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Potentiometric and Spectral Studies of Complex Formation of La(III), Pr(III) and Lu(III) with Aspartic Acid and Asparagine

Anna Wojciechowska, Lechosław Łomozik, and **Stanisław Zieliński***

Faculty of Chemistry, Adam Mickiewicz University, PL-60-780 Poznań, Poland

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The composition and stability of La^{3+}, Pr^{3+} and Lu^{3+} complexes with aspartic acid and asparagine were analysed. The formation of complexes of the type ML and MHL was determined for La^{3+} and Pr^{3+} with aspartic acid, and of the type MHL for Lu^{3+} with aspartic acid. For La^{3+}, Pr^{3+} and Lu^{3+} with asparagine the formation of $ML(OH)$ complexes was observed. By means of ^1H NMR and ^{13}C NMR studies the participation in the coordination of both —COOH groups was determined for aspartic acid, whereas for asparagine the participation of the —COOH group was determined in complexes with La^{3+}, Pr^{3+}, and of the —COOH and the —NH_2 groups in the complex with Lu^{3+}.

[*Keywords: Asparagine; Aspartic acid;* ^{13}C *NMR; Complexes; Coordination sites;* ^1H *NMR; Lanthanum(III); Lutetium(III); Praseodymium(III); Stability constants*]

Potentiometrische und spektroskopische Untersuchungen an La(III), Pr(III) *und* Lu(III)*-Komplexen von Asparaginsäure und Asparagin*

Die Zusammensetzung und die Stabilität von La^{3+}, Pr^{3+} und Lu^{3+}-Komplexen mit Asparaginsäure und Asparagin wurden untersucht. Es wurde die Bildung von La^{3+} und Pr^{3+}-Komplexen des Typs ML und MHL, und ein Lu^{3+}-Komplex des Typs MHL mit Asparaginsäure festgestellt. Für diese drei Lanthaniden wurde auch die Bildung von Komplexen des Typs $ML(OH)$ mit Asparagin beobachtet. Mit Hilfe von ^1H-NMR und ^{13}C-NMR-Untersuchungen wurde für Asparaginsäure die Teilnahme der beiden —COOH-Gruppen, für Asparagin die Teilnahme der —COOH-Gruppe in den Komplexen mit La^{3+}, Pr^{3+} und der —COOH und —NH_2-Gruppen in dem Komplex mit Lu^{3+} an der Koordinierung festgestellt.

Introduction

Studies of amino acid complexes are of great importance both to coordination chemistry and inorganic biochemistry. Amino acids possess at least two functional groups, amino and carboxylic ones, enabling the formation of a number of complex species. The reaction between amino acid and metal may be considered as a model reaction in the system protein-metal, reflecting the course of important processes taking place in living organisms. In this field of research it is useful to study amino acid-lanthanide systems. Lanthanides do not occur in living organisms, but when introduced into the system instead of other metals, they modify the spectroscopic characteristics of the complexes. Calcium for example, commonly found in biological systems, has unfavourable spectroscopic properties due to its completed electronic shells. However, when it is substituted by a lanthanide with its unpaired electrons in the shell f, the lanthanide makes the complex spectroscopically active yielding absorption bands of the complex that are distinct even in aqueous solutions. However, the lanthanide complex—before being used for spectroscopic studies instead of the calcium one—requires to be carefully studied. Such studies comprise determining the complex composition, stability and formation conditions as well as the coordination mode of the ligand with the lanthanide.

The present study discusses asparagine and aspartic acid complexes with La^{3+}, Pr^{3+} and Lu^{3+}. The results presented in the literature are often in disagreement. Some authors [1–6] determined only the occurrence of *ML* forms, others of *ML* and ML_2, and also *M*H*L*. In our paper we present results of studies on the composition and stability of complex bindings existing in the system asparagine-lanthanide and aspartic acid-lanthanide. Presented are also results of spectroscopic studies relating to the coordination sites in these ligands.

