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NMR Shifts Induced by Lanthanide Cations as an Aid to Phenyl ^{1}H Resonance Assignments

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Experimental

The lanthanide chlorides were prepared from the corresponding oxides (Fluka puriss). Glycyl-L-phenylalanine (Interchim puriss) was used without further purification. The pH (pD = pH + 0.4) was measured using a Metrohm EA 603 digital pH meter with a combined Metrohm EA 120-type microelectrode. For all samples, pH was adjusted to ±0.02 pH units with KOD and DCl.

All proton NMR spectra were recorded on a Bruker Fourier-transform spectrometer operating at 200 MHz.

Measurements were performed on samples containing 0.01 M glycyl-L-phenylalanine in D_2O in the presence of various amounts of $LnCl_3$.

Simulations were performed using the program LAOCOON III.

Line Attribution: Principles of Method

The induced chemical bound shift (IBS) of a ligand nucleus (*i*) in a paramagnetic lanthanide complex is related to the lanthanide-induced shift (LIS) δ by [1]:

$$\delta_i = (\delta_{\text{obs}} - \delta^0)_i = f\Delta_i$$

where f is the molar fraction of the complex in the solution, δ_{obs} is the observed chemical shift and δ^0 is the chemical shift in the free ligand for the same nucleus. The ratio of induced shifts for any pair of nuclei is constant regardless of the ratio C_M/C_L used:

$$\delta_i/\delta_{i'} = (\delta_{\text{obs}} - \delta^0)_i/(\delta_{\text{obs}} - \delta^0)_{i'} = \Delta_i/\Delta_{i'}$$

$\delta_{i'}$ and δ_{obs} are measured systematically for increasing concentration of metal, C_M, at constant concentration of total peptide, C_L, and at a fixed pH value throughout the range investigated.

After an arbitrary initial value of $\delta^0{}_i$ is introduced, all ratios $\delta_i/\delta_{i'}$ are established. At each $\delta^0{}_i$ value, the $\delta_i/\delta_{i'}$ mean ratio value and mean standard deviation value are computed successively. The retained $\delta^0{}_i$ value will correspond to the best mean standard deviation value.

Results and Discussion

The activity of a great number of biological molecules has been linked to the presence of various metal ions which induce conformational changes resulting in the formation of a physiologically active conformer [2]. The lanthanides, because of their spectroscopic properties, have been used as Ca or Mg analogues to probe the metal binding environment of peptides or proteins.

A serious problem with the proton NMR spectra of active molecules possessing aromatic residues is that there are so many overlapping lines in the aromatic region that very little information can be gleaned from the spectra.

Lanthanide cations have been used extensively for simplifying the complex absorption pattern of various classes of molecules in NMR spectroscopy. However, less attention has been given to the use of data for lanthanide-induced shifts as a tool to assign ^{1}H chemical shifts whose resonance lines are overlapped or unresolved.

We present a data-fitting procedure from lanthanide-shifted NMR spectra which avoids the more tedious and sophisticated line-shape analysis and allows the derivation of chemical shift values. This method is still applicable even if there is a possible conformational influence of the lanthanide cation.

When assignments of ^{1}H resonances of phenyl rings, which constitute either an AA′BB′C or A_2B_2C system, are not possible by a first-order analysis, proton NMR spectra are generally calculated and plotted with the aid of a computer and compared to experimental NMR spectra [3, 4]. Such a spectral analysis is time-consuming because of the great number of adjustable NMR parameters. Moreover, the validity of established results is questionable. Recently two-dimensional correlated spectroscopy has been used for such assignments [5–7].

Figure 1a shows the unresolved ^{1}H resonance lines in the downfield region of the phenyl residue of glycyl-L-phenylalanine (Fig. 2). The complex spectral patterns are not simplified through variation of pD and temperature. Successive addition of paramagnetic lanthanide cations (Dy or Yb) as shift reagent leads to a more important deshielding of protons and transforms the AA′BB′C system to the simplified and easily analysable AA′XX′Y system (Fig. 1b). By

