March, 1975] BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 48 (3), 853—856 (1975) 853

Lanthanide-Induced Nuclear Magnetic Resonance Shifts and Molecular Structure of L-Azetidine-2-Carboxylic Acid. I. Evaluation of the Shifts Intrinsic to the Lanthanide-Substrate 1:1 Complex

Fuyuhiko INAGAKI, Seizo TAKAHASHI, Mitsuo TASUMI, and Tatsuo MIYAZAWA
Department of Biophysics and Biochemistry, Faculty of Science,
The University of Tokyo, Bunkyo-ku, Tokyo 113

(Received November 5, 1974)

The lanthanide-induced PMR shifts (LIS) were observed in D_2O solution of L-azetidine-2-carboxylic acid using europium nitrate as shift reagent. Two series of measurements were carried out with the successive dilution and the incremental dilution methods. The analysis of observed data shows that the formation of 1: 2 complex between the lanthanide ion and the substrate molecule should be taken into account in addition to 1: 1 complex. The LIS's intrinsic to 1: 1 and 1: 2 complexes were evaluated. An experimental method appropriate for obtaining the ratio of the LIS's intrinsic to 1: 1 complex is proposed.

One of the most important features of the lanthanide-induced nuclear magnetic resonance shift (LIS) lies in its potentiality of giving quantitative information on the structure of molecules in solution. As is well known, however, there are several points to be checked before the observed shift data are utilized for the determination of molecular structures.[1–3] In the first place, it is necessary to evaluate the LIS intrinsic to 1: 1 complex formed between a lanthanide ion (or, more generally, a lanthanide shift reagent) and a substrate molecule. This is not a simple task, since the contributions of 1: 2 and other complexes to the observed LIS are expected to vary case by case. In this paper we treat the equilibrium between the europium ion and L-azetidine-2-carboxylic acid in aqueous solution and propose a method for evaluating the intrinsic LIS's and the shift ratios for 1: 1 complex. This method may be applicable to other systems as well. The results obtained here will be used in a subsequent paper for determining the molecular conformation of L-azetidine-2-carboxylic acid in aqueous solution.

Experimental

L-Azetidine-2-carboxylic acid was kindly provided by Mr. H. Sugano of Tanabe Pharmaceutical Company. Commercially available nitrate of Eu(III) (99.9%) was used without further treatments.

PMR spectra were recorded on a Hitachi R-22 (90 MHz) spectrometer in the frequency-sweep mode. The LIS measurements were performed for D_2O solution at a probe temperature of 37 °C using DSS as an internal standard. The shifts were determined by a frequency counter with the accuracy of ± 0.1 Hz. All the resonance lines shifted to high field in the presence of europium nitrate. The pH value of each sample solution was adjusted to be in the range between 4.6 and 5.0, so that the zwitter ion is the predominant species of the sample molecule.

The computation and curve plotting were carried out at the Computer Centre of the University of Tokyo using HITAC-8800/8700 computers and a Calcomp 563 curve plotter. The programs used for the spectral analysis were NMRTRY, NMRFIT, and NMRPLOT. The curve fitting for the LIS data was carried out with the program SHIFT. All these programs were prepared in our laboratory.

Results and Discussion

PMR Spectrum of L-Azetidine-2-Carboxylic Acid in D_2O Solution. The observed PMR spectrum of L-azetidine-2-carboxylic acid in D_2O solution is shown in Fig. 1, together with the simulated. The absorptions indicated as A, B, C, D, and E in this figure may be assigned to five protons, *viz.*, A and B to the β, C and D to the γ, and E to the α protons (see Fig. 2). The least-square adjustments gave the chemical shifts and spin-spin coupling constants listed in Table 1, which

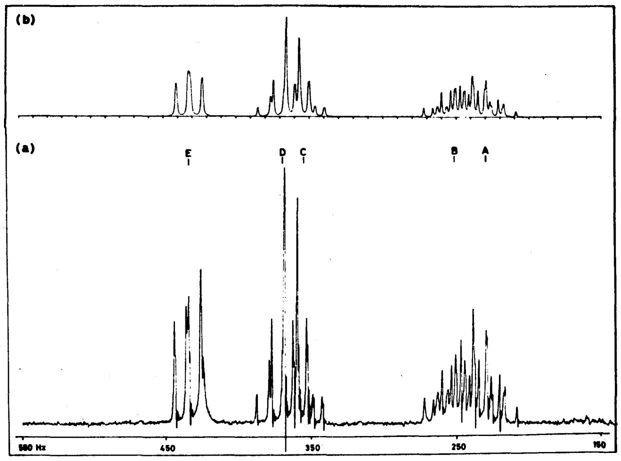

Fig. 1. PMR spectrum (90 MHz) of L-azetidine-2-carboxylic acid in D_2O; (a), observed spectrum; (b) simulated spectrum.