Experimental

In the studies *L*-asparagine and *L*-aspartic acid manufactured by Reanal (Budapest), chromatographically pure, were employed. Lanthanide solutions in 2 *M* HCl were prepared by dissolving their oxides: La_2O_3 99.9% prepared by the Laboratory of Rare Earth Elements, Faculty of Chemistry, A. Mickiewicz University, Poznań; Pr_2O_3 99.9% made by IEL England and Lu_2O_3 99.9% made by Fluka, Switzerland. Chloride solutions were evaporated until dryness and dissolved in redistilled water. Concentration of a lanthanide in the solution was determined by titration with *EDTA* in the presence of xylenol orange. In spectroscopic studies the following deuterated compounds were used: D_2O (IBJ, Poland), DCl (PAN, Poland), NaOD (VEB-Berlin-Chemie). The *pH* was measured with a Radiometer GK 2401 C electrode by using a Radiometer titrating set (*pH* meter 26c, autoburette ABU 1-c, titrator TTT-11). The *pH* meter was calibrated with two buffer solutions of $pH = 4.005 \pm 0.005$ and 9.24 ± 0.01. Measurements were carried out at 21 ± 1 °C in the inert atmosphere of argon. Ionic strength was adjusted with $NaClO_4$ and was $\mu = 0.1$. The titrant was a carbonate-free solution of NaOH of a concentration 0.173 *M*. Concentrations of the ligand in the studied systems were 0.01 *M* and 0.005 *M*, concentration of a metal from 0.02 M to 0.001 M. Samples for ^{1}H NMR and ^{13}C NMR measurements were prepared by dissolving lanthanide chlorides and asparagine or aspartic acid in D_2O. Measurements of pH were corrected according to the formula $pD = pH + 0.40$ [7]. Concentration of asparagine in the samples was 0.2 M, of aspartic acid 0.5 M. The metal—to—ligand ratio in the samples was 1 : 5. The ^{1}H NMR spectra were recorded on a Tesla BS-467 spectrometer with *DSS* as standard.

The ^{13}C NMR spectra were recorded on a NMR JEOL Fx 90 Q spectrometer with dioxan as an internal standard. All calculations were carried out on a RIAD-32 computer using local versions of the programs SCOGS [8], MINIQUAD [9] and HALTAFALL [10].

Results and Discussion

The dissociation constants of asparagine and aspartic acid were determined earlier [11]. In the present study the complexes of asparagine and aspartic acid with lanthanum, praseodymium and lutetium were analysed by means of the potentiometric method. Data obtained in potentiometric titration underwent a computer analysis using the programs SCOGS and MINIQUAD in order to determine the composition and stability of the complex compounds occurring in the studied systems. The results are presented in Table 1. Aspartic acid forms two types of complex species with the lanthanides under investigation. Lanthanum and praseodymium form the complexes $M\mathrm{H}L$ and ML with aspartic acid, lutetium only the $M\mathrm{H}L$ type. The stability constants of the studied complexes of both types increase in the series La^{3+}, Pr^{3+}, Lu^{3+}. Distributions of complex forms depending on pH in the system consisting of aspartic acid and La^{3+}, Pr^{3+}, Lu^{3+} were obtained with the help of the

Table 1. *Stability constants* of aspartic acid and asparagine complexes with proton, lanthanum, praseodymium and lutetium*

Aspartic acid	log	Asparagine	log
H_3L^+	15.25 (1)	H_2L^+	10.92 (1)
H_2L	13.50 (1)	HL	8.91 (1)
HL^-	9.76 (1)	$LaL(OH)^+$	—6.97 (17)
LaL^+	4.53 (9)	$PrL(OH)^+$	—6.31 (20)
PrL^+	5.14 (8)	$LuL(OH)^+$	—3.71 (6)
$LaHL^{2+}$	12.49 (8)		
$PrHL^{2+}$	12.62 (12)		
$LuHL^{2+}$	12.71 (20)		

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* For hydroxo complexes: equilibrium constants of reaction $Ln^{3+} + L^- + H_2O \rightleftharpoons LnL(OH)^+ + H^+$

