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Fast enantioselective amino acid quantitative ^{13}C NMR determination by a praseodymium chiral shift reagent

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ABSTRACT

The fast quantitative determination of enantiomers of amino acids by the use of a praseodymium-based water-soluble chiral shift reagent, utilizing natural abundance ^{13}C NMR spectra, is described.

1. Introduction

^{13}C NMR is rarely used for quantitative analytical purposes because of the long relaxation time, the relatively low natural isotopic abundance, low magnetic sensibility and the necessity of the elimination of NOE effect.[1] Herein we report a fast quantitative ^{13}C NMR method for the enantioselective determination of free amino acids utilizing the particular magnetic features of praseodymium.[2] This method is based on a water-soluble chiral shift reagent of Pr(III), which is an additional advantage for samples containing free amino acids.

2. Results and discussion

The chiral shift reagent used in our studies was generated in situ by reacting $PrCl_3$ (anhydrous) with an excess (*S*)-PDTA (PDTA = *N*,*N*,*N*′,*N*′-tetrakis[(hydroxycarbonyl)methyl]-1,2-diaminopropane), which was prepared by a published method.[3] The reaction of the Pr(III) ions with this polydentate ligand is fast and quantitative.[4] This reagent could be used in water containing D_2O (just enough for the lock signal). The possibility that normal water can be used in these experiments represents an advantage because less deuterated water would be required and the aqueous amino acid samples can be analyzed directly.

Pr(III), as with several other lanthanide (Ln) metal ions, has advantageous relaxation effects as described in the literature.[5] These relaxation properties are so efficient that the ^{1}H NMR spectra can only be interpreted with difficulty, since the fine structure usually gets totally lost. The situation radically changes in the ^{13}C NMR spectra, where the signals get only slightly broader than in the spectra without added Ln compounds (from 1–2 Hz to 5–10 Hz). An additional advantage is that ^{13}C NMR spectra extend over about a 200 ppm range, while the usual range of ^{1}H NMR spectra is approximately 10 ppm. Combining these attractive features with the chirality of (*S*)-PDTA ligand, excellent splitting of the signals corresponding to the enantiomers of natural amino acids can be obtained (Fig. 1).

Without an additive or derivatization, good quality ^{13}C NMR spectra can be obtained for water-soluble amino acids, in the solvent mentioned above, with an acquisition time of a few (3–5) hours, without chiral information. In the presence of the Pr(III)/(*S*)-PDTA reagent, however, the time is reduced to ~0.5 h (38 min), which corresponds to the lowest instrumentally determined possible acquisition time for natural amino acid samples. Even shorter measurement times, in the order of 5–6 min, can be used with a lower number of scans and these still enable to obtain spectra which can be suitably evaluated for quantitative purposes.

The peaks of the enantiomers of the water-soluble free amino acids (Tables 1 and 2) could be excellently resolved by the use of the Pr(III)/(*S*)-PDTA chiral shift reagent and the integrals of the corresponding spectral signals gave back very well the weighed ratio of enantiomers in synthetic L/D mixtures (Table 3). We also studied the linearity of the L/D ratio with respect to the weight ratio of the enantiomers in their mixtures (Table 4). This was found to be very good (R = 0.9997), as shown also in Figure 2.

The above results appear to be suitable as the basis of fast and routine analysis of samples containing free, water-soluble, amino acids.

Beyond the fast and simple analytical application enabled by the Pr(III)/(*S*)-PDTA reagent, we should point out another interesting possibility. The ^{13}C NMR spectra obtained by this reagent are of such a good quality, that these enable via a bidimensional analysis (Fig. 3), the trustworthy assignment of the ill-resolved ^{1}H NMR spectra. ^{1}H NMR spectra thus became a useful tool for the analysis of *more diluted* samples, with the proton resonances being ~6000 times[6] more sensitive than the (natural abundance) ^{13}C-signals.

We tried to obtain some insight into the mode of action of the chiral shift reagent described above by UV–vis/CD spectroscopy.

