DALTON
FULL PAPER

Nature of equilibrium shifts in racemic praseodymium(III) tris(2,2′-oxydiacetate) induced by interaction with chiral probes

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Received 12th October 2001, Accepted 8th February 2002
First published as an Advance Article on the web 19th March 2002

The effects of L-proline and its derivatives *N*-acetyl-L-proline, L-proline methyl ester, and L-proline benzyl ester on the equilibrium shift in the racemic $Pr(ODA)_3^{3-}$ complex, in which ODA is 2,2′-oxydiacetate, have been studied by means of circular dichroism (CD) and ^1H NMR spectroscopy. While *N*-acetyl-L-proline did not induce any optical activity in $Pr(ODA)_3^{3-}$, the addition of L-proline, L-proline methyl ester, and L-proline benzyl ester led to the appearance of the Pfeiffer effect in this lanthanide complex. The signs of the induced CD signals at 484 nm which correspond to the $^3P_0 \longleftarrow {}^3H_4$ transition implied that L-proline interacts more favourably with the Λ-enantiomer of the $Pr(ODA)_3^{3-}$, while L-proline methyl ester and L-proline benzyl ester preferably interact with the Δ-enantiomer of the same complex. The ^1H NMR studies suggested that L-proline and its esters adopt different conformations upon interaction with $Pr(ODA)_3^{3-}$. The dependence of the absorption dissymmetry factor, g_{abs}, on the concentration of the L-proline indicated that the Pfeiffer effect in this lanthanide complex is best described by the associated model. Measurements in the solution of various pH, ionic strength, and dielectric constant suggested that the chiral discriminatory interaction is a combination of electrostatic and hydrophobic forces, while the hydrogen bonding effects are less important.

Introduction

The interaction of an optically active probe with a labile racemic metal complex may induce a shift in the racemic equilibrium, resulting in an excess of one enantiomer over the other one. This phenomenon was first observed by Pfeiffer and has subsequently been named the "*Pfeiffer Effect*",[1] in honour of its discoverer. The confirmation that the effect was due to an equilibrium shift was provided by experiments with transition metal complexes which showed that for selected systems the resultant circular dichroism (CD) spectrum was identical to the CD of the resolved enantiomers.[2] The effect has been extensively studied in the case of transition metal complexes, and the induced optical activity associated with d–d bands is quite well understood.[3] However, the analogous studies regarding f–f optical activity of the lanthanide complexes have only been achieved more recently.[4–10] The method has proven to be useful to study f–f optical activity in trigonal lanthanide complexes, since the extremely fast racemization rates preclude resolution of these complexes by classical methods.

The development of circularly polarized luminescence (CPL), in which one measures the difference in emission of left and right circularly polarized light by optically active molecules, enabled monitoring of induced equilibrium shifts in labile luminescent complexes through excited state populations. Most extensive studies have been performed on 9-coordinate tris-terdentate lanthanide complexes with 2,6-pyridinedicarboxylate (DPA), since these complexes are highly optically active and luminescent.[4,7,11] Contrary to transition metal complexes, no comparison of the CPL spectra could be made to the pure or partially resolved system, because $Ln(DPA)_3^{3-}$ complexes racemize too quickly for chemical resolution. However, Hilmes *et al*, demonstrated that CPL could be measured from a photo-enriched excited state of $Tb(DPA)_3^{3-}$ complex if one used circularly polarized excitation and if the lifetime of the excited state was shorter than the time required for chemical racemization.[11] Comparison of the CPL spectra from the photo-enriched sample and the system perturbed by the addition of a Pfeiffer agent showed that the total emission and CPL line shape were identical, suggesting that the source of the increased optical activity was, in fact, due to an equilibrium perturbation in racemic $Tb(DPA)_3^{3-}$.

