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^{1}H and ^{13}C NMR Studies on Lanthanide Complexes with Proline and Hydroxyproline

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The formation and the mode of coordination of rare earth (Y, La, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Lu) complexes with proline and hydroxyproline have been investigated by ^{1}H and ^{13}C NMR spectral techniques. It has been established that the nitrogen and the carboxyl group of the ligands are involved in complexation, and that the OH$^-$ group of hydroxyproline does not participate in coordination.

(Keywords: ^{13}C NMR; Complexes; ^{1}H NMR; Hydroxyproline; Lanthanides; Proline)

^{1}H und ^{13}C NMR Untersuchungen an Lanthanid-Komplexen mit Prolin und Hydroxyprolin

Die Bildung und die Koordination von seltenen Erden (Y, La, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Lu) in Komplexen mit Prolin und Hydroxyprolin wurden mit Hilfe von ^{1}H- und ^{13}C-NMR-Spektroskopie untersucht. Es wurde festgestellt, daß in den Komplexen der Stickstoff und der Carboxylsauerstoff der Liganden koordinieren. Die OH$^-$-Gruppe von Hydroxyprolin nimmt keinen Anteil an der Koordinierung.

Introduction

The mode of coordination of complexes of metal ions with amino acids, peptides and proteins is of fundamental importance when the role of these ions in biological systems is considered. There are a number of functional groups in the above-mentioned compounds and most of them are potential coordination sites. This fact has been confirmed by the studies of bonding between metals and simple amino acids as well as

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their derivatives[1–3]. NMR spectroscopy plays a considerable role in the investigation of molecular problems, especially in the determination of coordination mode of certain metal complexes with amino acids and simple peptides[4–8]. Particularly useful in spectral studies is ^{13}C NMR spectroscopy. There are only single singals in ^{13}C NMR spectrum, separately for every non-equivalent atom of carbon. Moreover, the value of chemical shift is at last one order of magnitude higher than that in the ^{1}H NMR spectrum.

In this paper we present some results and conclusion from ^{1}H and ^{13}C NMR studies concerning complexes of proline and hydroxyproline with lanthanide ions (Y^{3+}, La^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Lu^{3+}). This research has been preceded by our potentiometric studies of composition and stability of proline and hydroxyproline compounds with lanthanides[9]. The latter studies together with results presented in this paper allow to draw some definite conclusions as to the mode of coordination in the analyzed compounds.

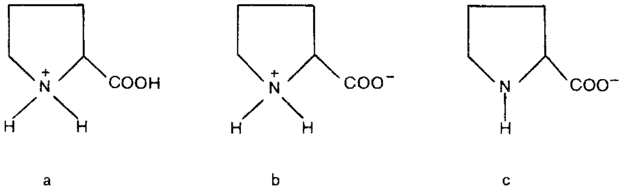
Experimental

Proline by Reanal (Hungary) and hydroxyproline by BDH (England) were purified by precipitation from ethanolic solution with the use of acetone. Precipitation of lanthanide solutions and procedures for potentiometric titrations of metal ions with proline and hydroxyproline have been described in the earlier report[9].

Samples for ^{1}H NMR and ^{13}C NMR measurements were prepared by dissolving the lanthanide chlorides and proline (hydroxyproline) in D_2O. The concentration of proline (hydroxyproline) in the samples for ^{1}H NMR measurements was $7 \cdot 10^{-1} M$, and lanthanide concentrations in subsequent samples were: $0 M$, $2 \cdot 10^{-2} M$, $4 \cdot 10^{-2} M$, $6 \cdot 10^{-2} M$, $10 \cdot 10^{-2} M$, and $12 \cdot 10^{-2} M$. Proline (hydroxyproline) concentration in the samples for ^{13}C NMR measurements was $1 M$ and lanthanide concentrations: $12 \cdot 10^{-2} M$, $5 \cdot 10^{-1} M$, and $8 \cdot 10^{-1} M$, respectively. A *pH* value of 7 was maintained in each sample with the use of NaOD or/and DCl. All solutions were prepared under argon as inert gas. ^{1}H NMR and ^{13}C NMR spectra were recorded with the use of NMR EM-360 60 MHz (Varian) and NMR Fx 90 Q JEOL spectrometers with *DSS* and dioxan, respectively, as standards.

