Potentiometric Studies on the Complex Formation of Lanthanides with Proline and Hydroxyproline

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

Institute of Chemistry, Adam Mickiewicz University, 60-780 Poznań, Poland

The formation of the lanthanide (La, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Lu) complexes with proline and hydroxyproline has been investigated by potentiometric methods as well as by a turbidimetric one which has provided some additional conclusions. It has been found that 1:1 and a slight amount of 2:1 complexes are formed. The deviation from the typical course of the formation function is discussed. It is suggested that the perturbations of complex formation in the systems are caused by hydrolysis. The stability constants of the complexes are reported.

Potentiometrische Untersuchungen an Lanthanid-Komplexen von Prolin und Hydroxyprolin

Die Bildung von Lanthanid-Komplexen (La, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Lu) mit Prolin und Hydroxyprolin wurde potentiometrisch und mit Hilfe von Trübungsmessungen untersucht. Es wurde festgestellt, daß sich Komplexe der Zusammensetzung 1:1 und in kleiner Menge auch 2:1 bilden. Die Abweichung von typischem Verlauf der Bildungskurven wurde untersucht. Es wurde dabei festgestellt, daß für die Störungen in der Komplexbildung die Hydrolyse verantwortlich ist. Die Stabilitätskonstanten wurden bestimmt.

Introduction

Complexes of metal ions with amino acids are of essential importance for life processes. However, lanthanides do not occur in living organisms but whenever they replace a metal (for example Ca^{2+}) in a complex, spectral properties of the system are modified, with no change in the functionality of the complex. Thus, the rare earth metals are used

as probes in the biochemistry of calcium. It seems worthwile then, to study the lanthanide—amino acid systems. Complexes of proline and hydroxyproline with lanthanides belong to that group of compounds.

Coordination compounds of proline with Pr(III) and Eu(III) were studied by *Katzin*[1,2], who postulated that complexes of molar ration 2:1 are formed and that nitrogen and oxygen are involved in coordination of the chelate ring formed. *Kriss*[3], *Rogozina, Popov* and *Ponikarova*[4,5] investigated the pro-line—lanthanide systems by potentiometric methods and established the formation of 1:1 chelate compounds. Polarographic studies of complexes of proline with Eu(III) were done by *Lal*[6], who found that complexes of molar ratio 2:1 are formed in solution.

Experimental

Proline (Reanal, Budapest) and hydroxyproline (BDH Ltd Poole England) were recrystallized from an absolute alcohol—aceton mixture.

The solutions of lanthanides were prepared by dissolving the appropriate oxides (Nd_2O_3, Y_2O_3, 99.9% obtained in the Rare Earth Laboratory, Institute of Chemistry UAM, Poznań; Pr_2O_3, Eu_2O_3, Gd_2O_3, Tb_2O_3, Dy_2O_3, Ho_2O_3, Er_2O_3, Tm_2O_3, 99.9% from IEL, England, spectrographically pure La_2O_3 and Sm_2O_3 from Johnson Matthey, England and Lu_2O_3 99.9% from Fluka, Switzerland) in $2\,M$-HCl. The solutions were then evaporated to dryness and dissolved in doubly distilled water. The titration curves were taken with the Radiometer automatic titration apparatus (pH-meter 26 c, titrator TTT 11, auto-burette ABU-1 c, recorder SBR-2 c). All potentiometric measurements were made under argon as inert gas. The constant ion strenght of 0.1 was maintained by addition of the required volume of $0.1\,M$-sodium perchlorate. Tetramethyl-ammonium hydroxide of $1 \cdot 10^{-2}\,M$ was used as titrant. Titrations of proline and hydroxyproline, in concentrations of $5 \cdot 10^{-2}\,M$, $4 \cdot 10^{-2}\,M$, $3 \cdot 10^{-2}\,M$, $2.5 \cdot 10^{-2}\,M$, $1.25 \cdot 10^{-2}\,M$ and $1 \cdot 10^{-2}\,M$ were performed. Then, titrations of proline—lanthanide and hydroxyproline—lanthanide mixtures were made for 1:1, 2:1, 2:1, 4:1 and 10:1 ratios of amino acids to metal ion concentration. (The initial concentrations of the ligands and metals were: $1 \cdot 10^{-2}\,M$, $8 \cdot 10^{-3}\,M$ and $4 \cdot 10^{-3}\,M$). *Schwarzenbach*'s method[7] was applied to calculate the dissociation constants of proline and hydroxyproline, and *Bjerrum*'s method[8] to determine the stability constants of the complexes. Computer programs were written for both methods. All computations were performed on an ODRA 1204 computer. The values of the dissociation constants were refined by the method of succesive approximations, and the stability constants by the least square method. The procedure involved also the titration of lanthanides with proline (hydroxyproline) in concentration of the reagents equal to $5 \cdot 10^{-3}\,M$. The initial pHs of the systems were: 6.0, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6 and 7.8. Acidity was adjusted with the use of HCl or NaOH. The measurements of turbidity of proline (hydroxyproline)—lanthanide mixtures of molar ratios 1:1, 4:1 and 10:1 were made with a spectral colorimeter Spekol (Carl Zeiss Jena, GDR) equipped with a TK attachement. Doubly distilled water and fully precipitated with NaOH lanthanide (of concentration as in experiments) were used as turbidity standarts (0% and 100% respectively). For each lanthanide a series of 11 samples was prepared in order to determine the relative degree of turbidity.