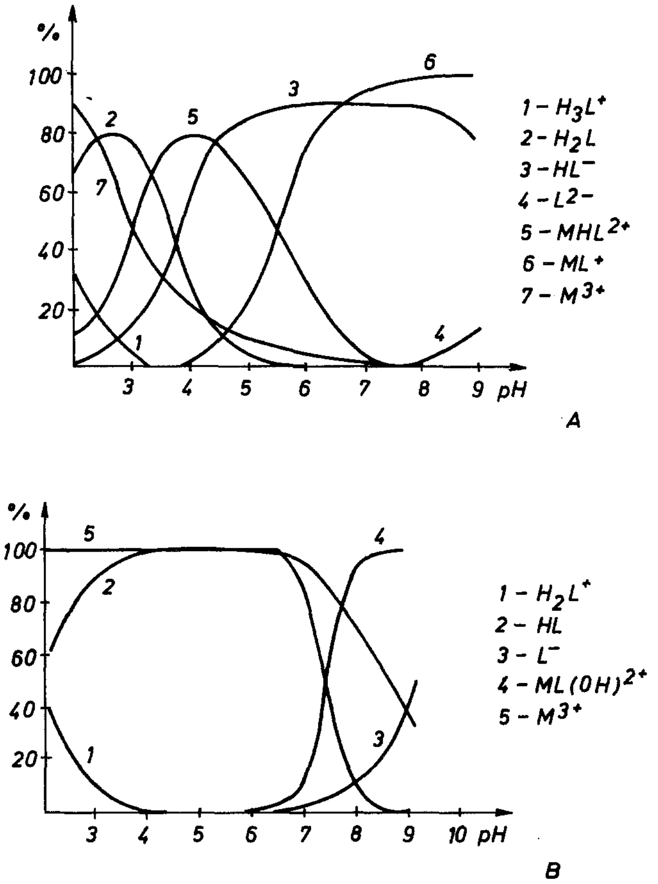

Fig. 1. Distribution diagram of the system *A* Asp/Pr^{3+}; *B* Asn/Lu^{3+}; percentages of the species refer to total metal except for the metal-free forms which refer to total ligand

HALTAFALL program (Fig. 1 A). These distributions show that aspartic acid forms the *M*H*L* type complex with La, Pr and Lu in the *pH* range where the H_3L and H_2L ligand forms occur. The most probable reactions giving rise to this complex are thus expressed by the equations:

$$H_3L^+ + M^{3+} \rightleftharpoons MHL^{2+} + 2\,H^+$$

and/or

$$H_2L + M^{3+} \rightleftharpoons MHL^{2+} + H^+$$

In the systems aspartic acid—La^{3+} and aspartic acid—Pr^{3+}, a complex of the *ML* type is formed in addition to that of the *M*H*L* type.

The *ML* complex formation results from the dissociation of a proton from the *M*H*L* complex, as illustrated by the equation:

$$MHL^{2+} \rightleftharpoons ML^{+} + H^{+}$$

In the aspartic acid—Lu^{3+} system no *ML* species was found. The most stable complex of the *M*H*L* type is formed by aspartic acid with Lu. Dissociation of this complex, giving rise to the *ML* type, can occur at such a high *pH* where hydrolysis of Lu is already observed, i.e. at *pH* 8 (which was observed experimentally).

The second ligand investigated in the complex-forming reaction with La, Pr and Lu, was asparagine. In these systems only the *ML*(OH) complex is formed. Stability constants of these complexes increase in the series La^{3+}, Pr^{3+}, Lu^{3+}. Differences in these stability constants show that the mode of coordination is different for the asparagine complex with La^{3+}, Pr^{3+} from that with Lu^{3+}. The distribution of complex forms in the system consisting of asparagine and La, Pr, Lu as dependent on *pH* (Fig. 1 B) manifests the participation of the H*L* form of asparagine in the complex formation reaction with metal.

$$M^{3+} + HL + OH^{-} \rightleftharpoons ML(OH)^{+} + H^{+}$$

Coordination sites of asparagine and aspartic acid in their complexes with La^{3+}, Pr^{3+}, Lu^{3+} were determined on the basis of observations of ^{1}H NMR and ^{13}C NMR signal shift changes.