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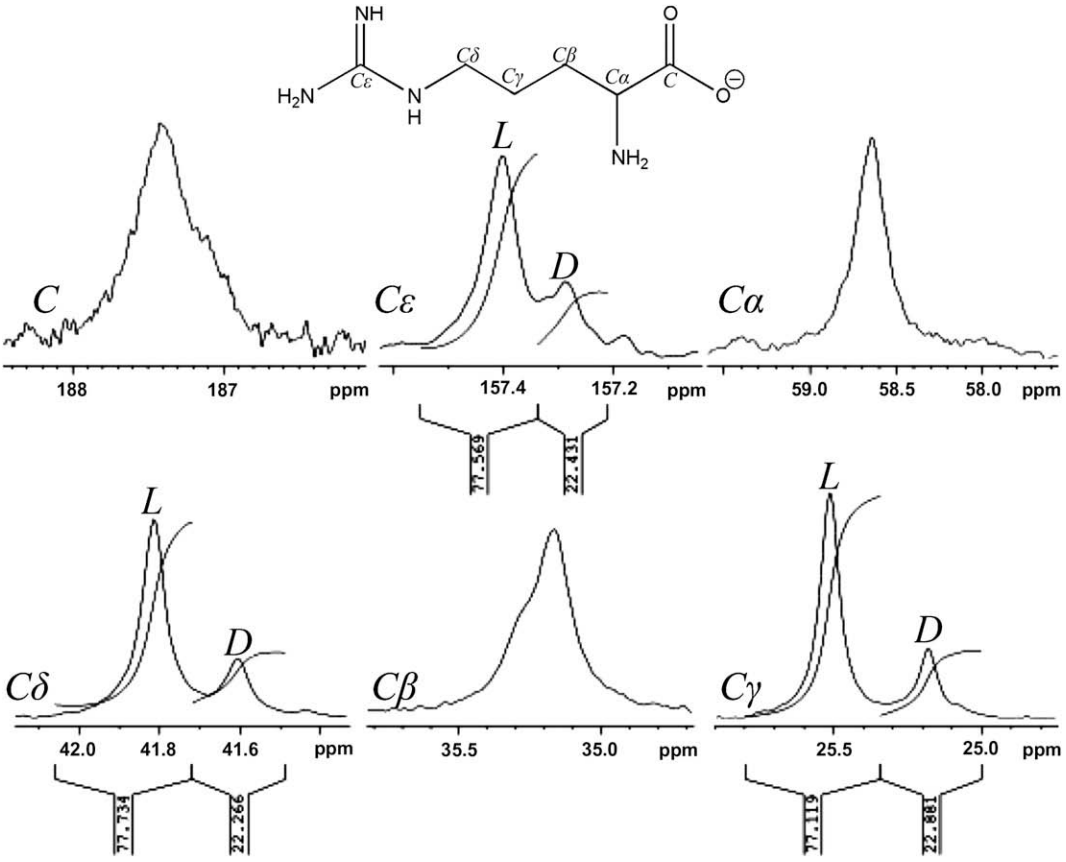

Figure 1. ^{13}C NMR spectrum of L/D Arg in the presence of the Pr(III)/(S)-PDTA in H_2O/D_2O solvent mixture.

Table 1
^{13}C NMR signals of L amino acids (natural abundance) in the presence of Pr(III)/(*S*)-PDTA

AA	Weighed L/D ratio (%)	Signal						
		COO	αCH	Lateral group				
Arg	77.185, 22.815	[a]	[a]	βCH_2:[a]	γCH_2: 77.12, 22.88	δCH_2: 77.18, 22.83	εC: 76.61, 23.39	
Asn	75.780, 24.220	[b]	74.22, 25.78	βCH_2:[b]	CO: 75.75, 24.25			
His	75.489, 24.511	[a]	75.98, 24.02	βCH_2: 76.20, 23.80	γCH_2: 76.48, 23.52	C3: 75.71, 24.29	δCH_2: 76.85, 23.15	
Met	68.557, 31.443	[a]	[b]	βCH_2: 68.42, 31.68	γCH_2: 69.30, 30.70	SCH_3: 68.84, 31.26		
Phe	65.817, 34.183	[a]	[a]	βCH_2:[a]	Ph, C1:[b]	C2/C6: 65.66, 34.34	C3/C5:[b]	C4: 65.52, 34.48
Pro	74.134, 25.866	[a]	[b]	βCH_2:[a]	γCH_2: 73.72, 26.28	δCH_2:[b]		

[a] No splitted signal.
[b] Shoulder signal.

Table 2
Splitting of the ^{13}C NMR signals of enantiomers of amino acids (natural abundance) in the presence of Pr(III)/(*S*)-PDTA

AA	$\Delta\delta$[a] Signal						
	COO	αCH	Lateral group				
Ala	0.312	[b]	CH_3: 0.347				
Arg	[b]	[b]	βCH_2:[b]	γCH_2: 0.336	δCH_2: 0.212	εC: 0.118	
Asn	[c]	−0.30	βCH_2: −2.02	CO: −1.89			
His	[b]	0.61	βCH_2: 0.51	γCH_2: 1.10	δCH_2: 0.316	εCH_2:[b]	
Met	[b]	[b]	βCH_2: −0.125	γCH_2: 0.309	SCH_3: 0.142		
Phe	[b]	[b]	βCH_2:[b]	Ph: C1:[c]	C2/C6: 0.11	C3/C5:[c]	C4: 0.06
Pro	[b]	[c]	βCH_2:[b]	γCH_2: 0.152	δCH_2:[c]		

[a] $\Delta\delta = \delta_L - \delta_D$.
[b] No split signal.
[c] Shoulder signals.

