ions with *AMP*, *ADP*, and *ATP* and other secondary ligands (catechols, ethanolamines, 2,2′-bipyridyl, ethylene.diamine, pyrocatecholate, biogenic amines, 1,10-phenanthroline, tyrosine, phenylalanine, glycine, histidine, imidazole, ammonia, and aliphatic dipeptides) have been investigated using several techniques [8–22]. For an improved understanding of the driving forces leading to mixed ligand complexes of the type Co(II)-nucleotide-polybasic carboxylic acid (Co(II)–*NU*–*CA*), where nucleotide = *AMP*, *ADP* or *ATP* and carboxylic acid = malic, maleic, succinic, tartaric, citric or oxalic acid, the title systems have been investigated by potentiometric titrations to determine the stability constants of the complexes formed.

Results and Discussion

To calculate the initial estimates of the stability constants of the ternary complexes of Co(II) with *AMP*, *ADP*, *ATP* and malic, maleic, succinic, tartaric or oxalic acid the following equations were used:

$$\mathrm{Co(II)}(NU) + CA \rightleftharpoons \mathrm{Co(II)}(NU)(CA) \tag{1}$$

$$K^{\mathrm{Co(II)}(NU)}_{\mathrm{Co(II)}(NU)(CA)} = \frac{[\mathrm{Co(II)}(NU)(CA)]}{[\mathrm{Co(II)}(NU)][CA]} \tag{2}$$

$$\mathrm{Co(II)} + NU \rightleftharpoons \mathrm{Co(II)}(NU) \tag{3}$$

$$K^{\mathrm{Co(II)}}_{\mathrm{Co(II)}(NU)} = \frac{[\mathrm{Co(II)}(NU)]}{[\mathrm{Co(II)}][NU]} \tag{4}$$

$$\mathrm{Co(II)} + CA \rightleftharpoons \mathrm{Co(II)}(CA) \tag{5}$$

$$K^{\mathrm{Co(II)}}_{\mathrm{Co(II)}(CA)} = \frac{[\mathrm{Co(II)}(CA)]}{[\mathrm{Co(II)}][CA]} \tag{6}$$

It is assumed, for convenience, that complexation of the secondary ligand (*CA*) starts after the complete formation of the Co(II)(*NU*) 1:1 complex. Thus, the overall stability constant $\beta^{\mathrm{Co(II)}}_{\mathrm{Co(II)}(NU)(CA)}$ may be represented by Eq. 8:

$$\mathrm{Co(II)} + NU + CA \rightleftharpoons \mathrm{Co(II)}(NU)(CA) \tag{7}$$

$$\beta^{\mathrm{Co(II)}}_{\mathrm{Co(II)}(NU)(CA)} = \frac{[\mathrm{Co(II)}(NU)(CA)]}{[\mathrm{Co(II)}][NU][CA]} = K^{\mathrm{Co(II)}(NU)}_{\mathrm{Co(II)}(NU)(CA)} \cdot K^{\mathrm{Co(II)}}_{\mathrm{Co(II)}(NU)} \tag{8}$$

Initial estimates of the formation constants of the binary complexes and protonation constants [25] were refined with the SUPERQUAD computer program [26]. All calculations were performed on an IBM XT 286 personal computer. The constants were refined by minimizing U, where E_{obs} and E_{calc} refer

to the measured potential and that calculated from the *Nernst* equation.

$$U = \Sigma W_i (E_{obs} - E_{calc})^2 \qquad 9$$

The weighting factor W_i is defined as the reciprocal of the estimated variance where σ_E and σ_V are the estimated variances of the potential and volume readings, respectively.

$$W_i = 1/\sigma^2 = 1/[\sigma_E^2 + (\delta E/\delta V)^2 \sigma_V^2] \qquad 11$$

The quality of the fit was judged by the values of the sample standard deviation, S, and the goodness of fit, X^2, (*Pearson*'s test). At $\sigma_E = 0.1$ mV (0.001 *pH* error) and $\sigma_V = 0.005$ ml, the values of S in different sets of titrations were between 1.0 and 1.8, and X^2 was between 12.0 and 13.0. The scatter of residuals ($E_{obs} - E_{calc}$) *vs. pH* was reasonably random, without any significant systematic trends, thus indicating a good fit of the experimental data.