We recently showed that an equilibrium shift could be induced in lanthanide tris complexes with 2,2′-oxydiacetic acid (ODA), $Ln(ODA)_3^{3-}$, and that resulting optical activity could simply be monitored by CD spectroscopy.[12] The examination of CD spectra of nine members of the $Ln(ODA)_3^{3-}$ series, upon addition of L-proline, enabled correlation of the absolute configuration of these complexes and their chiroptical properties. This was of particular importance, since numerous theoretical studies were done on these complexes in order to obtain information on the nature of $f^N \longrightarrow f^N$ transitions, but the correlation between the absolute configuration and the sign of the CD signal remained a difficult issue.[13] We also showed that addition of D-proline to the racemic mixture of any member of $Ln(ODA)_3^{3-}$ series results in an excess of the Δ-enantiomer, while addition of L-proline results in an excess of the Λ-enantiomer of the $Ln(ODA)_3^{3-}$ complex.

In the present study we mainly focus on the nature of the interactions between the $Pr(ODA)_3^{3-}$ complex and several derivatives of L-proline, and we try to determine the driving forces responsible for the recognition. Our system differs from the extensively studied $Ln(DPA)_3^{3-}$ in the respect that, in contrast to the DPA ligand, the ODA ligand does not contain aromatic groups, which appeared to be necessary for the Pfeiffer effect to be measured and which are speculated to play a crucial role in the recognition between the complex and various chiral probes.[7]

Experimental

L-proline (L-Pro), L-proline methyl ester hydrochloride (L-Pro-OMe) and L-proline benzyl ester hydrochloride (L-Pro-OBz),

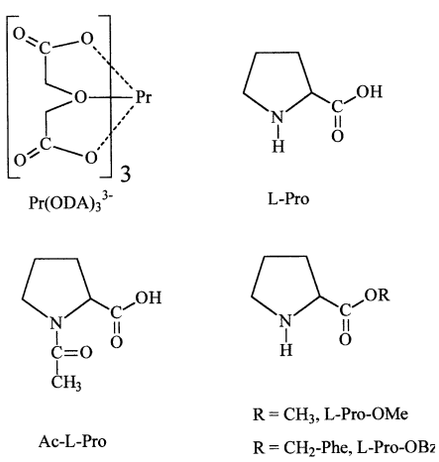
 DOI: 10.1039/b109334g

were obtained from Acros and used as received. *N*-acetyl-L-proline (L-Ac-Pro) was obtained by acetylation of L-proline by a standard method.[14] The complex $Pr(ODA)_3^{3-}$ was synthesised according to a literature procedure.[15] All common chemicals were of reagent grade. The CD spectra were recorded with an AVIV 62DS circular dichroism spectrometer. The UV–VIS spectra were recorded under the same conditions as the CD spectra with a Shimadzu UV-3100 spectrometer. All pH measurements were made with a Consort P602 pH meter, which was calibrated with buffer solutions of pH = 4.00 and 7.00. Proton NMR spectra of D_2O solutions were recorded with Bruker DRX 300 spectrometer.

Results and discussion

Mechanism of associations

The praseodymium(III) tris complex with the dianion of 2,2′-oxydiacetic acid (ODA), $Pr(ODA)_3^{3-}$, shown in Scheme 1, is



Scheme 1

chiral due to the D_3 symmetry arrangement of the 3 ODA ligands around the central metal ion. However, the complex exists in a racemic equilibrium that is not resolvable by classical methods.[15] Since the ODA ligand is achiral, the classical circular dichroism studies on these lanthanide complexes in solution have not been possible yet.