Results and Discussion

Like the majority of simple amino acids, proline and hydroxyproline have two functional groups, both of which are potential coordination sites. Additionally, the OH^- group in hydroxyproline may also be involved in coordination. In order to examine the possibility of nitrogen—lanthanide binding, titrations of lanthanide solutions have been performed with ligands. In the *pH* range under investigation (6.0-7.8), the following ion species occur in equilibrium:



The "b" species is predominant in the system: however, the anionic from "c" participates in complexation. The involvement of the anionic form of amino acids in coordination disturbs an equilibrium between the ionic species in solution. Thus, in order to maintain the equilibrium, a "zwitterion" undergoes dissociation and the anion produced thereby takes part in reaction with metal. Therefore, the creation of the bond between the metal and nitrogen from proline or hydroxyproline must be accompanied by a decrease of the *pH* value during the titration of the metal with ligand. This is so, because a proton is dissociated when a "zwitterion" converts into an anionic species suitable for complexation. The resulting titration curves[9] indicate that the binding occurs between a lanthanide and nitrogen of proline (hydroxyproline).

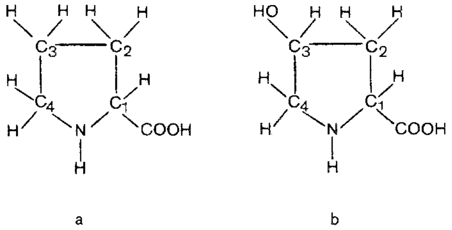


Fig. 1. Ligands: *a* proline; *b* hydroxyproline

Next, the site of coordination in proline and hydroxyproline has been studied with the use of ^{1}H NMR spectroscopy. There are three signals in the ^{1}H NMR spectrum of proline: a signal at 4.10 ppm due to C(1) proton, a signal at 3.14 ppm due to C(4) protons and two overlapping signals at 2.08 ppm from almost equivalent groups $-C(2)H_2$ and $-C(3)H_2$ (Fig. 1 *a*).

In the spectrum of hydroxyproline there are four signals due to the presence of a hydroxyl group in the molecule and non-equivalence of the protons at C(2) and C(3) positions: 4.72 ppm signal from the proton at C(3), 4.40 ppm signal from the proton at C(1), 3.47 ppm signal from the protons at C(4), and 2.35 ppm signal from the protons at C(2) (Fig. 1 *b*).

Spectra of proline and hydroxyproline complexes with lanthanide ions show a change in the chemical shift when compared with the signals of a metal-free ligand. The greatest change is observed for both the signals from the protons at C(1) and C(4) of proline—lanthanide systems [the changes at C(1) vary from 0.4 ppm for La^{3+} to 2.0 ppm for Tb^{3+}, and at C(4) from 0.25 ppm for La^{3+} to 1.2 ppm for Tb^{3+}] as well as the signals from analogous protons of hydroxyproline—lanthanide systems [in this case the changes of chemical shift for protons at C(1) range from 0.3 ppm for La^{3+} to 1.6 ppm for Tb^{3+} and at C(4) from 0.2 ppm for La^{3+} to 0.8 ppm for Tb^{3+}]. Among the investigated lanthanides lanthanum and lutetium form diamagnetic ions while the others form paramagnetic ions. As the result of complexation with paramagnetic ions the shifts of NMR signals of the protons run in the direction of the signals originating from the created lanthanide—proline (hydroxyproline) complex. The largest change is observed for the protons neighbouring with the coordination site of the ligand. In case of proline and hydroxyproline the greatest shift of the peak positions comes from the protons located near nitrogen and carboxyl groups. The dependence of the observed shifts of signals (for proline—Tb, and hydroxyproline—Tb systems) on concentration of the metal is presented in Fig. 2.