At the experimental *pH* values used in this work, the interfering effects of both hydroxo complexes and the *bis* complexes which may be formed between nucleotides and dicarboxylic acids are negligible. Thus the secondary ligand, *CA*, combines with the binary 1:1 complex in a similar manner as it interacts with aquated metal ions in solution. The initial estimates of the stability constants of the ternary complexes formed in solution have been determined using the *Rossotti* and *Irving* formula [25]. These values were then refined using the SUPERQUAD computer program [26]. The acid formation constants for *AMP* ($pK_{a1} = 3.81$, $pK_{a2} = 6.24$), *ADP* ($pK_{a1} = 3.93$, $pK_{a2} = 6.46$) and *ATP* ($pK_{a1} = 4.12$, $pK_{a2} = 6.54$) and the stability constants of their Co(II) complexes were determined from the titration curves and the results were found to cope with those reported in the literature [27–30].

In the case of *ADP* and *ATP*, the monoprotonated complexes, *i.e.* Co(H·*ADP*) and Co(H·*ATP*)$^-$, were taken into consideration. The calculated values $\log K^{Co}_{Co(H \cdot ADP)} = 2.03$ and $\log K^{Co}_{Co(H \cdot ATP)^-} = 2.64$ agree also with the literature [29]. The differences between our values of the stability constants of the binary Co(II)*CA* complexes and those reported in the literature [30] may be attributed to the different methods of calculation and the different ionic strengths.

It has been found that pK_{a1} values of 3.5–4.2 are associated with proton ionization from the protonated forms of *AMP*, *ADP*, and *ATP* [31–34]. Calorimetric work [35] provides evidence that proton ionization from protonated adenine and adenosine occurs at the N_1H^+ group. The second proton ionization was attributed to the phosphate groups.

The manner in which the Co(II) ion binds to *AMP*, *ADP*, and *ATP* has been discussed [36–38]. Coordination of Co(II) with the phosphate groups of ribonucleotides has been demonstrated in aqueous solutions for *AMP*, *ADP*, and/or *ATP* by ^{31}P NMR [36, 39] and potentiometric [40] investigations. Interaction with all available phosphates is indicated by ^{21}P NMR spectra in the cases of *AMP* [38], *ADP* [35], and *ATP* [36, 41]. A Raman spectral study [42] of Co(II)–*ATP* interactions shows the Co(II) to bind the base moeity and to promote intramolecular base phosphate interaction. Proton NMR data indicate that Co(II) coordinates to the adenine ring of *ADP* [36] and *ATP* [36, 43]. Since the C_8-H peak is broadened [36], coordination apparently occurs at the N_7 site of *ADP* and *ATP* with possible participation from the C_6-NH_2 group [38]. However, in an examination of the conformational possibilities for metal-nucleotide interaction [44], the author discounts the C_6-NH_2 group as a complexing site. He points out that the amino group in adenine is highly conjugated with the ring and has considerable double bond character with a resulting lowered basicity compared to the

amino groups of aniline or amino acids. Support for the binding of metal ions to the base moiety of *ATP* is found in a proton NMR and kinetic study [45]. The experimental data were accounted for by assuming that a water molecule forms a bridge between the Co(II) and the N_7 site. The remaining metal coordination sites were phosphate oxygen atoms. Thus, there is lack of agreement as to the assignment of the site of coordination. Our opinion in this point will be discussed later.

In Figs. 1–3, representative sets of experimental titration curves obtained according to the sequence described in the experimental section for the different Co(II)–*NU*–*CA* systems studied are displayed. It is observed that the Co(II)–*NU* titration curve (c) diverges from the nucleotide curve (b) in the lower *pH* range ($pH \sim 3.5$), denoting the formation of a Co(II)–*NU* complex. Generally, the complex titration curves show an inflection after addition of two moles of base per one mole of the nucleotide. This indicates the simultaneous dissociation of two protons from the nucleotide. Co(II)–*NU* complexes are quite stable up to high *pH* values, *i.e.* they show into tendency to form hydroxo complexes. With respect to the titration curves of the Co(II)-carboxylic acid binary complex solutions studied, one may deduce that these complexes begin to form at $pH > 4.0$. Generally, for all Co(II) carboxylic acid complexes studied, precipitation occurred at $pH > 10.5$. In all cases no calculations have been performed beyond the precipitation point, hence the hydroxo species likely to be formed after this point could not be investigated. For the titration curves of the ternary systems Co(II)–*NU*–*CA*, one observes that c and f are well separated at $pH = 4$. This behaviour reveals that in this *pH* range coordination of the secondary ligand with Co(II)–*NU* starts.