The shift of an initially racemic equilibrium upon adding a chiral probe, to a new equilibrium in which one of the enantiomers is produced in excess, occurs because the chiral probe interacts differently with the enantiomers forming the racemate. Such discriminating interactions may occur either because of diastereoisomer formation, or because of differences in non-covalent interactions between the chiral probe and the enantiomeric metal complex. In order to estimate the discrimination free energy, two extreme models were suggested, termed associated and dissociated, which describe the thermodynamics of the Pfeiffer effect.[16]

In the associated model, the definite aggregates consisting of the metal complex and *n* molecules of the chiral probe are being formed by stepwise association. In the dissociated model, however, it is assumed that the chiral probe can interact with more than one metal complex, *i.e.* the definite aggregates are not being formed. Assuming that the equilibrium shift is not large, one can derive the following expressions which relate the model to the experimental measurements:[17]

$$g_{abs} = -g^{\Lambda}_{abs}\Delta(P)N_P/2RTN \text{ (associated)} \quad (1)$$

$$g_{abs} = -g^{\Lambda}_{abs}\alpha\Delta(P)N_P/2RT \text{ (dissociated)} \quad (2)$$

where g_{abs} is the observed absorption dissymmetry factor, (calculated as a ratio $\Delta A/A$, A being the absorbance), g^{Λ}_{abs} is the absorption dissymmetry which one would observe for the pure Λ-enantiomer, $\Delta(P)$ denotes the chiral discrimination energy (in J mol^{-1}), N_P is the concentration of the chiral probe (in mol L^{-1}), and N is the total concentration of the metal complex (in mol L^{-1}).

In this work we monitor the CD signal of $Pr(ODA)_3^{3-}$ at 484 nm, which corresponds to the $^3P_0 \longleftarrow {}^3H_4$ transition, induced upon addition of L-Pro. $Pr(ODA)_3^{3-}$ was chosen because of its relatively high extinction coefficients compared to other lanthanide complexes in the series, and because its well-separated absorption lines enable accurate calculation of g_{abs}.

The dependence of g_{abs} on the concentration of the L-Pro is shown in Fig. 1. In agreement with eqn. 1 and 2, the linear

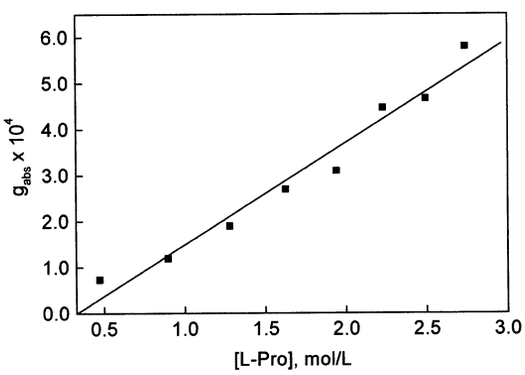


Fig. 1 Absorption dissymmetry factor, g_{abs} at 484 nm, *versus* the concentration of added L-Pro.

dependence of g_{abs} on the concentration of the L-Pro is expected to be observed in both the associated and dissociated models. In order to determine which model fits best to our system, we measured the dependence of g_{abs} on the ratio L-Pro/$Pr(ODA)_3^{3-}$ (which corresponds to N_P/N). According to eqn. 1, for the associated model this dependence should be linear, while according to eqn. 2 for the dissociated model, it should not. The linear dependence shown in Fig. 2 clearly suggests that our system exhibits the behaviour predicted by the associated model.

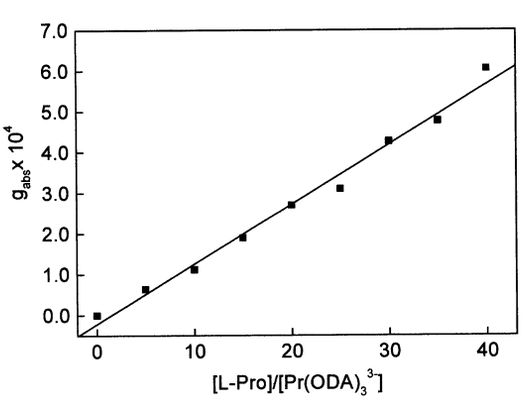
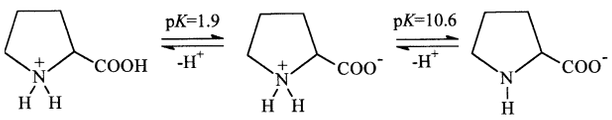


Fig. 2 Absorption dissymmetry factor, g_{abs} at 484 nm, *versus* the ratio [L-Pro]/[$Pr(ODA)_3^{3-}$]. A linear dependence corresponds to the associated model.

