^{35}Cl NMR STUDIES OF ZINC ADENOSINE DIPHOSPHATE COMPLEXES*

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Received July 28, 1967

The use of halide ions as chemical probes for nuclear magnetic resonance (nmr) studies of mercury-tagged biological marcromlecules has recently been reported by Stengle and Baldeschwieler (1966) and Haugland et al. (1967). We wish to indicate how this technique can also be applied fruitfully to the study of small chelates of biological interest, and in particular to the interaction of Zn^{2+} with adenosine diphosphate.

Since the nmr measurement is one of ^{35}Cl line width variations, a short description of the parameters which affect the line width is worthwhile. Chlorine-35 has a nuclear spin of 3/2 and thus a nuclear quadrupole moment, Q. The dominant relaxation mechanism for such a nucleus is the fluctuation in the orientation and magnitude of the electric field gradient, q, at the site of the nucleus. Thus for a ^{35}Cl ion at a single site, e.g., an aqueous environment, the nmr line width is given by

$$\Delta\upsilon = \left(\frac{2\pi}{5}\right)[e^2qQ]^2 \ \tau$$

where τ is the effective rotational correlation time. The line width for ^{35}Cl in a 0.5M-NaCl solution is about 14 cps (Fig. 1).

The addition of $ZnCl_2$ to a solution of 0.5M-NaCl produces a broadening of the ^{35}Cl nmr line (Fig. 1). This broadening varies linearly with zinc concentration over the range investigated, 0-0.025M, and is pH independent to

*This work was performed under the auspices of the U. S. Atomic Energy Commission.

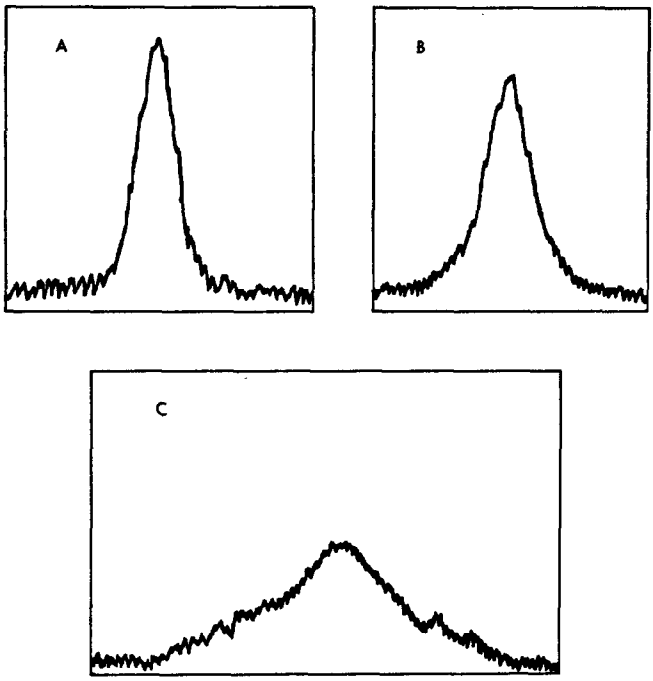

Fig. 1. ^{35}Cl nmr of (A) 0.5M NaCl, (B) 0.5M NaCl and 0.005M $ZnCl_2$, (C) 0.5M NaCl, 0.005M $ZnCl_2$, and 0.005M ADP, pH 7.

the point where zinc hydroxide begins to form. The linear relation between the ^{35}Cl nmr line width and zinc concentration defines a parameter, $\bar{\nu}$, the molar broadening, which is 1540 cps/M, i.e., $\Delta\nu_{obs} = \Delta\nu_{Cl^-} + \bar{\nu}_{Zn^{2+}}[Zn^{2+}]$. This parameter, $\bar{\nu}$, can be identified with the species in solution, which gives rise to the broadening and its physical significance and will be discussed more fully at a later date.