The solutions contained proline (hydroxyproline) and $0.01\,M$ lanthanide ions of $1:1$, $4:1$ and $10:1$ molar ratios. From $1\,cm^3$ to $11\,cm^3$ of $0.01\,M$-NaOH were added to each sample. Then, the samples were filled up to $17\,cm^3$ with distilled water. Immediately after preparing the sample, pH and the relative degree of turbidity were measured.

Results and Discussion

The dissociation constants determined for the ligands, namely, $pK_1 = 2.19$, $pK_2 = 10.68$ for proline and $pK_1 = 2.08$ and $pK_2 = 10.01$ for hydroxyproline are in good agreement with data cited in the litera-
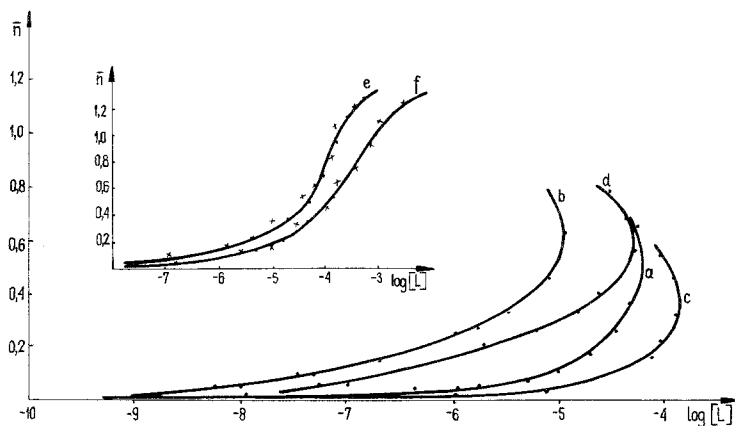

Fig. 1. Formation curves of complexes: a proline—La^{3+} (molar ratio $1:1$); b proline—Pr^{3+} ($1:1$); c hydroxyproline—La^{3+} ($1:1$); d hydroxyproline—Pr^{3+} ($1:1$); e proline—La^{3+} (o denotes $4:1$, x denotes $10:1$ molar ratio); f hydroxyproline—La^{3+} (o denotes $4:1$, x denotes $10:1$ molar ratio)

ture[9-12]. Subsequently, the stability constants of the complexes of proline and hydroxyproline with Y, La, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm and Lu were determined. The shape of the formation curves for proline—lanthanides and hydroxyproline-lanthanides indicates that some perturbations take place in $1:1$ and $2:1$ systems. Abnormal shape of the formation curves is observed for the values of $\tilde{n}$ greater than 0.5. It is presented for La^{3+} and Pr^{3+} complexes on Fig. 1 $a-d$. The perturbations increase in the series from La^{3+} to Lu^{3+}. This is apparent from the deviation from typical shape of curves (an example of typical formation curves for La^{3+} complexes is shown on Fig. 1 e,f). The perturbation is most probably caused by hydrolysis of the lanthanide ion. There is a possibility that formation of some types of mixed

complexes with OH⁻ions involved in coordination takes place. Al-
though, the shape of the formation curve indicates that $1:1$ complexes
form in the system, hydrolysis makes it impossible to obtain the correct
stability constants of proline (hydroxyproline) — Ln^{3+} compounds.