Some previous studies, which investigated the CPL of $Tb(DPA)_3^{3-}$ upon addition of L-histidine, disagree whether this complex interacts with the chiral probe in the associated or in the dissociated mode.[4,17] Compared to the studies with the $Tb(DPA)_3^{3-}$/L-histidine system, we extended the ratio of N_P/N under which g_{abs} was measured. While in the previous studies the ratio of N_P/N did not exceed 10, we show that up to a ratio of N_P/N of 40 our system exhibits the behaviour predicted by the associated model.

Effects of pH on the association

Since $Pr(ODA)_3^{3-}$ is negatively charged, one may expect that the charge of the chiral probe would be crucial for the interaction. The charge of the chiral probe may be very sensitive to the changes in pH, and therefore we examined the effects of pH on the magnitude of the Pfeiffer effect induced in $Pr(ODA)_3^{3-}$ upon addition of L-Pro. The ionization sequence of proline, shown in Scheme 2, is well established.[18] Scheme 2 shows that in



Scheme 2

the range of pH between 1.9 and 10.6 the proline is electroneutral overall, with a positively charged amino part and a negatively charged carboxylate group. Studying effects of pH outside this range unfortunately proved to be difficult for our system. At a pH below 2, the $Pr(ODA)_3^{3-}$ complex is being destroyed and the H_2ODA acid starts to precipitate. Increasing the pH above 10 tended to hydrolyze the complex, resulting in formation of various praseodymium(III) hydroxide species, which precluded accurate determination of g_{abs}. However, we were still able to gain some insights on the effects of pH by carefully measuring g_{abs} within the range in which no precipitation occurred.

Our measurements indicate that upon decreasing the pH from 10.0 to 9.0, g_{abs} increases from 3.9×10^{-4} to 4.8×10^{-4}, and remains practically constant upon further decrease of pH. Considering the protonation constants for proline shown in Scheme 2, one can easily calculate the ratio between the electroneutral form of L-Pro and $Pr(ODA)_3^{3-}$ for any pH value, assuming that at the high concentrations used in this study the tris-chelate $Pr(ODA)_3^{3-}$ is fully formed and that the bis-chelate $[Pr(ODA)_2(H_2O)_3]^{1-}$ is not present. The calculations indicate that under the conditions in which the concentrations of $Pr(ODA)_3^{3-}$ and L-Pro are 0.068 and 2.74 M, respectively, this ratio is *circa* 32 at pH 10.0, while at pH 9.0 it is *circa* 40, and this value remains practically constant when the pH is further decreased. The fact that g_{abs} increases as the concentration of the electroneutral form in the solution is increasing implies that the form in which nitrogen bears the positive charge interacts more favourably with the $Pr(ODA)_3^{3-}$ than the form in which nitrogen lost its charge. This is not surprising when one considers the pure electrostatic attraction of the negative $Pr(ODA)_3^{3-}$ complex with the positively charged amino group in L-Pro. However, our finding is in contrast with CPL studies done on $Tb(DPA)_3^{3-}$ in the presence of L-Pro, in which the maximum interaction was observed at pH 11, when proline is largely negatively charged.[4] This finding led to the conclusion that the main interaction between the complex and the probe involves specific bonding between the amino group of proline and the DPA π-orbitals. However, since the ODA ligand does not contain an aromatic functionality, this type of hydrogen bonding is not possible. We therefore propose that in our system some other interactions, likely electrostatic in nature, must be responsible for the recognition between the metal complex and the chiral probe.