The ^{1}H NMR and ^{13}C NMR shifts for aspartic acid are shown schematically in Fig. 2 and Table 2. The band originating from protons of the —CH_2— group appears at 1.03 ppm and from a proton belonging to —CH at 1.70 ppm. In the ^{1}H NMR spectrum of aspartic acid with La^{3+} the signal from the protons of —CH_2 group is shifted by 0.03 ppm as well as the proton signal from the —CH group undergoing a shift by 0.03 ppm.

Table 2. *Positions of* ^{13}C *NMR signals for aspartic acid and asparagine and their complexes with* La, Pr *and* Lu [*ppm*]

System	C_1	C_2	C_3	C_4
Asp	106.25	—15.82	—31.91	107.71
Asp + La^{3+}	106.09	—15.87	—31.86	108.09
Asp + Pr^{3+}	107.82	—13.76	—29.41	105.74
Asp + Lu^{3+}	107.44	—15.47	—31.47	108.82
Asn	106.68	—15.28	—32.07	107.87
Asn + La^{3+}	108.09	—14.62	—31.59	108.09
Asn + Pr^{3+}	109.49	—10.99	—30.12	108.51
Asn + Lu^{3+}	105.11	—14.95	—31.23	107.67

From the values of these shifts, which are identical for both signals, it can be concluded that two carboxylic groups of aspartic acid or one carboxylic group and one amino group take part in the coordination. Signals originating from C_1 and C_4 undergo a significant shift (by 0.16 ppm and 0.38 ppm, respectively) in the ^{13}C NMR spectrum of aspartic acid with La^{3+} in relation to the ligand alone. Shifts of this type exclude the possibility of amino group participation in the coordination, even more so as the C_2 signal shift (C_2 being the carbon atom at which the amino group occurs) is only 0.05 ppm. Coordination of the type described above is also confirmed by the sequence of proton dissociation in aspartic acid. Carboxylic groups undergo dissociation first, and only at a *pH* of approximately 9 the proton from the amino group dissociates.

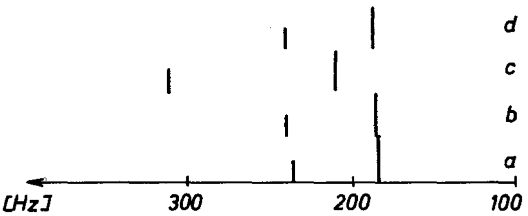

Fig. 2. Positions of the ^1H NMR signals of the systems *a* Asp; *b* Asp/La^{3+}; *c* Asp/Pr^{3+}; *d* Asp/Lu^{3+}