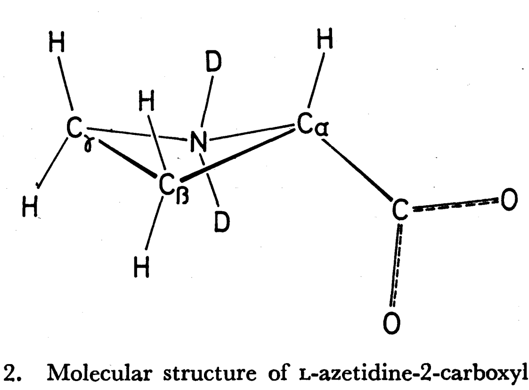

Fig. 2. Molecular structure of L-azetidine-2-carboxylic acid.

TABLE 1. CHEMICAL SHIFTS (ν) AND SPIN-SPIN COUPLING CONSTANTS (J) OF L-AZETIDINE-2-CARBOXYLIC ACID[a)]

ν_A	229.92±0.03	J_{AD}	9.34±0.05
ν_B	252.16±0.03	J_{AE}	10.02±0.04
ν_C	354.01±0.03	J_{BC}	9.91±0.05
ν_D	368.63±0.03	J_{BD}	8.02±0.05
ν_E	431.64±0.03	J_{BE}	7.96±0.04
		J_{CD}	−10.63±0.04
J_{AB}	−12.17±0.04	J_{CE}	0.79±0.04
J_{AC}	6.06±0.05	J_{DE}	0.23±0.04

a) All values are in Hz.

are essentially in agreement with the values obtained by Thomas and Williams.[4)]

Analysis of the Lanthanide-Induced Shifts. *(a) Coordination Site*: The measurements of LIS were made in the pH range between 4.6 and 5.0, where L-azetidine-2-carboxylic acid exists as a zwitterion. As LIS was found negligible below $pK_a(=1.7)$ of the carboxylate group, the coordination site for the lanthanide ion must be the carboxylate group of L-azetidine-2-carboxylic acid.

(b) Stoichiometry of the Lanthanide-Substrate Complex and the Evaluation of the LIS Intrinsic to 1:1 Complex: Much work has been done on the stoichiometry of the lanthanide-substrate complex and now the formation of not only 1:1 complex but also 1:2 complex has been established.[2,3)] The equilibria between the lanthanide ion (L) and the substrate molecule (S) may be described by the following schemes (two-step mechanism) under the assumption that the formation of the complex between one lanthanide ion and three (or more) substrate molecules can be neglected.

$$L + S \overset{K_1}{\rightleftharpoons} LS \tag{1}$$

$$LS + S \overset{K_2}{\rightleftharpoons} LS_2 \tag{2}$$

where K_1 and K_2 are the association constants given as

$$K_1 = [LS]/[L][S] \tag{3}$$

$$K_2 = [LS_2]/[LS][S] \tag{4}$$

If the LIS (with respect to the i-th nucleus) intrinsic to LS and LS$_2$ complexes are expressed as Δ^i_1 and Δ^i_2, respectively, the observed shift ($\Delta\delta^i_{obs}$) may be given as

$$\Delta\delta^i_{obs} = \frac{[LS]}{[S_0]}\Delta_1^i + 2\frac{[LS_2]}{[S_0]}\Delta_2^i \tag{5}$$

where $[S_0]$ refers to the total concentration of the substrate. Equations (3)—(5) are used to analyse the observed data described below to obtain Δ_1, Δ_2, K_1, and K_2. The following two series of measurements have been made.

(1) Successive Dilution Method:[5)] The changes of chemical shifts are observed with changing $[L_0]$ (total concentration of the lanthanide ion) and $[S_0]$ by successive addition of the solvent (D_2O) to the original solution which contains both the lanthanide ion and the substrate. Accordingly, the mole ratio $\rho(\equiv[L_0]/[S_0])$ is kept constant throughout the measurements. The shifts observed by this method for the five protons of L-azetidine-2-carboxylic acid are shown in Figs. 3 and 5, where ρ was set equal to 1.01.

