members in the $[(CH_2)_y\text{-}O\text{-}]_x$ series vary with y in a manner strikingly similar to that shown by the characteristic ratio in Figure 5.

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Nuclear Magnetic Resonance Studies on Pyridine Dinucleotides. 4.[1] Measurements of Correlation Times and the Binding of Oxidized Nicotinamide Adenine Dinucleotide and Adenosine 5′-Monophosphate to Lactate Dehydrogenase as Viewed by ^2H and ^{13}C Relaxation Times

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Abstract: By employing the method of Saito, Mantsch, and Smith, deuterium quadrupole coupling constants have been experimentally determined for NAD^+ and several purine ring systems. From a combination of ^{13}C T_1 determinations and ^2H line widths, the reorientational correlation times can be extracted from the well-known equations. The trends in the correlation times among the purines are discussed in terms of intermolecular interactions. As an application of this aproach, the binding of NAD^+ and 5′-AMP to M_4LDH was studied by observing the line widths of the ^2H and ^{13}C resonances of the nucleotides. Because both ligands were found to be undergoing rapid exchange with the protein, M_4LDH, their correlation times could be inferred from the line width data. It was concluded that NAD^+ binds differentially to the protein M_4LDH in the absence of a substrate. That is, the adenine portion of NAD^+ was found to have a longer correlation time when bound to the enzyme than the analogous correlation time for the pyridyl ring. The advantages of using ^2H Fourier transform NMR methods in studies of ligand binding to enzyme are briefly discussed.

With the advent of experimental techniques that allow the resolution of the contribution of proton j to the spin–lattice relaxation time of proton i,[2-4] relaxation methods are potentially a powerful probe with regard to the solution conformational dynamics of complex molecules. Reference to eq 1 illustrates this fact, i.e., one can calculate a conformationally averaged interatomic distance between atoms i and j without recourse to molecular theories of structure and bonding. This,

however, assumes one has a value for the reorientational correlation time, τ_{cij}, for the vector $\mathbf{r}_{ij}$.

$$\frac{1}{T_1(i, \text{atom } j\ ^2\text{H})} - \frac{1}{T_1(i, \text{atom } j\ ^1\text{H})} = \tfrac{3}{2}\gamma_H^4\hbar^2\langle r_{ij}^{-6}\rangle\tau_{cij} \quad (1)$$

We present herein a simple and direct method that allows the determination of reorientational correlation time for molecules at any concentration that is amenable to a modern Fourier transform (FT) NMR experiment. Furthermore, with a knowledge of these correlation times the binding of the ligands, NAD^+[5] and AMP, to the enzyme, chicken breast M_4LDH, is presented as an example of this approach.

Experimental Section

NMR Measurements. The ^2H and ^{13}C FT NMR measurements were made on a modified Varian XL-100 NMR spectrometer.[6] Spin-lattice relaxation times, T_1, were determined by the inversion recovery sequence of Freeman and Hill.[7] Transverse relaxation times, T_2, were measured by the Carr-Purcell/Meiboom-Gill (CPMG) T_2 sequence.[8] These experiments illustrated that for the ^2H resonances $T_2 = T_1 = T_2^*$ and, hence, T_2 or T_1 could be extracted from a simple line width determination. Here T_2^* is used to denote the transverse "relaxation time" which can be obtained from the line width of a resonance, i.e., $T_2^* = (\pi\Delta\nu_{1/2})^{-1}$.

Enzyme and Sample Preparation. The M_4LDH was isolated from chicken breasts using previously described procedures.[9] Enzyme samples were assayed[10] just prior to use and immediately after the NMR experiment. To within the experimental error of the assay, no loss of enzyme activity was noted. The experimental details on each sample are summarized in Tables II and III.

Materials and Synthesis. Adenosine, purine riboside, 5′-AMP, 3′-AMP, and 2′-AMP were purchased from Sigma Chemical Co. and NAD^+ from P-L Laboratories and used without further purification. The 99.8% D_2O was obtained from Columbia Organic Co.