Table 3
Comparison of the integrated areas and the corresponding weighed ratios of enantiomers in mixtures of L/D amino acids

AA	Weighed L/D ratio (%)	Signal						
		COO	αCH	Lateral group				
Arg	77.185, 22.815	[a]	[a]	βCH_2:[a]	γCH_2: 77.12, 22.88	δCH_2: 77.18, 22.83	εC: 76.61, 23.39	
Asn	75.780, 24.220	[b]	74.22, 25.78	βCH_2:[b]	CO: 75.75, 24.25			
His	75.489, 24.511	[a]	75.98, 24.02	βCH_2:76.20, 23.80	γCH_2: 76.48,23.52	C3: 75.71, 24.29	δCH_2: 76.85, 23.15	
Met	68.557, 31.443	[a]	[b]	βCH_2: 68.42, 31.68	γCH_2: 69.30, 30.70	SCH_3: 68.84, 31.26		
Phe	65.817, 34.183	[a]	[a]	βCH_2:[a]	Ph, C1:[b]	C2/C6: 65.66, 34.34	C3/C5:[b]	C4: 65.52, 34.48
Pro	74.134, 25.866	[a]	[b]	βCH_2:[a]	γCH_2: 73.72, 26.28	δCH_2:[b]		

[a] No split signal.
[b] Shoulder signal.

Table 4
Relative errors of the best integrated areas of the ^{13}C NMR signals with respect to the weighed L/D ratios of amino acid enantiomers in the presence of Pr(III)/(*S*)-PDTA

AA	ee (L/D)		Relative error (%)
	Weighed	Best measured	
Arg	54.37360	54.35	0.0434
Asn	51.55143	51.50	0.0998
His	50.97821	51.42	−0.8666
Met	37.11392	36.74	1.007
Phe	31.63418	31.32	0.993
Pro	48.26706	47.44	1.714

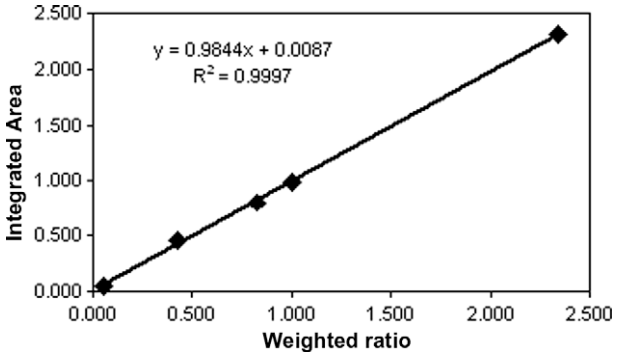

Figure 2. The linearity of the L/D ratio with respect to the weight ratio of the enantiomers in mixtures of L- and D-alanine.

The spectra of solutions containing (a) amino acid alone, (b) praseodymium chloride alone, (c) the (*S*)-PDTA ligand alone and combinations (b) plus (c) as well as (a) plus (b) plus (c) were studied (Fig. 4). The former two samples did not give significant CD bands in the range of 190–800 nm. The (*S*)-PDTA ligand gives a broad CD band at 310 nm, which is shifted to 260 nm by the addition of Pr(III). The last combination activates three CD bands between 400 and 500 nm, in the same range, where Pr(III) alone has 3 UV–vis bands. We interpret these observations as follows; the addition of Pr(III) to the polydentate chiral ligand causes only electron density changes in the ligand, while the addition of the amino acid activates new chiral transitions involving orbitals of the metal. This interesting situation may be the result of a fast exchange path in the absence of the amino acid, which gets closed or slowed down significantly by the coordination of the amino acid.

The addition of L-Ala or D-Ala gives exactly the same CD spectra thus supporting the above explanation.