Examination of the different formation constant values listed in Table 1 clearly reveals that the formation constands of the mixed ligand complexes increase in the order *AMP* < *ADP* < *ATP*. Though many studies in solution favoured the phosphate group rather than the base as the primary metal binding site, the simultaneous binding of Co(II) ion to base moiety and phosphate may be reported in the mixed ligand complexes formed in the present work. The Co(II) bound to the

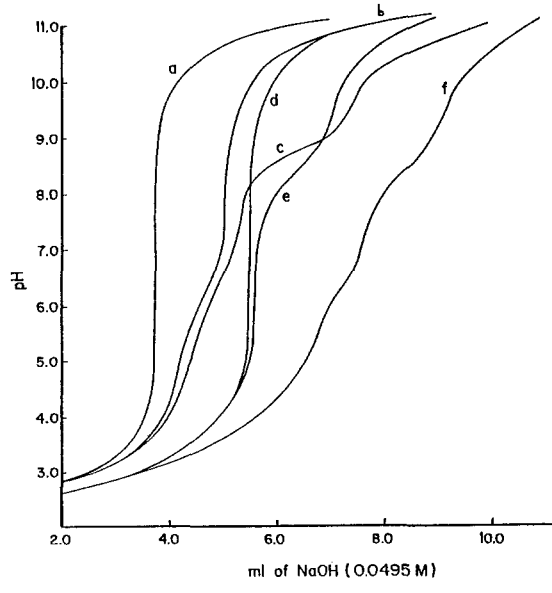

Fig. 1. Potentiometric titration curves for the Co(II)–*AMP*–tartaric acid system at 25 °C and $I = 0.1\,M$ KNO_3: (a) $0.0037\,M$ HNO_3; (b) solution (a) $+ 1 \times 10^{-3}\,M$ *AMP*; (c) solution (b) $+ 1 \times 10^{-3}\,M$ Co(II); (d) solution (a) $+ 1 \times 10^{-3}\,M$ tartaric acid; (e) solution (d) $+ 1 \times 10^{-3}\,M$ Co(II); (f) solution (e) $+ 1 \times 10^{-3}\,M$ *AMP*

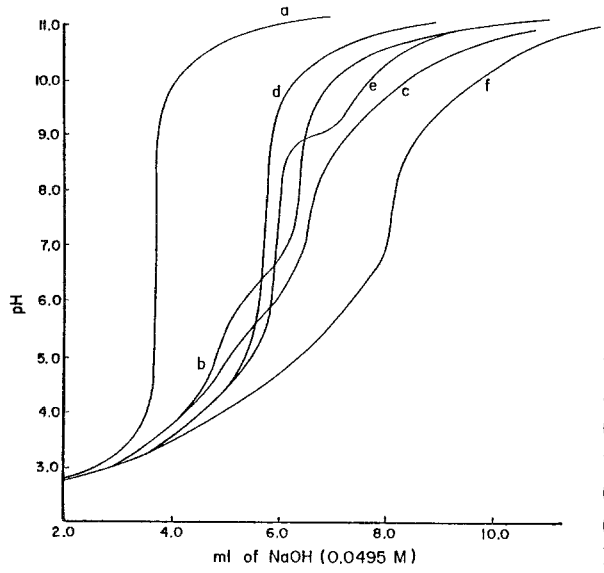

Fig. 2. Potentiometric titration curves for the Co(II)–*ADP*–malic acid system at 25 °C and $I = 0.1\ M\ KNO_3$: (a) $0.0037\ M\ HNO_3$; (b) solution (a) $+ 1 \times 10^{-3}\ M$ *ADP*; (c) solution (b) $+ 1 \times 10^{-3}\ M$ Co(II); (d) solution (a) $+ 1 \times 10^{-3}\ M$ malic acid; (e) solution (d) $+ 1 \times 10^{-3}\ M$ Co(II); (f) solution (e) $+ 1 \times 10^{-3}\ M$ *ADP*