^{2}H-A8-Adenosine. Adenosine was deuterated at the A8 position by heating 0.1 g of adenosine in 20 ml of D_2O at a pD of 13 for 1 h at 85 °C. The deuterated product was lyophilized and then recrystallized from 50 ml of warm water.

^{2}H-A8-Purine Riboside, ^{2}H-A8-5′ (or 3′ or 2′)-AMP and ^{2}H-A8-NADH. The corresponding purine containing compound was deuterated by a procedure analogous to that of ^2H-A8-adenosine. The appropriate nucleotide or nucleoside was placed in D_2O at a pD of 10. The solution was heated at 55 °C for 1 to 10 days. The deuterium incorporation was monitored by ^1H NMR. The reaction was considered complete when the deuterium incorporation was at least 99%. Furthermore, there was no evidence of decomposition (by ^1H NMR) during this time period at this temperature.

^{2}H-N4-NAD$^+$. The preparation of ^2H-N4-NAD^+ was accomplished by a method analogous to that previously reported by San Pietro.[11] The KCN/NAD^+ mixture was adjusted to a pD of 11 with NaOD. After a 2-h incubation at room temperature the NAD-CN complex diluted by the addition of 80 ml of D_2O to which dilute DCl was added until the final pD was 2. The solution was then lyophilized to a powder. Analysis by ^1H NMR showed that the resulting deuterated NAD^+ was at least 98% isotopically pure.

^{2}H-A8-NAD$^+$. A solution containing 110 mg of ^2H-A8-NADH was adjusted to a pD of 7. Pyruvic acid (33 mg, mol wt = 110) and 10 μl of a 1 mM solution of M_4LDH was added to this solution. Before the addition of the M_4LDH the absorbance of the solution at 340 nm was 0.67. Ten minutes after the addition of the M_4LDH the absorbance of the solution was 0.09. After this 10-min period the solution was acidified to a pD of 2 and then brought back to a pD of 6 by the addition of base. The solution was applied to a Dowex Ag 1-4X column in the formate form.[1] Analysis by ^1H NMR of lypholized powder from the column indicated that the deuterated NAD^+ was at least 98% isotopically pure.

Results and Discussion

Accurate knowledge of correlation times, τ_c, for molecules of biological importance is often difficult to obtain when working at low concentrations, i.e., 10^{-2} to 10^{-3} M. Direct calculation of correlation times when dealing with these systems usually requires ^{13}C labeling and a reasonable estimate of the C-H bond distance in order to abstract τ_c from the ^{13}C dipole-dipole relaxation expression,[12]

$$T_1^{-1}(^{13}\text{C}) = N\gamma_H^2\gamma_C^2\hbar^2 r_{CH}^{-6}\tau_c \quad (2)$$

Here N is the number of directly bonded hydrogens to the carbon of interest. Furthermore, this equation ignores the effects of other spins which are not directly bonded to the carbon, and assumes that the C-H vectors can be described by a single effective correlation time.[13]

Deuterium NMR offers an economical alternative to this dilemma, since in many cases labeling with ^2H is synthetically more facile. In the case of various adenosine derivatives, labeling the hydrogen attached to C-8 with ^2H is easily accomplished by heating in mildly basic D_2O.[14] For a quadrupolar nucleus, e.g., ^2H, under conditions of extreme narrowing $T_1^{-1}(^2H)$ is given by[12]

$$T_1^{-1}(^2\text{H}) = \frac{3}{8}\left(\frac{e^2qQ}{\hbar}\right)^2\tau_c \quad (3)$$

where $e^2qQ/\hbar$ is the quadrupole coupling constant. The quadrupole coupling constant can be extracted from ^2H and ^{13}C T_1 experiments in a fashion which is independent of the correlation time. This method, due to Saito, Mantsch, and Smith,[15] is embodied in eq 4, which is the result of the ratio of eq 2 and 3, converted to the units of kilohertz.

