Nuclear magnetic resonance relaxation studies of the interaction of ligands with the monomer and tetramer forms of formyltetrahydrofolate synthetase

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Previous work using n.m.r. spectroscopy to investigate the binding between formyltetrahydrofolate synthetase and its ligands was done using the catalytically active tetrameric form of the enzyme. By removal of specific monovalent cations the tetramer dissociates to four identical, catalytically inactive monomers, which are capable of binding nucleotides with affinities similar to those obtained with the tetramer. In the studies reported here, we examined the interaction of metal–nucleotide, formate and monovalent cations with the monomer using n.m.r. relaxation measurements. We were able to demonstrate that formate binds to the monomer. The spin-lattice relaxation rate ($1/T_1$) of the formate carbon in the monomer·Mn²⁺·ADP·formate complex is enhanced when Mg²⁺ is replaced by Mn²⁺. By assuming that the exchange of formate is not rate-limiting and that τ_c of the monomer is the same as that of the tetramer, the distance between the Mn²⁺ and the formate carbon was calculated and found to be similar in the monomer and tetramer complexes. The spin-lattice relaxation rates of [¹³C]trimethylammonium ion (an inactive monovalent cation), [¹³C]methylammonium and [¹⁵N]ammonium ions (both active monovalent cations), were measured in the presence of tetramer, MnADP and formate. The relaxation rates of methylammonium and ammonium ions were enhanced under these conditions whereas the relaxation rate of trimethylammonium ion was not. The results indicate that the active monovalent cations bind near the MnADP binding site. A distance from the Mn²⁺ to the ammonium nitrogen of between 0.5 and 0.6 nm was calculated.

INTRODUCTION

Formyltetrahydrofolate synthetase catalyses the reaction shown in eqn. (1):

In previous work two n.m.r. techniques have been used to study various substrate complexes of formyltetrahydrofolate synthetase from *Clostridium cylindrosporum*: (1) measurement of the water-proton relaxation, and (2) measurement of the relaxation rates of ligand nuclei, such as the ¹³C and ¹H of formate. In these studies, Mg²⁺ was replaced by the paramagnetic cation, Mn²⁺, and non-reactive enzyme–substrate complexes were used so that the lifetimes of the complexes would permit examination by n.m.r., e.g. ADP was used instead of ATP, particularly in the enzyme·MnADP·formate·H₄-folate quinary complex.

Using water-proton relaxation measurements Himes & Cohn [1] demonstrated that the true substrate is the metal–nucleotide complex and that the free metal ion (Mn²⁺) interacts with the enzyme only in a non-specific way. Buttlair *et al.* [2] studied the ternary complex, enzyme·MnADP, and found that upon addition of H₄-folate, the water-proton relaxation value was de-enhanced. It was concluded that there are three to four exchangeable water molecules ligated to Mn²⁺, but upon

addition of H₄folate, this is reduced to an apparent fractional number. These results were also confirmed by e.p.r. studies of Mn²⁺ in the same enzyme–substrate complexes [3], and led to a suggestion that addition of H₄folate to the ternary complex, enzyme·MnADP, causes a conformational change near the active site of the enzyme. In a later study, Wendland *et al.* [4] measured the relaxation rates of the ¹H and ¹³C nuclei of formate in enzyme–substrate complexes and estimated the distance from Mn²⁺ to the formate proton and carbon, respectively, to be 0.64–0.71 nm and 0.55–0.6 nm in the complex with ADP or 0.67–0.74 nm and 0.58–0.63 nm in the complex with ATP. Furthermore, the ¹³C relaxation rate of [¹³C]CH₃NH₃⁺, a cation which can replace K⁺, was used to find a distance, in a range of 0.74–1.2 nm, from Mn²⁺ to the methylammonium carbon. It was also found that addition of H₄folate to the enzyme–substrate complexes caused a decrease in the relaxation rates of the formate carbon and proton which could be interpreted as resulting from a decrease in the rate of exchange of free and bound formate. Thus, it was concluded that the addition of H₄folate either causes a conformational change at the active site or physically blocks the exchange of bound and free formate and CH₃NH₃⁺.