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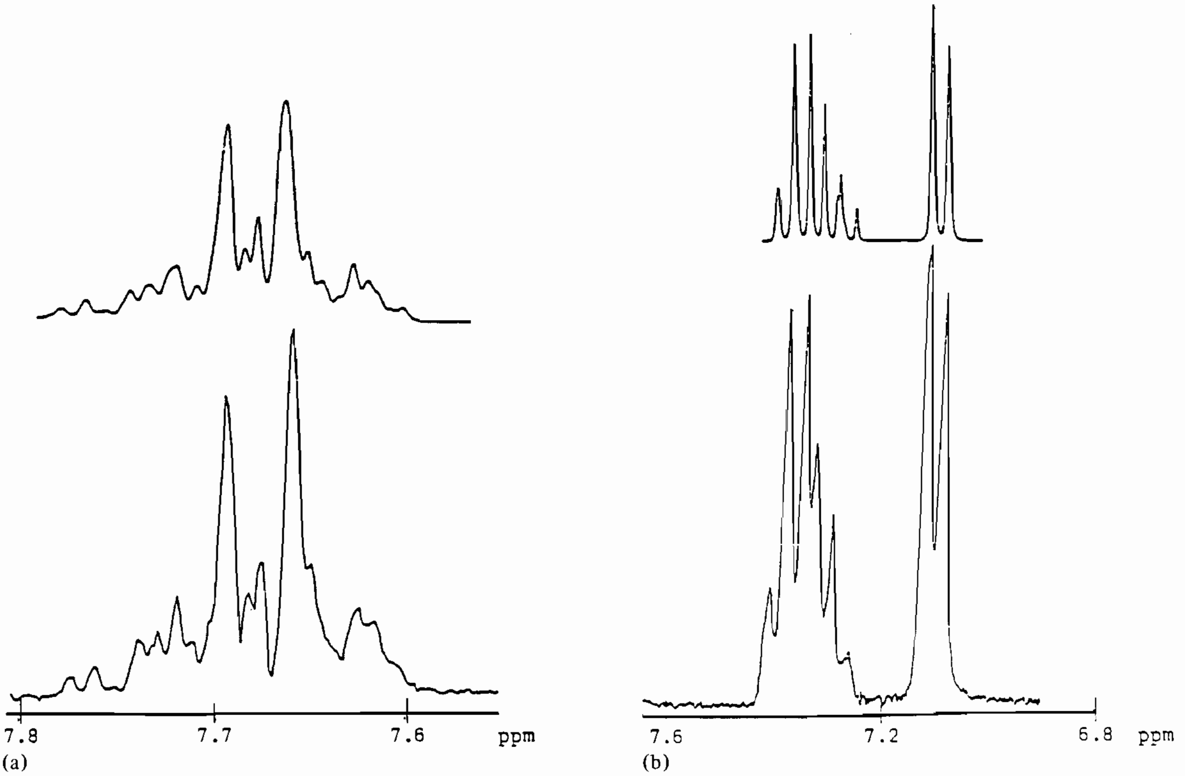

Fig. 1. Experimental (top) and computed (bottom) NMR spectra of glycyl-L-phenylalanine restricted to the aromatic region. (a) T = 298 K, C_M/C_L = 0. (b) In the presence of Yb(III); T = 348 K, C_M/C_L = 4.

TABLE I. ^{1}H NMR parameters of the Glycyl-L-phenylalanine Aromatic Residue

δ^0 (Hz)			$J_{AA'}$	$J_{AB} = J_{A'B}$	$J_{AB'} = J_{A'B}$	$J_{A'C} = J_{AC}$	$J_{BC} = J_{B'C}$	$J_{BB'}$
A = A'	B = B'	C						
1464.8	1479.0	1467.4	1.77	7.74	0.64	1.20	7.34	1.15

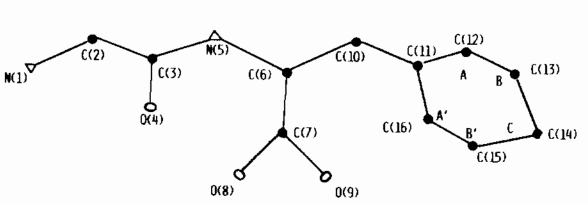

Fig. 2. IUPAC–IUB numbering of glycyl-L-phenylalanine.

this procedure, the coupling constants $J_{AA'}$, $J_{XX'}$, J_{XY} and $J_{X'Y}$ and $(\delta_{obs})_i$ have been measured for nearly all the C_M/C_L ratio values. Coherent δ^0_i chemical shift data for i nuclei, established by using an upfield inducing shift cation (Yb) and a downfield inducing shift cation (Dy) reveal the accuracy of measurements and resonance assignments (Table I). After minor refinements, the remaining coupling constants of the computed spectra were obtained. The observed and calculated ^{1}H NMR spectrum (Fig. 1b) match well, thus confirming the usefulness of this new method of assignment.

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