Temperature Dependence of ^{67}Zn NMR Spectra of Zn^{2+}–Ligand Complexes

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Applications of ^{67}Zn NMR spectroscopy to biological systems have shown its usefulness in the study of the dynamic or static behaviour of Zn^{2+} bound with peptides or proteins in aqueous solutions [1–3]. The NMR spectral character of the $^{67}Zn(I = 5/2)$ nucleus seemed to be similar to those of $^{43}Ca(I = 7/2)$ and $^{25}Mg(I = 5/2)$ nuclei on the whole [4–7]. However, ^{67}Zn NMR spectra of aqueous Zn^{2+} are different from ^{43}Ca and ^{25}Mg NMR spectra of aqueous Ca^{2+} and Mg^{2+} in some respects. For example, ^{67}Zn NMR spectra of aqueous Zn^{2+} have marked concentration dependences in terms of the half-band widths ($\Delta\nu_{1/2}$) compared with those of ^{43}Ca and ^{25}Mg NMR spectra of aqueous Ca^{2+} and Mg^{2+} [1–3]. In addition, the $\Delta\nu_{1/2}$ values in Hz of ^{67}Zn NMR spectra of diluted Zn^{2+} are much broader than those of ^{43}Ca and ^{25}Mg NMR spectra of diluted Ca^{2+} and Mg^{2+} [1–3]. In these respects, further analyses on ^{67}Zn NMR spectra are necessary for developing ^{67}Zn NMR spectroscopy. We have investigated for the first time the temperature dependence of the ^{67}Zn NMR spectra of natural abundant Zn^{2+} bound with various ligands in aqueous solutions. It was found that the temperature effects of the ^{67}Zn NMR are markedly different from each other depending upon the molecular weights of ligand molecules.

Experimental

Imidazole was purchased from Nakarai Chemicals Co. Bovine thyrocalcitonin and calf thymus DNA (Highly Polymerized, Type I) were purchased from Sigma. Three-crystallized thermolysin was purchased from Daiwa Kasei, Co. Linear and cyclic hexapeptides were synthesized by the standard procedures [8–10].

^{67}Zn NMR spectra were recorded on a Bruker CXP-300 FT NMR spectrometer operating at 18.774 MHz with internal D_2O (10% in volume) for the frequency lock as described previously [1–3]. The pH values were adjusted by adding HCl or NaOH. To reduce precipitates of the Zn^{2+} complex, 0.1 *M* HEPES (N-2-hydroxyethylpiperazine-N′-2-ethenesulfonic acid) buffer (pH 6.52) was used for the DNA complex.

Results and Discussion

The half-band width ($\Delta\nu_{1/2}$) of ^{67}Zn NMR of aqueous Zn^{2+} was 20 Hz, which was linearly increased by adding small amounts of imidazole, **cyclo**-[Cys(***S***-Acm)-***D***-Leu-His]$_2$, Boc-[Cys(***S***-Acm)-***D***-Leu-His]$_2$–OCH_3, calf thymus DNA, thyrocalcitonin or thermolysin. Figure 1 shows typical ^{67}Zn NMR spectra of aqueous Zn^{2+}(100 m*M*) (A), Zn^{2+}(100 m*M*)-imidazole (1 m*M*) (B) and Zn^{2+}(100 m*M*)-thermolysin (50 μM) (C) complexes. Adding larger amounts of these ligands to the aqueous Zn^{2+} solution made the $\Delta\nu_{1/2}$ much broader and thus ^{67}Zn NMR spectra of those Zn^{2+} complexes were not obtained under our experimental conditions due to the low signal/noise ratios.

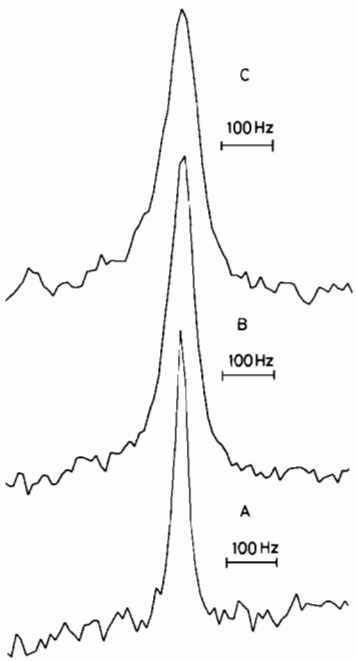

Fig. 1. ^{67}Zn NMR spectra of (A) $ZnCl_2$ (100 m*M*), pH 6.20; (B) $ZnCl_2$ (100 m*M*)–Imidazole (1.0 m*M*), pH 6.20; (C) $ZnCl_2$ (100 m*M*)–Thermolysin (50 μM), pH 5.61. The temperature is 292 K.