All the previous n.m.r. work on formyltetrahydrofolate synthetase involved the catalytically active tetrameric form of the enzyme in the presence of monovalent

Abbreviation used: H₄ folate, tetrahydrofolate.

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cation. Relatively little is known about the catalytically inactive monomer form of the enzyme which is produced upon dissociation of tetramer when specific monovalent cations are absent. The monomer is known to have a metal-nucleotide binding site, but it does not catalyse the formylation of H_4 folate, and it is reported not to bind H_4 folate [5]. The purpose of this study was to compare various monomer-ligand complexes with tetramer-ligand complexes using n.m.r. techniques. The following questions were posed at the outset of this study. Does the active site in the monomer bind formate and, if so, how does the Mn^{2+} -formate distance compare with that of the tetramer? Does H_4 folate have an effect on the exchange of formate with monomer-bound formate? Additionally, it was of interest to learn more about the monovalent-cation binding site in the tetramer. ^{13}C n.m.r. experiments involving $[^{13}C]CH_3NH_3^+$ indicated that there is a monovalent cation site near the divalent ion site of the enzyme [4]. However, the possibility of non-specific $CH_3NH_3^+$ binding could not be ruled out. Additional n.m.r. experiments with an amine cation such as $(CH_3)_3NH^+$, which is not an effective substitute for smaller monovalent cations, and $[^{15}N]NH_4^+$, which is the most effective cation, tested this possibility.

MATERIALS AND METHODS

Materials

$[^{13}C]HCOOH$, $[^{13}C]CH_3NH_2 \cdot HCl$, and $[^{13}C]-(CH_3)_3N \cdot HCl$, all at least 90% enriched in ^{13}C , and $[^{15}N]NH_4NO_3$ (99% enriched with $[^{15}N]NH_4^+$) were purchased from Merck Sharp & Dohme, St. Louis, MO, U.S.A. (6*RS*)- H_4 folate was prepared by the catalytic hydrogenation of folic acid in aqueous solution and was purified further by DEAE-cellulose column chromatography [6]. $[^3H](6*RS*)-H_4$ folate was prepared by the reduction of folic acid with $[^3H]NaBH_4$ as described by Curthoys *et al.* [7]. *C. cylindrosporum* was provided by the Biochemical Research Service Laboratory at the University of Kansas, KS, U.S.A.

Purification of formyltetrahydrofolate synthetase

Formyltetrahydrofolate synthetase was purified from *C. cylindrosporum* by combining two previously used methods. The preparation steps including cell extraction, protamine sulphate treatment, and the first two ammonium sulphate precipitations were as described by Buttlair [8] with the exception that the buffer used was 25 mM-Tris/ SO_4 /10 mM-KCl/50 mM-2-mercaptoethanol, pH 7.5. The impure enzyme was dialysed against 2 l of buffer for a total of 2 h with three changes of the buffer solution. The enzyme was finally purified on a Heparin-Agarose column [9] and crystallized in 60% ammonium sulphate. The specific activity of the enzyme was about 480 μ mol/min per mg. The purity of the protein was verified by SDS/polyacrylamide-gel electrophoresis.

Preparation of the monomer and tetramer for n.m.r. experiments

The enzyme was collected from a suspension in 60% ammonium sulphate by centrifugation and redissolved in a small volume of buffer containing 25 mM-Tris/HCl/50 mM-2-mercaptoethanol at pH 8.0 for the monomer, and the same buffer with 25 mM-KCl for the tetramer.

The enzyme solution was dialysed against four changes of 500 ml of the same buffer over a 16 h time period. In some experiments the enzyme was passed through a Bio-Gel P-6DG column in the same buffer. The protein solution was concentrated in a membrane cone (Amicon CF-25) to 60–120 mg/ml as determined using a specific absorption coefficient of 0.53 ml/mg per cm at 280 nm.