Effects of solvent

In order to further examine the importance of the electrostatic component on the recognition between $Pr(ODA)_3^{3-}$ and L-Pro, we examined the effect of the dielectric constant of the solution on g_{abs}. The dielectric constant of the aqueous solution is varied by using different ratios of formamide : water and DMSO : water. Compared to water, which has a dielectric constant of $\varepsilon = 78$, the dielectric constants of formamide and DMSO are $\varepsilon = 111$ and 47, respectively. As shown in Fig. 3,

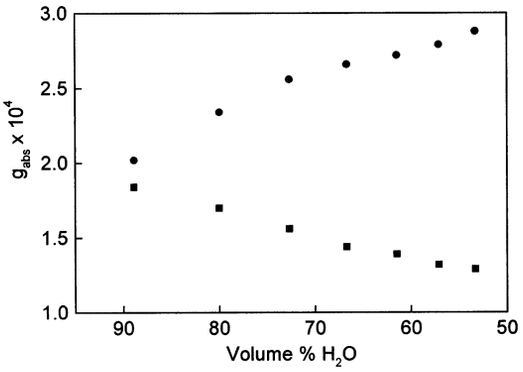


Fig. 3 Absorption dissymmetry factor, g_{abs} at 484 nm, *versus* mole fraction H_2O for DMSO : water (circles) and formamide : water (squares) solutions.

when the dielectric constant of the solution is increased by the addition of formamide, the magnitude of g_{abs} decreases, indicating a smaller equilibrium shift due to a mediation of the proline–lanthanide interactions. The opposite effect is observed when the dielectric constant of the solution is decreased by the addition of DMSO. In this case the magnitude of g_{abs} increases due to the enhanced interaction between proline and the praseodymium complex. A similar trend was previously observed in the $Tb(DPA)_3^{3-}$/L-histidine system in which the magnitude of g_{em} was measured.[17] Similar to that study, we did not observe any evidence for adduct formation between the DMSO and the lanthanide ion.

The importance of the electrostatic component was further demonstrated by examining the effects of ionic strength on g_{abs}. An increase in ionic strength, which was achieved by adding 1.5 M NaCl to an aqueous solution containing 81 mM $Pr(ODA)_3^{3-}$ and 1.62 M L-Pro resulted in a decrease of g_{abs} from 2.9×10^{-4} to 1.9×10^{-4}, presumably because of the reduction of the electrostatic interactions between the complex and the chiral probe.

Some transition metal studies have proposed that hydrogen bonding plays an important role in the Pfeiffer effect. In our system, the only possible direct hydrogen bonding is between the amino protons on proline and the carboxyl oxygen on the ODA ligand. Our studies in H_2O/DMSO solutions do not support the existence of this hydrogen bonding. Upon increasing the concentration of DMSO, g_{abs} increased although DMSO is devoid of hydrogen bonding. The opposite effect would be expected if the hydrogen bonding does play an important role. However, one cannot completely exclude the existence of hydrogen bonding, because it is also possible that the data shown in Fig. 3 represent the composite effect of DMSO on g_{abs}, with the electrostatic component clearly dominating over the hydrogen bonding interactions.

Effects of the nature of the chiral probe

Although the electrostatic component seems to play an important role in the recognition between the praseodymium complex and L-proline, it can not be solely responsible for the association. The fact that other amino acids such as alanine, lysine and glutamine, which also contain a positively charged amino group (and in the case of lysine even an additional positively charged amino group in the side chain), do not induce CD in the $Pr(ODA)_3^{3-}$ implies that van der Waals interactions between the praseodymium complex and L-proline are also important for the recognition.

Scheme 1 shows derivatives of proline in which functional groups have been varied in a systematic way. Addition of up to 15 molar excess of L-Ac-Pro to a 0.05 M solution of $Pr(ODA)_3^{3-}$ did not induce observable CD signal at 484 nm. L-Ac-Pro differs from the proline in the respect that the proton in the amino group is converted into the bulkier acetyl group.

This could lead to the preclusion of the optimal hydrophobic contacts between the $Pr(ODA)_3^{3-}$ and the chiral probe, since the probe may be too large to "enter" the pockets formed between the three ODA ligands. However, it is more likely that the inclusion of the positive charge on the amide group of L-Ac-Pro, removes much of the driving force responsible for the association.