Table 1. *Stability constants of proline and hydroxyproline complexes with
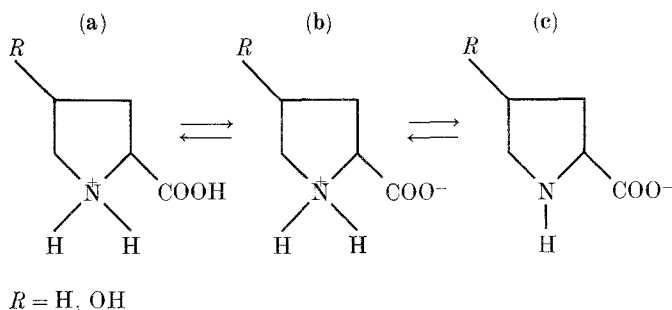lanthanides*

Ln	Proline $\log K_1$	Hydroxyproline $\log K_1$
Y	5.50	5.10
La	4.26	3.90
Pr	5.10	4.55
Nd	5.18	4.63
Sm	5.53	4.95
Eu	5.57	5.03
Gd	5.60	5.08
Tb	5.62	5.20
Dy	5.67	5.31
Ho	5.75	5.38
Er	5.90	5.47
Tm	6.08	5.55
Lu	6.15	5.63

Thus, the complex formation was subsequently investigated using
solutions with greater excess of ligand. The following findings confirm
the hypothesis about the importance of hydrolysis:

1. The deviations from the typical shape of formation curves are
smaller for the systems of molar ratio $2:1$ than those of $1:1$ ratio. If the
perturbation is indeed caused by hydrolysis, a greater excess of the
ligand should shift the equilibrium of the hydrolysis reaction to the left.
A study of the systems of molar ratios $4:1$ and $10:1$ was made to
confirm this assumption. The typical shape of formation curves was
obtained already for $4:1$ systems of both proline and hydroxyproline
(Fig. 1*e,f*). The stability constants determined from these results are
presented in Table 1.

2. Titrations of lanthanides with ligand provided further evidence
on the importance of hydrolysis.

The initial values of pH: 6.0, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6 and 7.8
were chosen for the experiment. The following equilibrium exists
between the species of ligands:

(a)   (b)   (c)

$R = H, OH$

The species **c** of proline (hydroxyproline) is involved in complexation. Thus, the formation of complexes is easier if the pH values increase. Consequently, the greater increase of the hydrogen ions
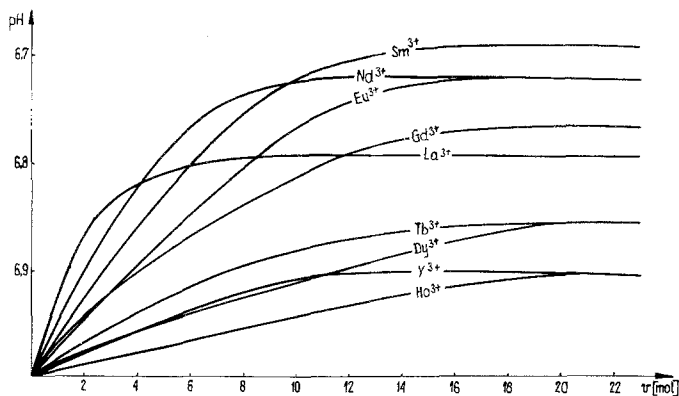

Fig. 2. Titration curves of lanthanides with proline

concentration ought to be found when the initial values of pH of the solution are higher. This is so, because in order to maintain the equilibrium of the system, the dissociation of the **b** species is necessary. From the data for titration of lanthanides with ligand one can see, however, that if the initial pH of the metal—ligand mixture is smaller then 6.0-6.2 very small changes of acidity are observed. These changes increase in the series from La^{3+} to Lu^{3+}. On the other hand, if the initial pH value is greater than 7.4-7.6 significant changes of acidity occur, and they decrease from La^{3+} to Lu^{3+} (Fig. 2).

There are two possible explanations for the latter case:

a) the strength of the metal-nitrogen bond decreases from La^{3+} to Lu^{3+};

b) there is some additional factor (hydrolysis of the lanthanide ion) which makes complexation difficult.