Similar curves have been obtained for other paramagnetic lanthanides. The changes of chemical shift indicate that binding occurs at nitrogen and carboxyl group of proline and hydroxyproline. The *trans*-location of the peaks originating from hydroxyproline is smaller than the shift of the analogous peaks of proline. The shift of the signals from protons at C(2) as well as C(3) of proline and hydroxyproline occurs also as the result of a pseudocontact interaction. Due to the fact, that the distance of these protons from the lanthanide ion is much greater than the distance of the ligand protons at C(1) and C(4), the corresponding shifts of peaks are small.

Only for the lanthanides Eu and Tb which cause strong shifts of signals, splitting of the peaks assigned to C(2) and C(3) protons has been observed (Fig. 3). There is only one multiple signal originating from C(2) and C(3) protons of metal-free proline (Fig. 4).

The pseudocontact interaction between the ions and the protons at C(2) and C(3) of hydroxyproline causes an even smaller translocation of signals.

The band due to protons at C(3) is shifted more than the signal of protons at C(2) (0.3 ppm and 0.15 ppm for Tb^{3+}, respectively). The shift

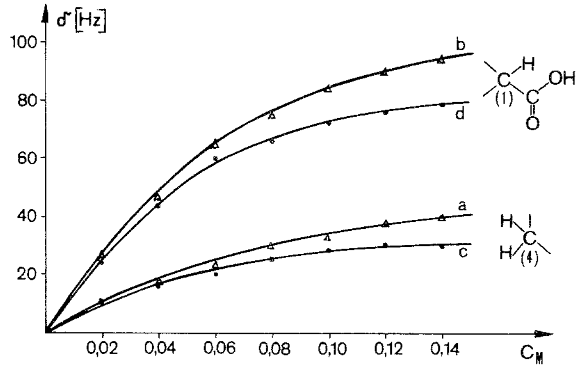


Fig. 2. The observed shifts of signals in ^{1}H NMR spectra: *a*, *b* proline—Tb^{3+}; *c*, *d* hydroxyproline—Tb^{3+}

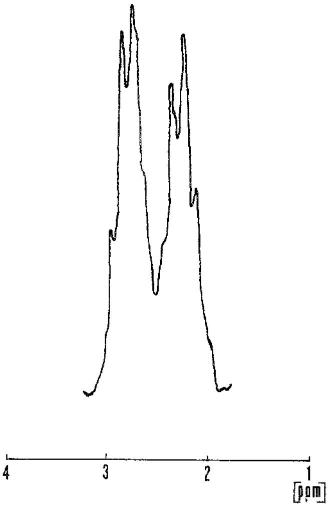


Fig. 3. ^{1}H NMR spectrum of proline—Tb^{3+} (metal concentration of 0.04 *M*)

of signal assigned to protons directly neighbouring with the hydroxyl group of hydroxyproline makes one to consider a possibility of coordination between the OH$^-$ group and the lanthanide ions. A considerable translocation of signals of hydroxyproline protons at C(2) and C(3) has been observed (0.1 ppm and 0.2 ppm for Eu^{3+}, 0.15 ppm and 0.3 ppm for Tb^{3+}, 0.15 ppm and 0.2 ppm for Dy^{3+}, respectively).

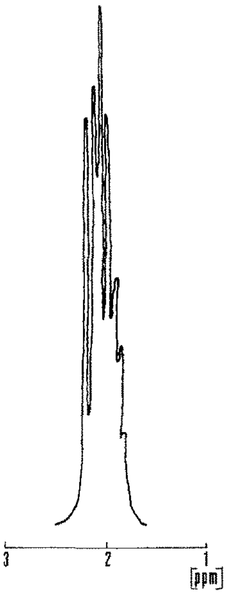


Fig. 4. ^{1}H NMR spectrum of metal-free proline

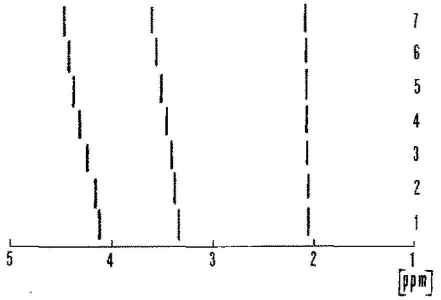


Fig. 5. Positions of the ^{1}H NMR peaks of the proline—La^{3+} system. Concentration of La^{3+}: *1* metal-free system; *2* 0.02 *M*; *3* 0.04 *M*; *4* 0.06 *M*; *5* 0.08 *M*: *6* 0.10 *M*; *7* 0.12 *M*.