An interesting effect was observed upon addition of L-Pro-OMe to the $Pr(ODA)_3^{3-}$ complex (Fig. 4). Compared to the CD

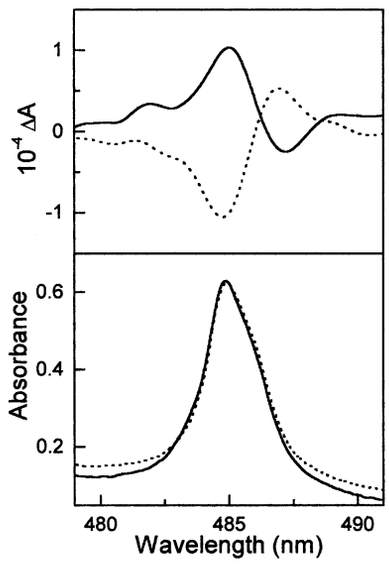


Fig. 4 Absorption spectrum (a) and CD-spectrum (b) of the $^3P_0 \longleftarrow {}^3H_4$ transition of 81 mM $Pr(ODA)_3^{3-}$ in the presence of 1.62 M L-Pro (full line) and L-Pro-OMe (dotted line).

spectrum obtained with L-Pro, the spectra obtained with L-Pro-OMe have identical shape and intensity, but an opposite sign. This implies that contrary to L-Pro, which interacts more favourably with the Λ-enantiomer of the $Pr(ODA)_3^{3-}$ complex, L-Pro-OMe preferably interacts with the Δ-enantiomer of the same complex. This fact is quite surprising if one keeps in mind that the configuration in all three compounds is the same, namely L. Similarly to L-Pro-OMe, L-Pro-OBz also preferably interacts with the Δ-enantiomer of the $Pr(ODA)_3^{3-}$ yielding the negative CD signal at 484 nm. The question is why does the simple conversion of the carboxylate group into an ester cause such a significant change in the affinities toward different enantiomers of $Pr(ODA)_3^{3-}$?

Proton NMR measurements provided some clues to answer this question. It has been known that Pr^{III} complexes induce a shift in proton resonances in various organic compounds, and therefore are often used as shift reagents.[19] The magnitude of this shift varies with the nature of the probe (reflecting the degree of complexation) and with the particular proton resonance being studied. In principle, the protons closest to the Pr^{III} ion shift to the greatest degree. The effects of 10 molar excess of $Pr(ODA)_3^{3-}$ on proton resonances in L-Pro-OMe are shown in Fig. 5. While the methyl group at 3.82 ppm remained unaffected by the addition of the praseodymium complex, Hα and Hδ resonances, at 4.47 and 3.40 ppm respectively, which are closest to the amino group shifted upfield by approximately 0.1 ppm. In contrast, the NMR measurements performed under the same conditions with L-Pro showed that the protons which shifted most upon addition of $Pr(ODA)_3^{3-}$ were Hα and Hβ while Hδ remained practically unaffected. This implies that conformations of L-Pro and L-Pro-OMe in which they interact with the $Pr(ODA)_3^{3-}$ are different, which can explain why they exhibit different preferences toward the two enantiomers of $Pr(ODA)_3^{3-}$.

An interesting finding which resulted from NMR measurements is shown in Fig. 5b. Due to the fast exchange with H_2O, the amine proton could not be observed in either L-Pro or L-Pro-OMe. However, upon addition of $Pr(ODA)_3^{3-}$ a broad resonance at 8.75 ppm, corresponding to the amino proton, appeared in both cases, indicating that this exchange is slowed down. It is very likely that the interaction of the positively charged amino group with the negatively charged $Pr(ODA)_3^{3-}$ complex "locks" the amino proton so that it could be observed by NMR.