For those systems in which the chloride nucleus can exist in various sites and for which the exchange of the chloride between these sites is very rapid compared to line width of the broadest line, a composite signal is observed. The line width of this composte signal is $\Delta\nu = \Sigma(\Delta\nu_i)P_i$ where $\Delta\nu_i$ is the line width for site i and P_i is the probability that chloride is at i. Thus for solutions where the chloride exchange rate is "rapid" the ^{35}Cl line width is a function of the following parameters for each site: (1) the accessibility

of zinc to the chloride ions, (2) the electric field gradient at the chlorine nucleus, and (3) the rotational correlation time for the chloride bond.

The addition of a small amount of chelating agent such as EDTA or citrate ion to a solution of Zn^{2+} in 0.5M NaCl at pH 7 decreases the width of the ^{35}Cl line. The addition of an excess amount of the chelating agent reduces the ^{35}Cl line width to that of aqueous chloride ion. This narrowed line is the result of the reduced accessibility of zinc to chloride ions.

A solution of 0.005M Zn^{2+} and 0.005M ADP at pH 7, however, exhibits a much larger broadening of the ^{35}Cl resonance than the same concentration of Zn^{2+} by itself (Fig. 1). This broadening is both concentration and pH dependent. Since P_i can only decrease for Zn^{2+} this enhanced broadening must result from changes in Q or τ. We have observed that addition of pyrophosphate to a solution of Zn^{2+} ions reduces the ^{35}Cl line width. Since metal ions preferentially bind to the phosphate groups of ADP, and it appears reasonable that the electric field gradients at the site of the Zn-Cl bond should be similar in Zn ADP and Zn pyrophosphate, we tentatively attribute the enhanced broadening of the ^{35}Cl line width by Zn ADP to an increase in τ. This interpretation is, of course, subject to the limitation that the chloride exchange time between sites remains rapid.

Since our interpretation of ^{35}Cl line widths is based on the assumption that the rate of chloride exchange is not a contributing factor in our line width measurements, it is important to consider this point. We have examined the zinc-adenosine diphosphate system by means of ^{81}Br nmr and have observed essentially the same results as with ^{35}Cl nmr. The ^{81}Br line widths are at least 50 times larger than those of ^{35}Cl, thus the possibility of having the same relation between exchange rate and width of broadest nmr line for the two systems seems remote. We therefore conclude that the rate of halide exchange is probably not the determining factor in our line width measurements. We plan, however, to investigate this point more fully.

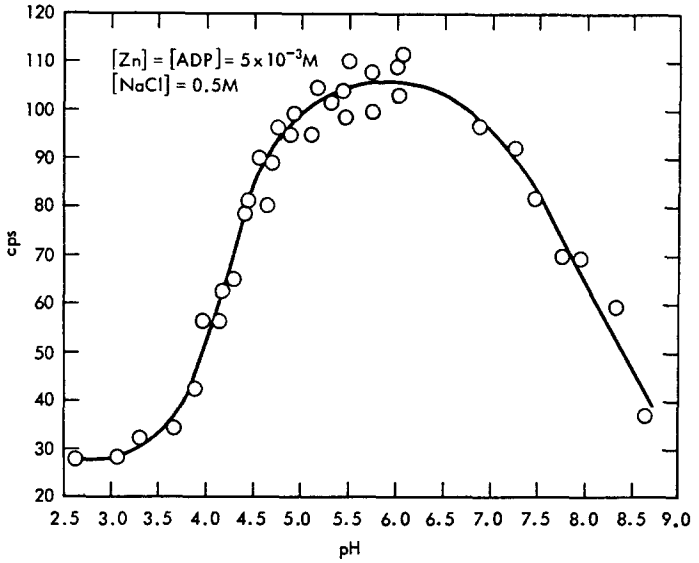

Fig 2. ^{35}Cl nmr line width as a function of pH for a solution 0.5M NaCl, $ZnCl_2$ = ADP = 5×10^{-3}M. This curve is a composite of three separate titrations. Each point is the average of six line width measurements and the deviation from the average was 5% or less.