$$\frac{e^2qQ}{\hbar} = \frac{1}{2\pi}\left[\frac{N8\hbar^2\gamma_{^{13}C}^2\gamma_{^1H}^2 T_1(^{13}\text{C})}{3r^6 T_1(^2\text{H})}\right]^{1/2} \quad (4)$$

If one further realizes that ^2H line widths are sufficiently broad enough, for the systems of interest to us here, to ensure that

$$T_1 = T_2 = T_2^*$$

a simple three-step procedure can be formulated to allow the determination of τ_c at any concentration which is amenable to a FT NMR experiment. The first two steps are the measurement of ^2H and ^{13}C spin-lattice relaxation times at a convenient concentration and a known temperature. Utilizing eq 4 the quadrupole coupling constant can be determined. The final step is a measurement of the line width of the ^2H resonance at the lower concentration and the *same* temperature. Then with the use of eq 3, the correlation time for the ^2H nucleus can be determined.

The quadrupole coupling constant as it is expressed by eq 4 is sensitive to the choice of the value of r, the CH bond distance. For example, if a CH bond distance of 1.10 ± 0.03 Å is employed to calculate the quadrupole coupling constant for ^2H-A8-5′AMP [$T_1(^{13}C)$ = 213 ms and $T_1(^2H)$ = 9.6 ms], then the resulting quadrupole coupling constant is 174 ± 14 kHz. Hence, a relative uncertainty in the bond distance of 3% produces a relative uncertainty of 8% in the coupling constant. This "error" is, of course, transmitted to the calculated correlation time.

For the calculated correlation time of the ^2H atom to be extended to that of the molecule as a whole, the molecule must reorientate isotropically.[13] An equally important consideration is the validity of the assumption that $T_1 = T_2 = T_2^*$. Reference to the ^2H NMR spectrum of ^2H-A8-5′-AMP in Figure 1 supports this assumption. The line width of the ^2H resonance is 33 Hz, which corresponds to a T_2^* of 9.6 ms. Independent T_1 and CPMG T_2 experiments confirmed that $T_1 = T_2 = T_2^*$.

As an example of the first two stages of this procedure, we have determined the ^2H quadrupole coupling constants for ^2H-A8-5′-AMP and ^2H-N4-NAD^+. These values and the other necessary relaxation and structural information are summarized in Table I. It was further assumed that the ^2H quadrupole coupling constant for the A8 position in 5′-AMP

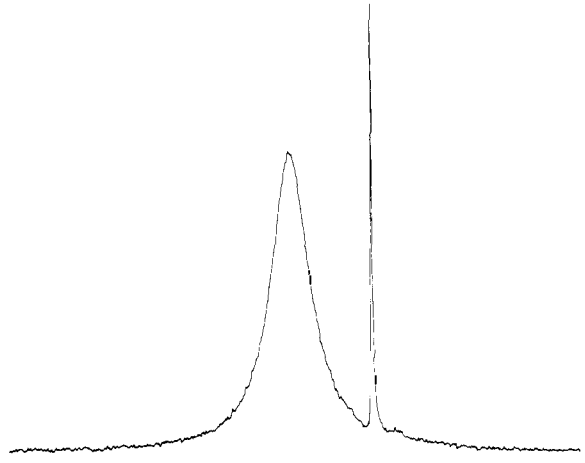

Figure 1. The FT ^2H NMR spectrum of 5′AMP ^2H enriched in the A8 position in isotopically normal water. The flip angle was 90° and this spectrum is the result of 1000 accumulations. The spectral width depicted is 400 Hz. The sharp resonance to the right is that of natural abundance HDO.