Conclusions

We showed that the Pfeiffer effect may be induced in a nine-coordinate lanthanide complex which differs from the extensively studied $Ln(DPA)_3^{3-}$. The nature of the outer-sphere interaction is rather complex, but it seems that the recognition between the lanthanide complex and the chiral probe consists

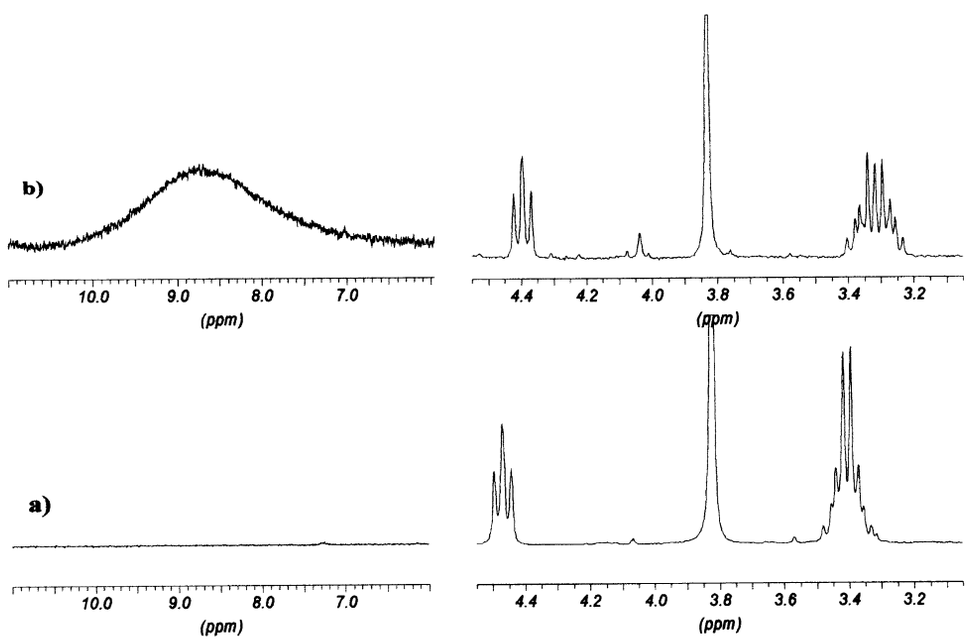


Fig. 5 Proton NMR spectrum of 5mM L-Pro-OMe in the absence (a) and in the presence (b) of 50 mM $Pr(ODA)_3^{3-}$. The right portion of the spectrum corresponds to part of the aliphatic region in L-Pro-OMe, while the left portion corresponds to the amino region of the same amino acid.

of a combination of electrostatic and hydrophobic forces, while the hydrogen bonding effects are less important. The induced optical activity arises from the associative mechanism in which definite aggregates between the lanthanide complex and the chiral probe are formed. Further proof that an association between $Ln(ODA)_3^{3-}$ and L-Pro takes place (thus demonstrating that the induced CD is not due to the creation of a dissymmetric environment) was provided by using $Pr(ODA)_3^{3-}$ to induce NMR shifts in proton resonances of this amino acid. Different affinities of the L-Pro and its esters, L-Pro-OMe and L-Pro-OBz, toward Δ and Λ-enantiomers of $Pr(ODA)_3^{3-}$ are probably due to the different conformations which these substrates adopt to achieve maximal interaction with the praseodymium complex. This shows that simply choosing either free amino acid L-Pro or one of its esters can determine which enantiomer of $Pr(ODA)_3^{3-}$ is produced in excess in the solution. Given the extreme lability of the $Pr(ODA)_3^{3-}$ complex, the Pfeiffer effect may be the only method by which one can study the optical activity of this complex in the solution.

Acknowledgements

K. Binnemans is a Postdoctoral Fellow of the F.W.O.-Flanders (Belgium). Financial support by K.U. Leuven (GOA 98/03) and by the F.W.O.-Flanders (G.0243.99) is gratefully acknowledged.

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