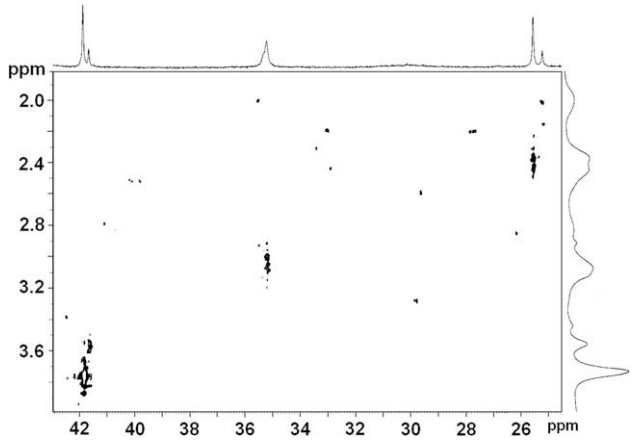

Figure 3. ^{13}C NMR spectra: bidimensional analysis of L/D Arg spectrum in the presence of the Pr(III)/(*S*)-PDTA in H_2O/D_2O solvent mixture.

3. Conclusion

The special target of enantiomeric analysis of water-soluble amino acids enables the use of ^{13}C NMR for quantitative purposes with the aid of the praseodymium(III) complex of the most simple chiral complex, PDTA. These possibilities are experimentally demonstrated in the present work. The mode of action of the Pr/PDTA chiral shift reagent was rationalized by UV–vis/CD spectroscopy. This new method for the enantiomeric analysis of amino acids gains particular importance through the increasing interest in D-amino acids biochemistry.[6]

4. Experimental

4.1. General

Reagents were of commercial origin except for the (*S*)-PDTA ligand, which was prepared as described earlier.[3] Measurements were performed with a Bruker FT-NMR Avance 400 MHz spectrometer using 100.62 MHz for the ^{13}C measurements. ^{13}C NMR spectra were obtained using a zgpg (standard decoupling sequence and NOE growth during D_1) sequence in which the acquisition parameters were 90° pulse length of 13.90 μs with 16k data point, spectral width 200 ppm, scan number 4k, receiver gain 32k and D_1 50 ms. All spectra were zero filled with 32k data point and have 2 Hz of line broadening. 2D HETCOR experiment (channel f1 = ^{13}C) was obtained using a HXCOQF sequence in which the acquisition parameters were CNST2 145.00 Hz, CNST11 3.00 Hz,

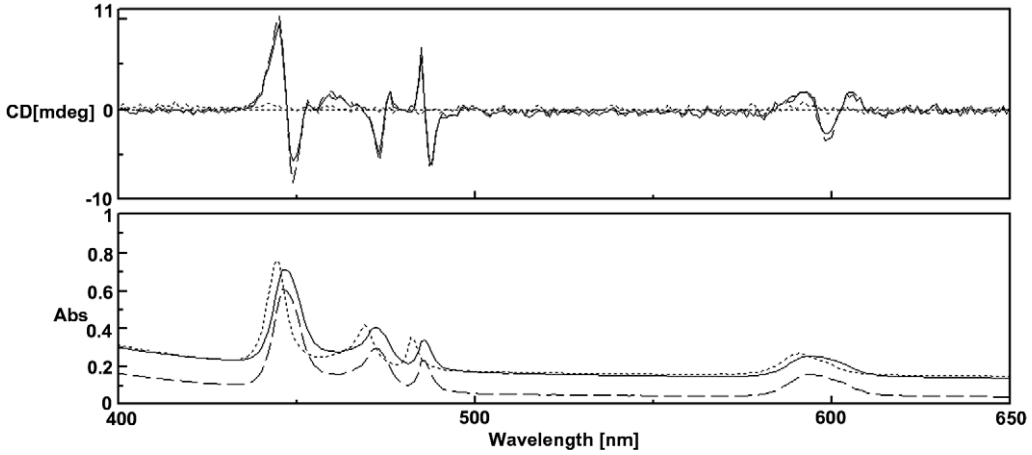

Figure 4. UV–vis/CD spectra of Pr/(*S*)-PDTA (dashed line), L-Ala/Pr/(*S*)-PDTA(continuous line) and D-Ala/Pr/(*S*)-PDTA (dotted line).

D1 50 ms, TD 4k, ns 64; f1: P1 13.90 μs, SFO1 of 100.61 MHz; f2: P3 5.80 μs, SFO2 400.1316 MHz.

4.2. Sample preparation

At first, 0.101 g (0.268 mmol) of H_4PDTAx2HCl, and amino acid (AA) (0.4 mmol) were fed into a test tube with (in order) 200 μL of D_2O, 300 μL of H_2O, 400 μL of 10 M NaOH and 0.040 g (0.16 mmol) of $PrCl_3$. Normally a clear solution is obtained. If a precipitate is formed, 3 M (aq) HCl was added until pH 1 was reached and then the pH was adjusted to 10.3 by the addition of small portions of 10 M (aq) NaOH. This treatment gave homogeneous solutions which were suitable for measurements.

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