The values of ^{13}C NMR signal shifts originating from C_1 (by 0.16 ppm) and C_2 (by 0.38 ppm) show that the participation of the two carboxylic groups in the coordination is not the same. The most probable mode of coordination of aspartic acid with La^{3+} is the participation of both carboxylic groups, where the group with C_1 is coordinated by the metal via one oxygen atom, and the carboxylic group with C_4 via two oxygen atoms. Conclusions drawn from our studies are in agreement with those presented in Refs. [12] and [13]. An identical coordination type occurs in the aspartic acid complex with praseodymium. In the ^1H NMR spectrum of aspartic acid with Pr^{3+} (Fig. 2) there is a signal shift of proton from —CH_2 group (of approximately 0.33 ppm) and much larger shift (in comparison with the former one) of the signal from the proton of —CH group (approximately by 0.67 ppm). Shifts of this type point to the possibility of coordination by the carboxylic group and amino group or by both carboxylic groups. However, in the first coordination mode, the larger shift of protons from the —CH_2 group compared to that of the proton from the —CH group (where the amino group is located) gives rise to certain doubts. More definite data concerning the coordination type in the discussed complex are supplied by the ^{13}C NMR spectrum. It shows shifts of C_1 and C_4 signals, which in turn point to a participation of both carboxylic groups in coordination. The C_1 signal undergoes a shift of 1.57 ppm, that of C_4 changes by 1.98 ppm. The position of signals from C_2 and C_3 is also changed (by 2.05 ppm and 2.98 ppm, respectively). A larger shift is observed for the C_3 signal, which exclude the participation of the amino group in coordination and confirms the participation of both carboxylic groups. The coordination of aspartic acid with Lu^{3+} is different. In the ^{1}H NMR spectrum the proton signals from the —CH_2 group as well as the proton signal from the —CH group undergo a change (in both cases by 0.05 ppm). These shifts are larger than respective shifts in the system of aspartic acid with La^{3+}. In the ^{13}C NMR spectrum of aspartic acid with Lu^{3+} the signals from C_1 and C_4 undergo shifts by 1.29 ppm and 1.11 ppm, respectively and values of these shifts are of the same order for both carbon atoms. There are thus two carboxylic groups which participate in the coordination of aspartic acid with Lu^{3+}, and the coordination mode of both functional groups is identical. Considering the values of signal shifts in the spectra of aspartic acid—Lu^{3+} system in relation to the similarly diamagnetic La^{3+}, we can conclude that both oxygen atoms in both carboxylic groups participate in the coordination.

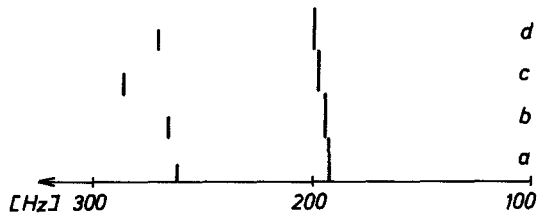

Fig. 3. Positions of the ^{1}H NMR signals of the systems *a* Asn; *b* Asn/La^{3+}; *c* Asn/Pr^{3+}; *d* Asn/Lu^{3+}

The most probable coordination sites in the other studied ligand, i.e. asparagine, are the carboxylic group and the amino group. ^{1}H NMR and ^{13}C NMR shifts are presented for asparagine in Fig. 3 and Table 2. In the ^{1}H NMR spectrum of the asparagine complex with La^{3+}, there is a shift of the signal originating from protons at the —CH group, and a minute signal shift from the protons of the —CH_2 group. Such a small shift of proton signals from the —CH_2 group indicates that the amino group does not participate in coordination. In the ^{13}C NMR spectrum of the studied system signals from all carbon atoms in the aspartic acid molecule undergo a shift. The shift is 1.41 ppm for C_1 and these shifts become gradually smaller when going towards C_4. The most probable coordination mode for this system is coordination by the carboxylic group. An identical coordination type is observed in the complex of asparagine with Pr^{3+}. The ^1H NMR and ^{13}C NMR spectra of asparagine with Lu^{3+}, however, indicate a different coordination mode. In the ^1H NMR spectrum, the signal from group —CH as well as the proton signal from group —CH_2 undergo shifts. These shifts are 0.13 ppm and 0.07 ppm, respectively, and are much larger than analogous values in the case of La^{3+}; and in the case of the proton signal from —CH_2, also larger than the analogous shift in Pr^{3+}. Shifts of this type result from coordination by the carboxylic and amino group of asparagine. In the ^{13}C NMR spectrum of the system asparagine—Lu^{3+}, the shifts of C_1 and C_2 signals are comparable with analogous shifts in the spectrum of the asparagine complex with La^{3+}. A clear increase in a C_3 signal shift (by 0.49 ppm for La^{3+} and 0.75 ppm for Lu^{3+}) manifests the participation of the asparagine amino group in the coordination. Thus in the case of the asparagine complex with La^{3+} and Pr^{3+} the carboxylic group participates in the coordination. The mode in which this group is coordinated by the central atom is identical with that in the asparagine complex with Lu^{3+} (in both cases values of C_1 signal shifts are comparable). In the complex of asparagine with Lu^{3+}, apart from the carboxylic group, also the amino group participates in the coordination.

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