Table I. Spin–Lattice Relaxation Data for the Determination of the Deuterium Quadrupolar Coupling Constant in 0.5 M 5′-AMP and NAD^+ [a]

	NOE[b]	$T_1(^2H)$	$T_1(^{13}C)$	r, Å	e^2qQ/h, kHz
^2H-A8-5′-AMP	3.07 ± 0.1	9.6	213	1.10[c]	175 ± 14
^2H-N4-NAD^+	3.0 ± 0.1	7.8	171	1.08	183 ± 15

[a] T_1's are in s × 10^{-3}. The precision of the T_1 measurements is approximately 5%; see the discussion in the text concerning the accuracy of the quadrupole coupling constants. [b] NOE is defined as $1 + \gamma_H/2\gamma_C$. These values demonstrate that the indicated carbon is relaxed via dipole-dipole processes. [c] This value was taken from x-ray data by P. L. Johnson, C. L. Maier, and I. C. Paul, *J. Am. Chem. Soc.*, **95**, 5370 (1973).

would be constant for a series of adenosine derivatives, e.g., 2′-AMP, adenosine, the adenyl portion of NAD^+. Utilizing this assumption, we have also determined the reorientational correlation times for the following series of molecules: purine riboside, adenosine, 2′-AMP, 3′-AMP, 5′-AMP, and NAD^+. These results are summarized in Table II.

The calculated τ_c values in Table II show that rotational reorientation times for the rigid purine ring systems vary by a factor of 2 over the range of compounds studied. On the basis of molecular weight and ionic character one would intuitively expect adenosine and purine riboside to reorientate faster than the various AMP derivatives. This view is confirmed by comparing the τ_c values of adenosine with 2′-AMP or 3′-AMP. The three AMP compounds studied show a small difference in correlation times. The fact that the reorientation rate of 5′-AMP is approximately 1.3 times slower than that of 2′-AMP suggests that 5′-AMP is more associated in solution than the former at 0.005 M and 5 °C.[16,17] One possible explanation for the variation in extent of association of these nucleotides might be the difference in the conformational preference of the purine ring with respect to its contiguous ribose. This notion is supported by the fact that 5′-AMP is predominantly anti while 2′-AMP and 3′-AMP are substantially more syn in character.[2,16,17]

The τ_c values for NAD^+ at 0.005 M for the A8 and N4 positions are shown to be nearly the same. Carbon-13 relaxation studies by Hamill and co-workers[18] at higher concentrations (≥0.2 M) have yielded similar results. The conclusion that this corroborates the base stacking model of Sarma et al.[19,20] does not necessarily follow since there is no reason to believe a priori that the two aromatic rings would reorient at different rates for different conformations.

Table II. Comparison of the Deuterium T_1 and τ_c for Purine Derivatives and NAD^+ at 0.005 M[a] at 5 °C

	$T_1(^2H)$[b]	τ_c[c]
^2H-A8-Purine riboside	17.1	1.3
^2H-A8-Adenosine	12.9	1.7
^2H-A8-2′-AMP	9.6	2.3
^2H-A8-3′-AMP	9.4	2.4
^2H-A8-5′-AMP	7.7	2.9
^2H-A8-NAD	8.0	2.8
^2H-N4-NAD	7.8	2.6 (2.3)[d] (1.9)[e]

[a] The samples were prepared in 12 mm NMR tubes pH 7. The samples were 0.68 M in phosphate buffer and 10^{-4} M in EDTA. [b] The relaxation times are in s × 10^{-3}. The T_1's were inferred from line width measurements. The accuracy of the line width measurements is estimated to be ±2 Hz. [c] The correlation times are in s × 10^{-10}. [d] The correlation time obtained from ^1H relaxation data on the N4 proton. See ref 4 and footnote *e* below. [e] The correlation time obtained from ^{13}C enriched N6-NAD^+; this value for τ_c appears to be long because the x-ray distance for the C–H bond distance was in all probability too short; footnote *b* from Table I.