Temperature dependences (temperature range: 292–342 K) of $\Delta\nu_{1/2}$ of these Zn^{2+}–ligand complexes are shown in Fig. 2, where $\Delta\nu_{1/2}$ is plotted against the reciprocal of absolute temperature (1/T). The $\Delta\nu_{1/2}$ of aqueous Zn^{2+} did not show clear temperature dependence. The $\Delta\nu_{1/2}$ of the Zn^{2+}–imidazole complex decreased at higher temperatures, while $\Delta\nu_{1/2}$ of the Zn^{2+}–DNA, thyrocalcitonin and thermolysin

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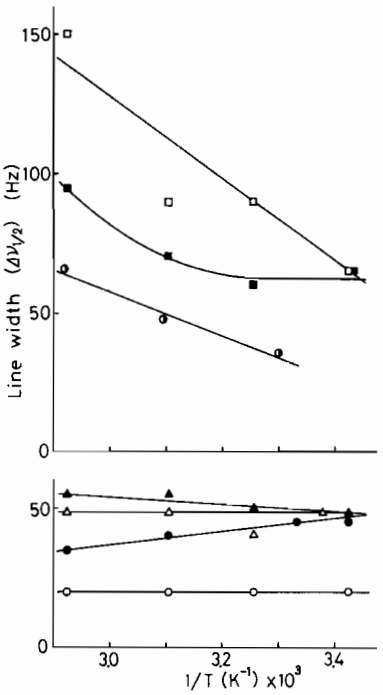

Fig. 2. ^{67}Zn NMR line width ($\Delta\nu_{1/2}$) of Zn^{2+} recorded at 18.774 MHz as a function of reciprocal temperature. The experimental conditions are: (○) $ZnCl_2$ (100 m*M*), pH 6.20; (●) $ZnCl_2$ (100 m*M*)–Imidazole (1.0 m*M*), pH 6.20; (△) $ZnCl_2$ (100 m*M*)–cyclo-[Cys(*S*-Acm)-*D*-Leu-His]$_2$ (3.5 m*M*), pH 4.48; (▲) $ZnCl_2$ (100 m*M*)–Boc-[Cys(*S*-Acm)-*D*-Leu-His]$_2$–OCH_3 (3.5 m*M*), pH 4.62; (◑) $ZnCl_2$ (50 m*M*)–DNA (0.33 m*M*, estimated per phosphate concentration), pH 6.52 (0.1 *M* HEPES buffer); (■) $ZnCl_2$ (100 m*M*)–Thermolysin (50 μ*M*), pH 5.61; (□) $ZnCl_2$ (100 m*M*)–Thyrocalcitonin (1.0 m*M*), pH 3.45. Abbreviations: Boc, N-tert-Butyloxycarbonyl; *S*-Acm, *S*-Acetamidomethyl.

complexes increased at higher temperatures*. The Zn^{2+}–hexapeptide complexes did not show clear temperature dependence of $\Delta\nu_{1/2}$. It is suggested from these findings that the cause of the ^{67}Zn NMR broadening of the Zn^{2+}–imidazole complex is different from those of the Zn^{2+}–thyrocalcitonin, DNA and thermolysin complexes. It seems likely that molecular weights of ligands are closely correlated with the mechanism of the ^{67}Zn NMR of Zn^{2+} complexes. Thus, the ^{67}Zn NMR temperature dependence observed for the complex composed of small molecules such as imidazole (molecular weight of imidazole is 68.1) is in contrast with that observed for the complexes composed of macromolecules such as thyrocalcitonin, DNA and thermolysin (molecular weights of thyrocalcitonin and thermolysin are 8,700 and 34,600 respectively). The $\Delta\nu_{1/2}$ of the Zn^{2+}–imidazole complex will be donimated by a correlation time which describes the reorientation of the entire molecule, while those of the Zn^{2+}–macromolecule complexes will be dominated by the chemical exchange under the temperature region we studied [6, 7, 13]. The ^{67}Zn NMR of the Zn^{2+}–hexapeptide complexes will be dominated by both mechanisms. The temperature dependences of ^{67}Zn NMR spectra of Zn^{2+} complexes are as a whole similar to those observed for ^{43}Ca and ^{25}Mg NMR spectra [1, 4, 6, 7, 12]. The principal mechanisms of the NMR of these quadrupole nuclei may not be very different from each other.

In conclusion, the present paper describes the first successful experiment with temperature dependences of ^{67}Zn NMR spectra. It was found that the size of the ligand is closely correlated with the main origins of the ^{67}Zn NMR line width of natural abundant Zn^{2+} in aqueous solution. Since the significant role of Zn^{2+} in biological systems has been noted, applications of ^{67}Zn NMR spectroscopy are promising for studying the dynamic and static characters of Zn^{2+} in biological systems.

*Sharp decrease of $\Delta\nu_{1/2}$ was seen for the DNA complex above 363 K due to the unfolding of the double helix of DNA, followed by recovery of $\Delta\nu_{1/2}$ of the DNA complex at 292 K, suggesting that the double helix was again formed at lower temperature [11].

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