As noted, the broadening of the ^{35}Cl nmr line in the presence of Zn-ADP is pH dependent (Fig. 2). This pH dependence agrees with what is known concerning the affinity of zinc for various protonated species of ADP. We have also observed similar results for Zn-ATP complexes; however, the enhancement of broadening is less. We are presently interpreting Fig. 2 and similar results for other nucleotide di and triphosphate zinc complexes in terms of molar broadenings, and will report our results at a later time. It appears that the ZnADP complex has a molar broadening, $\overline{\nu}$, of approximately 12,000 cps/M.

Interesting stoichiometry can be obtained by titrating Zn^{2+} with ADP and using the ^{35}Cl nmr line width as the measured parameter. The curve in Fig. 3 is the result of such a titration. Whereas the maximum ^{35}Cl broadening[1] is obtained at a molar ratio of Zn: ADP of about 1, the fact that the

[1]The association constant data of Khan and Martell (1962) and esr data with Mn^{2+} substituted for Zn^{2+} yield an estimate of 80-90% for the amount of bound zinc in our equimolar solution.

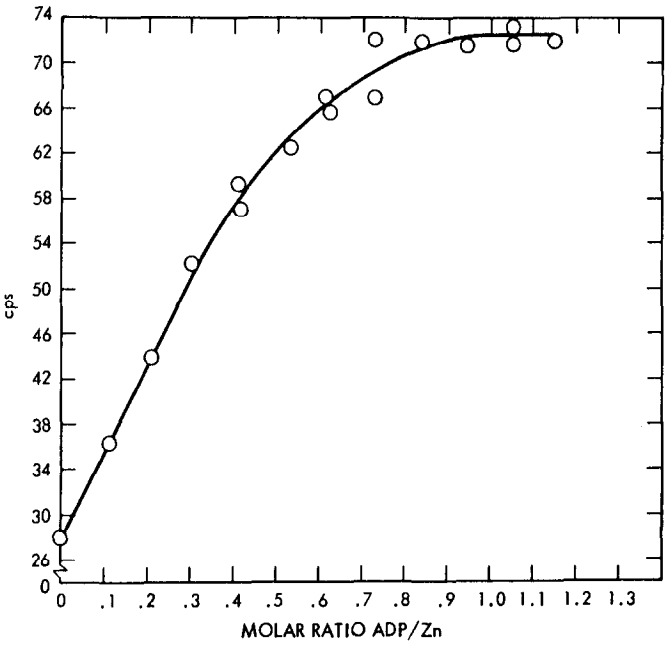

Fig. 3. ^{35}Cl nmr line width as a function ADP/Zn molar ratios, 0.1M triethanolamine, 0.5M NaCl, pH 7.0, $ZnCl_2 = 4.76 \times 10^{-3}$M.

initial slope of the titration curve is definitely greater than that predicted for the formation of ZnADP alone clearly indicates the formation of the species Zn_2ADP in the presence of excess Zn. If the titration, not shown, is continued past the 1:1 species by addition of relatively large excess ADP, the line width is observed to decrease from its maximum value to a value $\leq$ that for the corresponding solution containing Zn^{2+} alone. This decrease can be attributed to the formation of $Zn(ADP)_2$. We thus have evidence for the formation of Zn_2ADP, ZnADP, and $Zn(ADP)_2$ all in moderately dilute solution.

This report indicates the usefullness of ^{35}Cl nmr for the study of small zinc complexes in dilute concentrations. The observed enhanced broadenings are characteristic of the species which gives rise to the broadenings, and should be an excellent tool for studies of conformations in solution. Studies of a variety of nucleotide di and triphosphate-zinc complexes, presently in

progress, should yield some information on the interesting question of metal interactions with nucleotide rings.

Acknowledgment

This work was supported in part by the Bio-Medical Division of the Lawrence Radiation Laboratory.

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