In case of diamagnetic ions (La^{3+}, Lu^{3+}) the observed shift of ^{1}H NMR peaks is a result of changes in the screening of proton caused by a change of electron density around nitrogen and carbon. The series of La^{3+}—proline spectra are presented in Fig. 5. A slightly greater shift has been observed for lutetium [C(1)—0.5 ppm, C(4)—0.34 ppm].

Table 1. *Positions of* ^{13}*C NMR signals of the proline*—Pr^{3+} *system* [Hz] *(dioxan as standard)*

Proline $c = 1\,M$	C(1)	C(4)	C(5)	C(3)	C(2)
$c_{\mathrm{Pr}} = 0\,M$	−5.417	−20.562	+107.968	−37.595	−42.822
$c_{\mathrm{Pr}} = 0.12\,M$	−1.631	−20.235	+108.838	−37.469	−42.560
$c_{\mathrm{Pr}} = 0.4\,M$	+7.245	−19.517	+108.292	−37.142	−41.973
$c_{\mathrm{Pr}} = 0.6\,M$	+9.268	−19.256	+108.621	−37.011	−41.842

Table 2. *Positions of* ^{13}*C NMR signals of the hydroxyproline*—Pr^{3+} *system* [Hz] *(dioxan as standard)*

Hydroxyproline $c = 1\,M$	C(1)	C(4)	C(5)	C(3)	C(2)
$c_{\mathrm{Pr}} = 0\,M$	−6.723	−13.642	+107.640	+3.459	−29.113
$c_{\mathrm{Pr}} = 0.12\,M$	−3.328	−13.381	+107.640	+3.589	−29.113
$c_{\mathrm{Pr}} = 0.4\,M$	+5.482	−12.728	+108.031	+4.047	−28.787
$c_{\mathrm{Pr}} = 0.6\,M$	+7.767	−12.533	+108.422	+4.242	−28.591

Similar changes of chemical shifts for hydroxyproline—lanthanum system [C(1)—0.2 ppm, C(4)—0.15 ppm] and for hydroxyproline—lutetium system [C(1)—0.4 ppm, C(4)—0.2 ppm] as well as the absence of any translocation of the peaks assigned to the protons at C(3) (for La^{3+} and Lu^{3+} complexes) neighbouring with OH^{-} allow to rule out a supposition of the involvement of the hydroxyl group in coordination. This conclusion has been confirmed by ^{13}C NMR spectra of proline—Pr^{3+} and hydroxyproline—Pr^{3+} systems. There are no considerable differences in ^{1}H NMR spectra of proline (hydroxyproline)—paramagnetic lanthanide ion systems. This finding allows to generalize results of ^{13}C NMR study obtained for proline (hydroxyproline)—Pr^{3+} system to other investigated paramagnetic ions. Additionally, similar values of the stability constants of complexes indicate that the structure of these compounds must be alike.

The band positions of the obtained spectra (with dioxan as standard) are presented in Table 1 and 2. The largest change in chemical shift has been observed in the signal from the C(1) atom which is located between nitrogen and carboxyl group. The smaller shifts occur in case of C(5) and C(4) signals. Of special importance is the translocation of C(3) and C(2) signals in the hydroxyproline—lanthanide system. The chemical shift is in this case of the same order of magnitude as in the proline—lanthanide system, what indicates that the hydroxyl group of hydroxyproline does not participate in coordination. The results of the spectral study presented above show that nitrogen and carboxyl group are the coordination sites in proline (hydroxyproline)—lanthanide complexes. In addition, the possibility of the involvement of the OH^- group in complexation can be ruled out.

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