Because of the sensitivity of ^2H line widths to subtle changes in correlation times, ^2H Fourier transform NMR offers an excellent means to study the binding of substrates to enzymes.[21] Deuterium, which is a quadrupolar nucleus ($I > \frac{1}{2}$), has relaxation times which are short relative to spin ½ nuclei which are reorientating with the same correlation time. This phenomenon arises because of the efficient interaction of the nucleus with the electric field gradients generated by the reorientating quadrupole moment.[12] In exchange processes in which a ^2H nucleus is rapidly exchanging between two sites of different correlation times, i.e., the substrate exchanging on and off the surface of an enzyme, the difference in correlation time between the free substrate and the substrate bound to the enzyme is reflected in the increased line width observed relative to the line width of the free material as follows,[22-24]

$$\Delta\nu_{1/2(\text{obsd})} = \frac{3}{\pi 8}\left(\frac{e^2qQ}{\hbar}\right)^2\left[\tau_f(1-\chi) + \frac{1}{20}\left(6\tau_b + \frac{10\tau_b}{1+\omega^2\tau_b^2} + \frac{4\tau_b}{1+4\omega^2\tau_b^2}\right)\chi\right] \quad (5)$$

Here $\Delta\nu_{1/2(\text{obsd})}$ is the observed line width at half-height, τ_f and τ_b are the effective correlation times of the free and bound substrate, respectively, ω is the Larmor frequency in radians per second, and χ is the mole fraction of enzyme binding sites. In eq 5 it is evident that the increased efficiency of the ^2H quadrupolar relaxation process serves to amplify the difference in effective correlation times for the free and bound substrate.[24] This amplification is reflected by the increased sensitivity of $\Delta\nu_{1/2(\text{obsd})}$ to the amount of protein present. Figure 2 illustrates this point by showing plots of line width vs. protein mole fraction for ^1H, ^2H, and ^{13}C samples. The boundary conditions employed in the plots are outlined in the figure caption. In addition to the increased sensitivity, deuterium has a further advantage over proton studies of this kind in that the observed proton line widths of the substrate are often obscured by the proton resonances of the enzymes.[25] The use of deuterium labels also offers a substantial economic advantage over ^{13}C enrichments.

As a practical example of the utility of the method, and because of our continued interest in pyridine dinucleotides, we chose to study the interaction of NAD^+ and 5′-AMP with

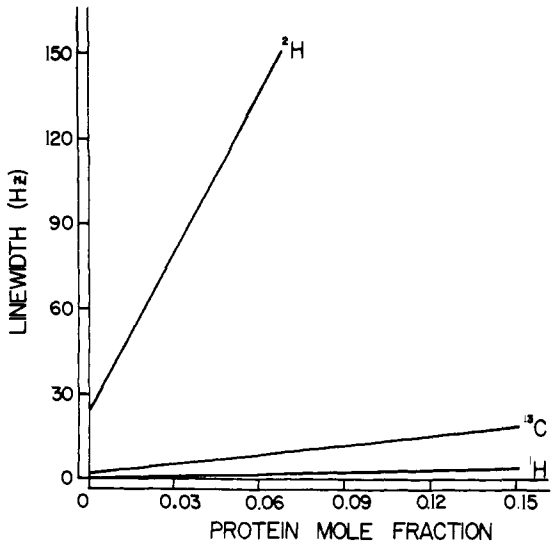

Figure 2. These are the plots for the calculated line widths of ^{1}H, ^{2}H, and ^{13}C resonances as a function of the mole fraction M_4LDH binding sites. The τ_f for the free material was 1.65×10^{-10} s and the τ_b employed was 40.0×10^{-9} s. The quadrupole coupling constant for the ^{2}H used was 174 kHz, the C-H bond distance used was 0.97 Å, and the H-H bond distance used was 2.27 Å.

