^{67}Zn NMR Spectral Studies of Aqueous Zn^{2+} and Zn^{2+}–Insulin Complexes

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Following our qualitative ^{67}Zn NMR studies on Zn^{2+}–biological molecule complexes [1], we present here the first quantitative studies on correlation time to interpret ^{67}Zn NMR spectra of the *naturally abundant* Zn^{2+}–insulin complex.

Typical ^{67}Zn NMR spectra are shown in Fig. 1. An aqueous solution of $ZnCl_2$ (2 *M*), pH 4.0, has a very broad ^{67}Zn NMR resonance having a half-band width ($\Delta\nu_{1/2}$) of 170 Hz. By decreasing pH to 0.50 the $\Delta\nu_{1/2}$ of $ZnCl_2$ (2 *M*) was reduced to 128 Hz, still much larger than those (<10 Hz) of ^{25}Mg NMR of 2 *M* Mg^{2+} [2] and ^{43}Ca NMR of 2 *M* Ca^{2+} [3]. Dilution of the $ZnCl_2$ solution to 50 m*M* led to a narrower ^{67}Zn NMR resonance with $\Delta\nu_{1/2}$ of 12 Hz. By adding nearly 1 m*M* bovine insulin (Sigma, 25.5 international units per mg protein) to the 50 m*M* Zn^{2+} solution, pH 2.95, the $\Delta\nu_{1/2}$ of ^{67}Zn NMR increased three-fold. The pH of the solution was very crucial for observing the resonance of the Zn^{2+}–insulin complex in that the ^{67}Zn NMR resonance at pH 3.5 or more was very hard to be observed for the Zn^{2+}–insulin complex, even after 2×10^5 transients. Addition of more than 1 m*M* insulin to the 50 m*M* Zn^{2+} solution also made the ^{67}Zn NMR spectrum very obscure, due to the pronounced broadening of the resonance. The longitudinal relaxation times, T_1, of the $ZnCl_2$ and the Zn^{2+}–insulin complex were first measured. T_1 values obtained by the inversion recovery method ($180° - \tau - 90°$ pulse sequences) and T_2 values estimated by the relation $T_2 = 1/\pi\Delta\nu_{1/2}$ are summarized in Table I. The

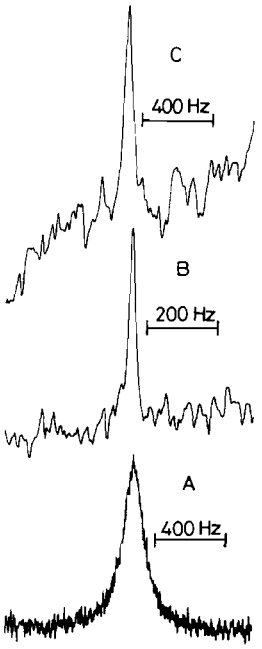

Fig. 1. ^{67}Zn NMR spectra of (A) $ZnCl_2$ (2 *M*), pH 0.50; (B) $ZnCl_2$ (50 m*M*), pH 3.00; (C) $ZnCl_2$ (50 m*M*)–Insulin (0.99 m*M*), pH 2.95.

determined T_1 value of the 2 *M* $ZnCl_2$ solution is almost the same as the T_2 value, suggesting that the extreme narrowing case can be applicable to this system and that the nucleus is isotropically tumbling. The same is true for the diluted $ZnCl_2$ solution. For the extreme narrowing case, the quadrupolar relaxation can be written as [4]:

$$1/T_{1q} = 1/T_{2q} = \frac{3\pi^2(2I+3)}{10I^2(2I-1)}\left(1+\frac{\eta}{3}\right)\chi^2\tau_c \qquad (1)$$

When it is assumed that the asymmetry parameter (η) is less than 0.5 and that the quadrupole coupling constant (χ) is 1 MHz [4], the correlation time (τ_c) describing an isotropic tumbling motion of the

TABLE I. ^{67}Zn NMR Spectra of $ZnCl_2$ and Zn^{2+}–Insulin Complexes.

Species	$\Delta\nu_{1/2}$/Hz	T_2/ms	T_1/ms	T_1/T_2
$ZnCl_2$ (2 *M*), pH 0.50	128	2.49	2.50	1.00
$ZnCl_2$ (50 m*M*), pH 3.00	12	26.54	28.10	1.06
Zn^{2+} (50 m*M*)–Insulin (0.99 m*M*), pH 2.95	38	8.34	11.47	1.37
Zn^{2+} (50 m*M*)–Insulin (0.95 m*M*), pH 2.84	30.5	10.40	14.13	1.36

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nucleus of the 50 m*M* $ZnCl_2$ solution is estimated from eqn. (1) to be 0.068 (±0.002) ns. This τ_c value of the 50 m*M* $ZnCl_2$ solution is much larger than those of other nuclei, which are usually in the range 1 ~ 10 ps for non-viscous liquids [4]. τ_c of the 2 *M* $ZnCl_2$ solution is estimated to be 0.76 (±0.02) ns under the same assumptions. The relatively large τ_c values of the aqueous Zn^{2+} nuclei and the difference of T_1 or T_2 values between the 2 *M* and 50 m*M* solution may be ascribed to the aggregated structures of Zn^{2+} ions. Observing the resonance of aqueous Zn^{2+} is practically unfeasible, even at pH 5.0. Precipitates are easily formed, even at pH 6.3 [1]. Those findings also suggest that aqueous Zn^{2+} has a tendency to form the aggregated structure. The aggregation of Zn^{2+} would influence the symmetry around the nucleus relating to the quadrupole coupling constant, and/or the correlation time of the nucleus.