(2) Incremental Dilution Method:[2)] The changes of chemical shifts are observed with changing $[L_0]$ and ρ by successive addition of the substrate solution to the original solution. The concentration of the substrate solution is made equal to that of the substrate in the original solution, so that no change in $[S_0]$ occurs

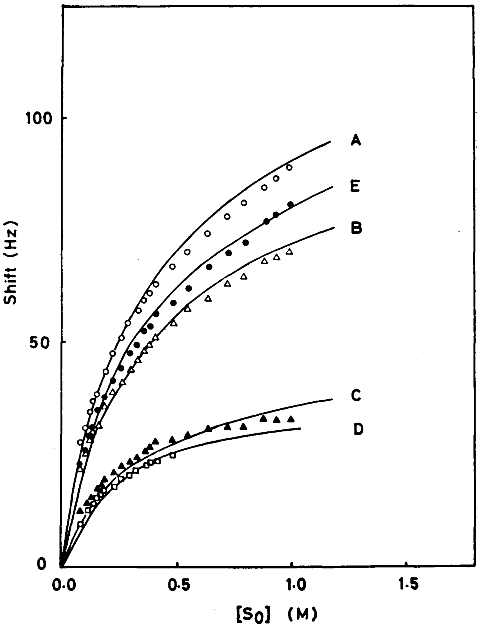

Fig. 3. Eu(III)-induced shifts ($\Delta\delta_{obs}$) of L-azetidine-2-carboxylic acid observed by the successive dilution method *vs.* total substrate concentration ($[S_0]$). D_2O solution, 37 °C, $\rho=1.01$. ○, A; △, B; ▲, C; □, D; ●, E. The curves were obtained by theoretical fitting based on the one-step mechanism.

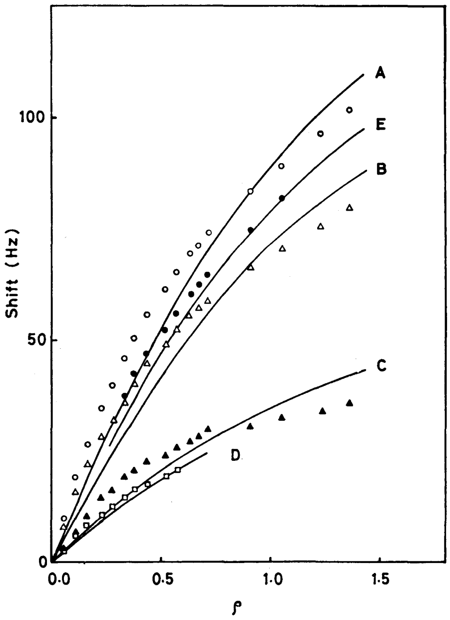

Fig. 4. Eu(III)-induced shifts ($\Delta\delta_{obs}$) of L-azetidine-2-carboxylic acid observed by the incremental dilution method *vs.* the mole ratio (ρ). D_2O solution, 37 °C, $[S_0]=0.991$ M. ○, A; △, B; ▲, C; □, D; ●, E. The curves were obtained by theoretical fitting based on the one-step mechanism.

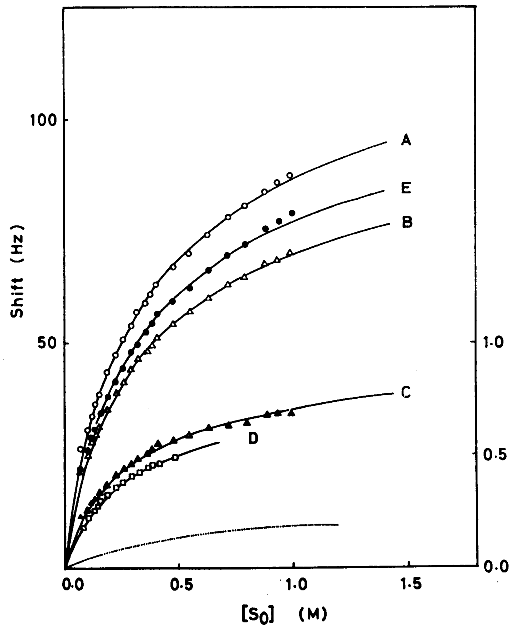

Fig. 5. Eu(III)-induced shifts ($\Delta\delta_{obs}$) of L-azetidine-2-carboxylic acid observed by the successive dilution method *vs.* total substrate concentration ([S$_0$]). D$_2$O solution, 37 °C, ρ=1.01. ○, A; △, B; ▲, C; □, D; ●, E. The full curves were obtained by theoretical fitting based on the two-step mechanism. The dotted curve indicates the dependence of the ratio [LS$_2$]/[LS] upon [S$_0$].