M_4LDH. In Table III, the calculated correlation times from ^{2}H NMR, for the interaction of 5′-AMP and NAD^+ respectively with M_4LDH at 10 and 25 °C, are presented. From examination of Table III it is found that the A8 position in 5′-AMP has an effective correlation time that is longer than the analogous correlation time for A8-NAD^+ or N4-NAD^+. This observation could be rationalized as a difference in the exchange rate of 5′-AMP with the protein as compared with the exchange rate of NAD^+ and M_4LDH, i.e., 5′-AMP is in the rapid exchange limit whereas NAD^+ has an intermediate exchange rate with the protein.[22,26] In order to establish which exchange region NAD^+ is in when interacting with M_4LDH, a determination of τ_b was made from ^{13}C NMR experiments on NAD^+ enriched at position N6.[27] Table III shows that with the experimental error the correlation times determined by either ^{13}C or ^{2}H NMR experiments are identical. This finding confirms that NAD^+ is in rapid exchange with M_4LDH. Such a conclusion follows because of the T_2 time scale differences between ^{13}C and ^{2}H, i.e., the $T_{2,b}$ for ^{13}C-N6-NAD^+ is an order of magnitude greater than the $T_{2,b}$ of ^{2}H-N4-NAD^+ for the same correlation time.[28] From this analysis one can see that if the condition for rapid exchange was not achieved, i.e., the ^{2}H $T_{2,b}$ was not sufficiently greater than the lifetime of NAD^+ on the protein, then for the ^{13}C $T_{2,b}$ this condition would be more nearly achieved. Clearly, if the rapid exchange conditions were not fulfilled, the correlation times deduced from the ^{2}H and ^{13}C experiments would be different.

The above argument now enables one to make a comparison between the various calculated τ_b values for 5′-AMP and NAD^+ on M_4LDH. Table III shows that at 10 and 25 °C the calculated τ_b for N4-NAD^+ is substantially shorter than that of A8-NAD^+. We feel that this can best be rationalized as differential binding of NAD^+ to M_4LDH with the adenine portion of NAD^+ being immobilized to a greater extent by the enzyme than the pyridyl moiety. This result clearly suggests that for the oxidized coenzyme, NAD^+, the pyridyl ring does not interact with the protein to the extent that the adenine ring does in the absence of a substrate.[29] At 25 °C it is evident that the adenine portion of 5′-AMP has its motion restricted to a greater extent by the protein than the adenine portion of NAD^+. This result is now compared with the τ_b values at 10 °C where one can see that the adenine portions of 5′-AMP and

Table III. Calculated Correlation Times[a] for NAD^+ and 5′-AMP Bound to M_4LDH[b]

	10 °C	25 °C
^{2}H-A8-5′-AMP[c,d]	89	60
^{2}H-A8-NAD[c,e]	99	32
^{2}H-N4-NAD[e,f]	26	12
^{13}C-N6-NAD[g,h]	26	15

[a] The correlation times are in s $\times 10^{-9}$. The estimated error for the calculated correlation times is ±20%. [b] The samples were 10^{-2} M in NAD^+ or 5′-AMP and were prepared in 12-mm NMR tubes in 0.3 M phosphate buffer with 10^{-4} M EDTA. The mole fractions of enzyme binding sites used are listed below; further it was assumed that the concentration of binding sites was four times the concentration of the protein. [c] The quadrupole coupling constant used here was 174 kHz; see Table I. [d] The calculated τ_b was determined using two different mole fractions of M_4LDH, 5.0×10^{-3} and 3.3×10^{-3}. [e] The mole fraction of binding sites used was 5.0×10^{-3}. [f] The quadrupole coupling constant used here was 183 kHz; see Table I. [g] The τ_b for ^{13}C-N6-NAD^+ was calculated from an expression similar to eq 5 for ^{13}C. See J. R. Lyerla, Jr., and D. M. Grant, *MTP Int. Rev. Sci.: Physiol., Ser. One*, **4** (1972). The ^{13}C–H bond length used was 0.97 Å. [h] The mole fraction of binding sites used was 3.7×10^{-2}.

NAD^+ have the same effective correlation time within experimental error. One possible explanation of these data is that the strengths of the respective adenine binding interactions to the protein are not the same. This difference can be further amplified by the possibility that at 25 °C the adenine portion of NAD^+ does not have a lifetime on the enzyme which is long enough for it to fully develop the correlation time of the protein, whereas at 10 °C, the binding for the adenine portion of NAD^+ is more efficient, and hence is able to remain on the enzyme long enough to develop its full correlation time. It should be emphasized that this conclusion is tentative and that further investigation into the nature of this difference between the binding of the adenine portion of 5′-AMP and NAD^+ is currently underway in these laboratories.