The extreme narrowing case cannot be applied to the ^{67}Zn NMR of the Zn^{2+}–insulin complex since T_1/T_2 is not unity [4, 6, 7]. By considering that two Zn^{2+} are bound to an insulin hexamer [5] and that 0.5% Zn is contained in the purchased bovine insulin, T_1 and T_2 for the Zn^{2+}–insulin complex (2:6 in molar ratio) are estimated to be 126 μs and 79.9 μs respectively. According to the fast exchange two-state model [6, 7], T_1 and T_2 are described even in non-extreme narrowing case as follows:

$$1/T_1 = \frac{3\pi^2}{10}\chi^2\frac{2I+3}{I^2(2I-1)}\left[\frac{0.2\tau_c}{1+(\omega\tau_c)^2}+\frac{0.8\tau_c}{1+(2\omega\tau_c)^2}\right] \quad (2)$$

$$1/T_2 = \frac{3\pi^2}{10}\chi^2\frac{2I+3}{I^2(2I-1)}\times \left[0.3\tau_c+\frac{0.5\tau_c}{1+(\omega\tau_c)^2}+\frac{0.2\tau_c}{1+(2\omega\tau_c)^2}\right] \quad (3)$$

These equations are good for I = 5/2 nuclei as ^{67}Zn, as well as for I = 7/2 nuclei such as ^{43}Ca. The T_1/T_2 value gives $\omega\tau_c$ (τ_c is the correlation time describing the reorientation of the electric field gradients at the nucleus) to be nearly 0.6 according to Andersson *et al.* [7], from which τ_c and the quadrupole coupling constant (χ) are evaluated to be 5.1 (±0.2) ns and 1.86 (±0.05) MHz respectively. The τ_c value of ^{67}Zn in the Zn^{2+}–insulin complex is close to that of Ca^{2+} in Ca^{2+}-binding proteins [7]. The quadrupole coupling constant, 1.86 (±0.05) MHz, is relatively large compared with that of Ca^{2+} in Ca^{2+}-binding proteins [7] and to those of isotropically tumbling quadrupolar nuclei [4]. From these findings it is suggested that the reorientation of ^{67}Zn in the Zn^{2+}–insulin complex is fairly fast and that an environment around Zn^{2+} in insulin at acidic pH is not very symmetric. Protonation of Zn^{2+}-bound imidazole may reduce the symmetry of Zn^{2+}.

We first offered macroscopic quantitative information on the ^{67}Zn nucleus of aqueous Zn^{2+} and the Zn^{2+}–insulin complex by ^{67}Zn NMR spectroscopy. Since the significance of Zn^{2+} in biological structure-function relationships has been noted, the application of ^{67}Zn NMR spectra to the biological system is promising.

Experimental

^{67}Zn NMR spectra were accumulated on a Bruker CXP-300 FT NMR spectrometer at 18.774 MHz in a spinning 10 mm sample tube with external D_2O for the frequency lock. A transmitter provided 90-degree pulse widths of 80 μs for the nucleus at a peak-to-peak voltage of 300 V. Typical spectra consisted of 40000 transients to obtain signal/noise > 6 using 2 k or 4 k data points over 5000 Hz sweep widths in quadrature detection model [1]. The signal/noise ratio was improved by exponential multiplication which introduced 2 ~ 8 Hz line broadenings. The acquisition time was 2 s for the $ZnCl_2$ solution and 250 ms for the Zn^{2+}–insulin solution. Temperature was kept at 298 ± 0.5 K.

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References

1 T. Shimizu and M. Hatano, *Biochem. Biophys. Res. Commun., 104,* 1356 (1982);
T. Shimizu, M. Kodaka and M. Hatano, *ibid., 106,* 988 (1982).
2 T. Shimizu and M. Hatano, *Biochem. Biophys. Res. Commun., 104,* 720 (1982).
3 O. Lutz, A. Schwenk and A. Uhl, *Z. Naturforsch., 30a,* 1122 (1982).
4 K. Harris and B. E. Mann, eds., "NMR and the Periodic Table", Academic Press, London (1978).
5 J. H. Bradbury, V. Ramesh and G. Dodson, *J. Mol. Biol., 150,* 609 (1981).
6 B. Halle and H. Wennerström, *J. Mag. Res., 44,* 89 (1981).
7 T. Andersson, T. Drankenberg, S. Forsén, E. Thulin and M. Swärd, *J. Am. Chem. Soc., 104,* 576 (1982).