Explanation a. is doubtful. Increase of the pH value results in an increase of the concentration of anionic species of amino acid, where

Table 2. *Relative degree of hydrolysis of lanthanide—proline* (1:1) *and lanthanide—hydroxyproline* (1:1) *systems*

NaOH cm³	Proline				Hydroxyproline			
	Lanthanum		Lutetium		Lanthanum		Lutetium	
	pH	%	pH	%	pH	%	pH	%
1	2.8	0	3.3	0	2.7	0	3.4	0
2	3.3	0	4.7	0	3.4	0	3.6	0
3	5.3	0	5.5	0	5.2	0	5.1	0
4	7.7	0	6.0	0	7.8	0	6.4	6
5	8.4	0	6.4	6	8.2	0	7.1	10
6	8.5	0	6.6	10	8.3	0	7.3	15
7	8.6	5	6.8	20	8.4	8	7.5	20
8	8.7	10	6.9	24	8.6	15	7.7	25
9	9.4	20	7.0	32	9.8	35	8.3	30
10	9.9	30	7.5	35	10.5	40	8.5	35
11	10.5	45	9.9	38	11.0	50	9.9	40

nitrogen is not protonated and the electron pair can participate in coordination. It seems than, that explanation b. is correct, namely, it is hydrolysis of the lanthanide ion that causes the perturbation of the complexes formation.

3. The above conclusion has been confirmed by the results of the measurements of the relative degree of hydrolysis (the values of turbidity were taken as relative degree of hydrolysis). The results obtained for 1:1 proline (hydroxyproline)—Ln^{3+} systems indicate that the pH values at which the start of hydrolysis is observed decrease in the series from La^{3+} to Lu^{3+}. This does not agree with the increases of stability constants. No formation of precipitate has been observed for 4:1 and 10:1 proline (hydroxyproline)—Ln^{3+} systems in the investigated range of acidity. The beginning of turbidity for the hydroxyproline—lanthanide system is observed at lower pH than for the proline—lanthanide system. Thus, complexes of proline are more stable

than those of hydroxyproline with lanthanides. This agrees with results of our potentiometric investigations. On the basis of the finding presented above one can finally conclude that it is hydrolysis of the Ln^{3+} ions that causes the perturbations of the complexation in proline (hydroxyproline)—lanthanide systems.

It has been found from a potentiometric study that 1:1 complexes are formed in the 1:1, 2:1, 4:1 and 10:1 proline (hydroxyproline)—lanthanide systems. Changes in the concentration of the metal as well as the ligand do not cause any shift of the formation curves. Thus the conclusion may be drawn that only one kind of complexes occurs. Taking under consideration the formation function values of 4:1 and 10:1 ligand—metal systems, one can conclude that slight amounts of 2:1 complexes exist in solution (Fig. 1 e, f). The stability constants of proline (hydroxyproline) with lanthanide complexes increase in the series from La^{3+} to Lu^{3+}, what is in agreement with the ionic radius change. The decrease of the stability of hydroxyproline—lanthanide complexes relative to proline complexes is probably caused either by steric effects or by an interaction of the hydroxyl group of the ligand molecule. The first conjecture ought to be ruled out because the 1:1 complex predominates in solution and no difficulty should be expected in steric arrangement of ligands around metal ions.

There is a possibility that the hydroxyl group is involved in coordination but no evidence has been found that such a complex exists in solution. The results of the potentiometric study indicate, that no polynuclear complexes are formed in the system. The relatively small differences in the stability constants between proline and hydroxyproline complexes indicate that the mode of coordination of both ligands is similar. The important factor determining the stability of amino acid—metal complexes is the basic dissociation constant (pK_2) of ligand. For proline this constant $(pK_2 = 10.68)$ is greater than for hydroxyproline $(pK_2 = 10.01)$ and that fact is reflected in the values of the stability constants of the complexes. The mechanism of the reaction should also be taken under consideration as the reason for the lower stability of hydroxyproline—lanthanide complexes. The difference in the reaction rates between proline and hydroxyproline complexes with transition metals supports this assumption[11]. There is a possibility that the water molecule from aquo-complex forms a hydrogen bond with the hydroxy group of the hydroxyproline. In consequence, the arrangement of ligand in relation to metal makes formation of the complex difficult, what decreases stability of the complex compared to that of proline. Investigation of the mode of coordination resulting from the experiments presented here as well as NMR (^1H, ^{13}C) measurements will be published in a subsequent paper.

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