In conclusion we would like to emphasize that the method described above should be a powerful tool to elucidate substrate-enzyme interactions when dealing with such systems where the enzyme is limited by concentration. It should also prove to be a useful approach to examining substrate-enzyme interactions where direct observation of either enriched or natural abundance ^{13}C resonances on the protein is not feasible. These conditions may arise because of the high molecular weight of the protein which will in turn generate prohibitively broad resonances due to its long correlation times.

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Structure of Ethylene Oxide Oligomer Complexes. 5. A Complex of Tetraethylene Glycol Dimethyl Ether with Cadmium Chloride[1]

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Abstract: The molecular and crystal structure of the 1:2 complex of tetraethylene glycol dimethyl ether (TGM), CH_3O-$(CH_2CH_2O)_4CH_3$, and $CdCl_2$ has been determined by x-ray diffraction. The crystal has a monoclinic unit cell of the space group $P2_1/a$, containing four TGM and eight $CdCl_2$, and the cell constants are $a = 14.877$, $b = 7.468$, $c = 17.621$ Å, and $\beta = 103.73°$. One TGM is coordinated to two Cd atoms, having the conformation III. Four $CdCl_2$ are combined to one another through Cl bridges. Interatomic distances are 2.409–2.735 Å for the O··Cd and 2.425–2.682 Å for the Cd–Cl distances.

Introduction

Blumberg et al. first reported complex formation of polyethylene oxide (PEO) with $HgCl_2$ on the basis of the observation of the infrared spectrum and x-ray diffraction pattern.[2] Iwamoto et al. found the existence of two kinds of complexes that have different compositions and determined the molecular and crystal structures of the complexes by x-ray diffraction.[3,4] PEO was found to have conformations of

$$\begin{array}{llllllll} -CH_2-CH_2-O-CH_2-CH_2-O & \text{and} & -CH_2-CH_2-O \\ T \quad T \quad T \; T \quad G \quad T & & T \quad G \quad G' \end{array}$$

where G and T mean gauche and trans,[3] respectively, and G′ is a torsional angle of about 90°,[4] for the repeating unit in the two complexes, whereas PEO consists of a succession of

$$\begin{array}{lll} -CH_2-CH_2-O \\ T \quad G \quad T \end{array}$$

in the pure polymer.[5] According to conformational studies of ethers consisting of CH_2CH_2O units, a gauche form is about 400 cal/mol more stable than a trans form for a CH_2–CH_2 bond,[6] whereas a trans form is 1100 cal/mol more stable than a gauche form for a CH_2–O bond.[7] This implies that coordination to Hg has the dominating influence on the molecular conformation of PEO in the $HgCl_2$ complexes. Iwamoto investigated further the effect of coordination on molecular conformations in a series of structural studies of the complexes of ethylene oxide oligomers $RO(CH_2CH_2O)_mR$ with $HgCl_2$.[8-10] He found that the

$$\begin{array}{llllllll} -CH_2-CH_2-O-CH_2-CH_2-O \\ T \quad G \quad T \; T \quad \bar{G} \quad T \end{array}$$

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form ($\bar{G}$: gauche opposite to G), which is considered most stable on the basis of what was mentioned above, is at the same time most suitable for coordination to a Hg atom, so long as the degree of polymerization $m \leq 4$.[9] This is just the case of a 1:1 complex of tetraethylene glycol dimethyl ether (TGM)[8] or tetraethylene glycol diethyl ether (TGE)[9] with $HgCl_2$, in which five coplanar oxygen atoms of the circular molecule are coordinated inwardly to one Hg. If $m > 4$, the molecule is too long to surround just one Hg atom and is coordinated to more than one Hg, and consequently G′ O–CH_2 bonds enter into the molecular chain to bring about intimate coordinations to Hg.[10] This is the case with a 1:2 complex of hexaethylene glycol diethyl ether (HGE) with $HgCl_2$.[10] In this complex one HGE is coordinated to two Hg and a

$$\begin{array}{lll} -CH_2-O-CH_2- \\ G' \quad G' \end{array}$$