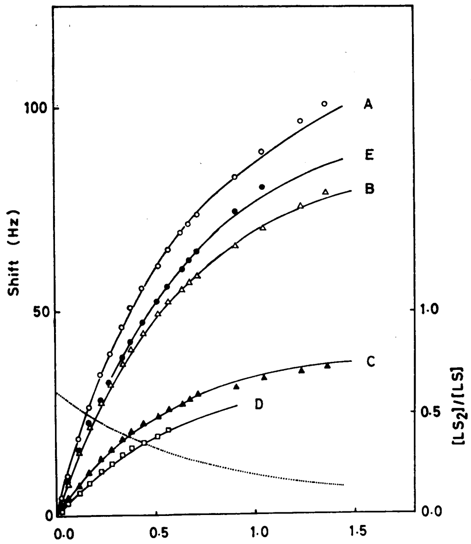

Fig. 6. Eu(III)-induced shifts ($\Delta\delta_{obs}$) of L-azetidine-2-carboxylic acid observed by the incremental dilution method *vs.* the mole ratio (ρ). D$_2$O solution, 37 °C, [S$_0$]=0.991 M. ○, A; △, B; ▲, C; □, D; ●, E. The full curves were obtained by theoretical fitting based on the two-step mechanism. The dotted curve indicates the dependence of the ratio [LS$_2$]/[LS] upon ρ.

throughout the measurements. The shifts observed by this method are shown in Figs. 4 and 6, where [S$_0$] was chosen to be 0.991 M.

Table 2. Lanthanide-induced shifts[a] intrinsic to the LS copmlex (Δ_1) and LS$_2$ complex (Δ_2) and the association constants[b] for the step L+S⇄LS (K_1) and LS+S⇄LS$_2$ (K_2)

Proton	One-step mechanism[c]	Two-step mechanism[d]	
	Δ_1, Hz	Δ_1, Hz	Δ_2, Hz
A	160.5	120.7	155.9
B	128.5	97.3	123.2
C	63.5	52.0	50.1
D	58.9	49.8	34.9
E	142.6	110.0	125.2
	K_1=3.2	K_1=3.6	K_2=0.6

a) Shifts for the probe frequency of 90 MHz. b) In the unit of M^{-1}. c) Mechanism involving only L+S⇄LS. d) Mechanism involving L+S⇄LS and LS+S⇄LS$_2$.

At first the shift data obtained above were analyzed by assuming that only the LS complex was present (one-step mechanism). In this case, $\Delta\delta^i_{obs}$ is simply given by the first term in the righthand side of Eq. (5). The best-fit curves obtained by the least-squares adjustments are shown in Figs. 3 and 4. The parameters obtained (Δ_1's and K_1) are given in Table 2. It is noted that in Fig. 3 the calculated curve tends to deviate from the observed data in the high [S$_0$] region, whereas in Fig. 4 the deviation is found both in the high and low ρ regions. These results indicate the inadequacy of the assumption of one-step mechanism. Then, the analysis in terms of the two-step mechanism has been carried out and the best-fit curves obtained are shown in Figs. 5 and 6. The agreement between the calculated curves and the observed data is now much better, giving a strong support for the LS$_2$ formation. The parameters obtained (Δ_1's, Δ_2's, K_1, and K_2) are listed in Table 2. The dotted curves in Figs. 5 and 6 indicate the calculated mole ratio [LS$_2$]/[LS]. The contribution of LS$_2$ is smaller for the successive dilution method under the present experimental conditions. The contribution of LS$_2$ decreases with decreasing [S$_0$] in the successive dilution method (Fig. 5), whereas it increases with decreasing ρ in the incremental dilution method (Fig. 6). These ratios [LS$_2$]/[LS] explain very well the origin of deviation between the calculated curves and the observed data in Figs. 3 and 4.