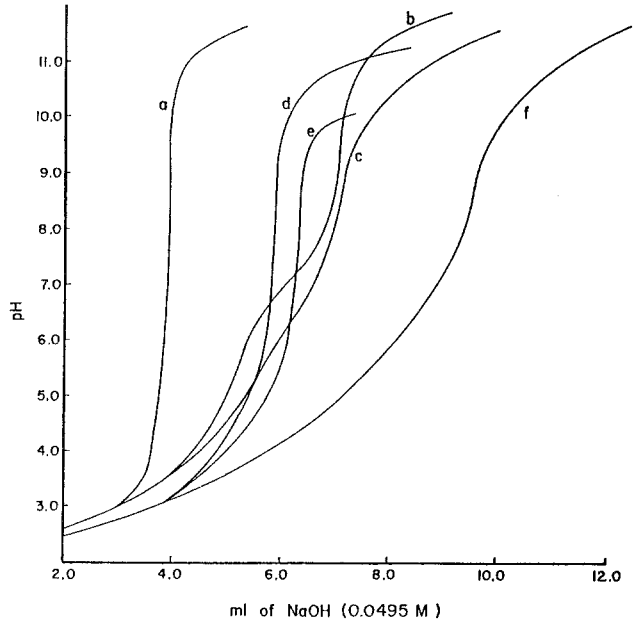

Fig. 3. Potentiometric titration curves for the Co(II)–*ATP*–malic acid system at 25 °C and $I = 0.1\ M\ KNO_3$: (a) $0.0037\ M\ HNO_3$; (b) solution (a) $+ 1 \times 10^{-3}\ M$ *ATP*; (c) solution (b) $+ 1 \times 10^{-3}\ M$ Co(II); (d) solution (a) $+ 1 \times 10^{-3}\ M$ malic acid; (e) solution (d) $+ 1 \times 10^{-3}\ M$ Co(II); (f) solution (e) $+ 1 \times 10^{-3}\ M$ *ATP*

base moiety may promote intramolecular base-phosphate interaction. Thus, the mixed ligand complexes studied may be considered as a relatively simple model from which information may be gained about the properties of nucleotides and their base moieties regarding the strength of their interactions with important metabolic intermediates.

With respect to the secondary ligands, the formation constants of the mixed ligand complexes decrease in the following order: succinic > maleic > tartaric > malic > citric > oxalic acid. This behaviour can be interpreted in terms of the basicities ($pK_{a1} + pK_{a2}$) of the carboxylic acids used. It is well known that the increase in basicity of a ligand increases the stability of its metal complexes.

Table 1. Formation constant values for the binary Co(II)-nucleotide or -carboxylic acid complexes and for the mixed ligand complexes Co(II)-nucleotide-carboxylic acid at 25 °C and $I = 0.1\ M\ KNO_3$

Ligand	$\log K^{Co(II)}_{Co(II)(Nucleotide)}$ or $\log K^{Co(II)}_{Co(II)(CA)}$	$\log K^{Co(II)(AMP)}_{Co(II)(AMP)(CA)}$	$\log k^{Co(II)(ADP)}_{Co(II)(ADP)(CA)}$	$\log K^{Co(II)(ATP)}_{Co(II)(ATP)(CA)}$	$\log \beta^{Co(II)}_{Co(II)(AMP)(CA)}$	$\log \beta^{Co(II)}_{Co(II)(ADP)(CA)}$	$\log \beta^{Co(II)}_{Co(II)(ATP)(CA)}$	$\Delta \log K_1$	$\Delta \log K_2$	$\Gamma \log K_3$
AMP	2.61 ± 0.03	–	–	–	–	–	–	–	–	–
ADP	4.41 ± 0.02	–	–	–	–	–	–	–	–	–
ATP	5.11 ± 0.02	–	–	–	–	–	–	–	–	–
oxalic acid	3.87 ± 0.04	3.490 ± 0.03	3.720 ± 0.03	4.402 ± 0.04	6.100	8.130	9.512	−0.380	−0.150	+0.532
succinic acid	5.303 ± 0.02	4.369 ± 0.02	4.920 ± 0.04	5.386 ± 0.02	6.979	9.330	10.470	−0.934	−0.383	+0.083
tartaric acid	4.082 ± 0.03	4.035 ± 0.03	4.538 ± 0.02	4.812 ± 0.03	6.645	8.948	9.922	−0.047	+0.456	+0.730
malic acid	3.983 ± 0.02	3.752 ± 0.04	4.142 ± 0.03	4.563 ± 0.02	6.362	8.552	9.673	−0.231	+0.159	+0.0580
maleic acid	4.670 ± 0.03	4.140 ± 0.02	4.773 ± 0.03	4.923 ± 0.03	6.750	9.183	10.033	−0.530	+0.103	+0.253
critic acid	3.962 ± 0.03	3.526 ± 0.03	3.948 ± 0.04	4.482 ± 0.03	6.136	8.358	9.592	−0.436	+0.014	+0.520