The procedures described above gives the LIS intrinsic to the LS complex quite straightforwardly. The simultaneous use of two series of experimental data, *viz.*, those obtained by the successive dilution and the incremental dilution methods, enhances the reliability of the calculated parameters. However, this method requires much experimental work followed by computer analysis of the data. So it seems desirable to introduce a method convenient for evaluating the LIS intrinsic to the LS complex by an experimental procedure. Taking into account that the shift ratio is a quantity more useful than the absolute shift in treating the structural problem, we show here that the shift ratio for the LS complex can be obtained by extrapolating the ratio of successive dilution data to [S$_0$]=0. The ratio of the LIS of the *i*-th nucleus and that of the

s-th nucleus may be expressed from Eqs. (3)—(5) as

$$\frac{\Delta\delta^i_{\mathrm{obs}}}{\Delta\delta^s_{\mathrm{obs}}} = \frac{\Delta_1{}^i+2K_2[\mathrm{S_0}]\Delta_2{}^i+2K_2(\Delta_1{}^i-\Delta_2{}^i)[\mathrm{LS}]}{\Delta_1{}^s+2K_2[\mathrm{S_0}]\Delta_2{}^s+2K_2(\Delta_1{}^s-\Delta_2{}^s)[\mathrm{LS}]} \tag{6}$$

Then, extrapolating the ratio of successive dilution data to zero substrate concentration, we get

$$\lim_{[\mathrm{S_0}]\to 0}(\Delta\delta^i_{\mathrm{obs}}/\Delta\delta^s_{\mathrm{obs}}) = \Delta_1{}^i/\Delta_1{}^s \tag{7}$$

The ratios of LIS's obtained by the successive dilution method are shown in Fig. 7, where the LIS of the proton A is taken as reference. In Table 3 the observed shift ratios are compared with the values calculated using the parameters for the two-step mechanism listed in Table 2. Satisfactory agreement between the observed and calculated values provides evidence for the consistency between the curve fitting analysis and the shift-ratio extrapolation method. The method of extrapolating the shift ratio obtained by successive dilution to zero substrate concentration would be useful also in other systems for evaluating the ratio of LIS's intrinsic to 1: 1 complex, particularly in the case with a rather small association constant (K_1). This point will be treated in a separate paper by comparing the two cases with small and large association constants.

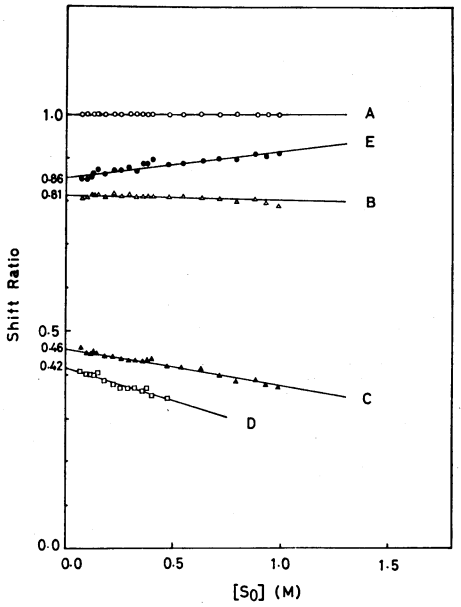

Fig. 7. The ratios of Eu(III)-induced shifts of L-azetidine-2-carboxylic acid observed by the successive dilution method. The shift of proton A was chosen as reference. ○, A; △, B; ▲, C; □, D; ●, E.

TABLE 3. SHIFT RATIOS

Proton	Obsd[a]	Calcd[b]
A	1.00[c]	1.00[c]
B	0.81	0.81
C	0.46	0.43
D	0.42	0.41
E	0.86	0.91

a) Extrapolated to zero substrate concentration ($[\mathrm{S_0}]=0$). b) Calculated using the parameters for the two-step mechanism listed in Table 2. c) Taken as reference.

References

1) B. Bleaney, C. M. Dobson, B. A. Levine, R. B. Martin, R. J. P. Williams, and A. V. Xavier, *Chem. Commun.*, **1972**, 791.

2) B. L. Shapiro and M. D. Johnston, Jr., *J. Amer. Chem. Soc.*, **94**, 8185 (1972).

3) B. L. Shapiro, M. D. Johnston, Jr., R. L. R. Towns, A. D. Godwin, H. L. Pearce, T. W. Proulx, and M. J. Shapiro, "Nuclear Magnetic Resonance Shift Reagents," ed. by R. E. Sievers, Academic Press, New York, N. Y. (1973), pp. 227—245.

4) W. A. Thomas and M. K. Williams, *Org. Mag. Resonance*, **4**, 145 (1972).

5) T. A. Wittstruck, *J. Amer. Chem. Soc.*, **94**, 5130 (1972).