$\Delta \log K_1 = \log K^{Co(II)(AMP)}_{Co(II)(AMP)(CA)} - \log K^{Co(II)}_{Co(II)(CA)}$; $\Delta \log K_2 = \log K^{Co(II)(ADP)}_{Co(II)(ADP)(CA)} - \log K^{Co(II)}_{Co(II)(CA)}$; $\Delta \log K_3 = \log K^{Co(II)(ATP)}_{Co(II)(ATP)(CA)} - \log K^{Co(II)}_{Co(II)(CA)}$

$\Delta \log K$, defined by Eq. 12, is a measure of the stability of the ternary complexes with respect to the binary complexes

$$\Delta \log K = \log K^{Co(II)(NU)}_{Co(II)(NU)(CA)} - \log K^{Co(II)}_{Co(II)(CA)} \qquad 11$$

In the case of Co(II)–*NU*–*CA* systems, $\Delta \log K$ is found to be slightly positive or negative (Table 1) in accordance with statistical expectations [46]. $\Delta \log K$ values for Co(II)–*AMP*–*CA* systems are negative in accordance with statistic, steric, and electrostatic factors which result in a lower stability constant for the ternary complexes as compared with those for the binary systems [47]. The higher stability constant of Co(II)–*ATP*–*CA* ternary complexes compared with the binary systems may be attributed to interligand interactions or to some cooperativity between the ligands, possibly H-bond formation.

Experimental

Materials and solutions. Adenosine-5′-monophosphoric acid disodium salt ($C_{10}H_{12}N_5Na_2O_7P \cdot H_2O$, $Na_2AMP \cdot H_2O$), adenosine-5′-diphosphoric acid disodium salt ($C_{10}H_{13}N_5Na_2O_{10}P_2 \cdot 2H_2O$, $Na_2ADP \cdot 2H_2O$), and adenosine-5′-triphosphoric acid disodium salt ($C_{10}H_{14}N_5Na_2O_{13}P_3 \cdot 3H_2O$, $Na_2ATP \cdot 3H_2O$) were purchased from Sigma Chemical Co. and were used without purification. The amount of free phosphates initially present in the nucleotides was determined [23]. To account for this and to prepare metal ion nucleotide solutions of an exactly 1:1 ratio, we also determined the molecular weight of the purchased nucleotides. $Co(NO_3)_2 \cdot 6H_2O$, nitric acid, NaOH, and organic carboxylic acids (malic, maleic, succinic, tartaric, citric, and oxalic acid) were of *p.a.* grade. The concentration of NaOH used for the titrations was determined with potassium hydrogen pathalate (Merck AG). The concentrations of the metal ion stock solutions were determined with *EDTA*.

Apparatus. Potentiometric measurements were performed in a double-walled glass vessel using a Beckman 4500 digital *pH* meter with a precision of ± 0.1 mV. The potentiometric system was equipped with a glass electrode (Metromh 1028) calibrated against a double junction reference electrode (Orion 9020). The titrant was delivered by an Amel 882 dispensor, readable to 1 µl. The measurement cell was kept at a temperature of 25 °C constant within ± 0.1 °C, and a magnetic stirrer was used. Purified nitrogen was bubbled through the solutions during titrations.

Procedure. The test solution was titrated with standard CO_2-free potassium hydroxide. The electrodes were calibrated, in both the acidic and alkaline regions, by titrating 0.01 *M* nitric acid with standard potassium hydroxide under the experimental conditions. Carbonate free KOH was standardized against standard potassium hydrogen phthalate with the help of a *Gran* plot. The data so obtained were analysed by the non-linear least-squares computer program ESAB2M [24] to refine E° and the autoprotolysis constant of water.

In order to avoid hydrolysis prior to potentiometric measurements, samples of the nucleotides were weighted out as the solid and added to the reaction vessel just before the titration. The solutions titrated were as follows: HNO_3 (a); HNO_3 + nucleotide (b); HNO_3 + nucleotide + Co(II) (c); HNO_3 + polybasic carboxylic acid (d); HNO_3 + polybasic carboxylic acid + Co(II) (e); HNO_3 + nucleotide + polybasic carboxylic acid + Co(II) (f). A constant ionic strength was obtained with 0.1 *M* KNO_3 and the total volume was kept constant at 50 ml.

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