N.m.r. measurements

^{13}C spin-lattice relaxation times, T_1 , were measured at 20 MHz using a Bruker WP-80. The fast inversion-recovery method with pulse sequence $(T-180^\circ-\tau-90^\circ)_n$, with $T < 5T_1$ was used [10]. T_1 and s.d. values were calculated using a non-linear least-squares routine. In all cases, a 5 Hz line-broadening was applied to the accumulated free-induction decays. For T_1 measurements of ^{13}C , 0.5 ml portions of enzyme-ligand solutions were prepared and transferred into cylindrical n.m.r. tube inserts which fit into standard 10 mm n.m.r. tubes. 2H_2O for the field-frequency lock was contained in the outside compartments of the same tubes. The final concentrations of components in the n.m.r. samples are given in the individual Tables. The concentrations of various complexes such as enzyme-ADP, enzyme-MnADP and MnADP were calculated using known association constants as described previously [4]. When formate, $CH_3NH_3^+$, $(CH_3)_3NH^+$ or NH_4^+ was present, their concentrations were 100 mM and it was assumed that enzyme complexes were saturated with these components. The sample temperature was maintained at 5 $^\circ C$.

^{15}N spin-lattice relaxation times for $[^{15}N]NH_4^+$ were measured at 30 MHz using the Varian XL-300 spectrometer and 5 mm sample tubes. The temperature was maintained at 20 $^\circ C$.

N.m.r. theory and calculations

The theoretical principles underlying this work have been described by a number of investigators in review articles and have been summarized in a previous publication from this laboratory [4]. Calculations of the distances from Mn^{2+} to substrate and cation nuclei are based upon the Solomon and Bloembergen equation, modified for the experimental conditions employed here (eqn. 2):

$$\frac{1}{T_{1M}} = \frac{2}{15} \frac{\gamma_I^2 g^2 S(S+1) \beta^2}{r^6} \left(\frac{3\tau_c}{1 + \omega_I^2 \tau_c^2} \right) \quad (2)$$

In this equation, T_{1M} is the relaxation time of nuclei in substrate molecules or cation bound to the paramagnetic complex, γ_I is the nuclear magnetogyric ratio, S is the electron spin quantum number, β is the Bohr magneton, ω_I is the precession frequency of the nucleus, τ_c is the correlation time of the dipolar interaction, and r is the distance between the nucleus and Mn^{2+} in the complex. Under conditions of fast exchange between free and bound substrate, T_{1M} is related to the observed paramagnetic contribution to the spin-lattice relaxation rate, T_{1p} , by the equation:

$$T_{1M} = p T_{1p} \quad (3)$$

where p is the mol fraction of the substrate bound to the enzyme-Mn-nucleotide complex and was calculated as described previously [4]. T_{1p} was obtained by subtracting the relaxation rate for the diamagnetic Mg^{2+} complex

Table 1. [^{13}C]Formate relaxation times of various solutions

Component concentrations were: 25 mM-Tris/HCl, pH 8.0, 50 mM-2-mercaptoethanol, 1 mM-ADP; 0.1 M-sodium [^{13}C]formate; 2 mM- H_4 folate, 0.1 M-KCl (only when the tetramer was present); M^{2+} as indicated; protein, 0.33–1.17 mM. All measurements were made at 20 MHz and 5 °C. T_1 values are in the presence of Mg^{2+} or Mn^{2+} . Numbers in parentheses each represent one S.D.

Components	$[\text{M}^{2+}]$ (μM)	$T_1(\text{Mg}^{2+})$ (s)	$T_1(\text{Mn}^{2+})$ (s)	T_{1p} (s)	$10^4 \times pT_{1p}$ (s)
M^{2+} , ADP, formate	20	12.2 (0.9)	6.2 (0.3)	12.6 (2.2)	–
M^{2+} , ADP, formate, H_4 folate	20	12.2 (0.9)	6.4 (0.5)	13.5 (3.3)	–
M^{2+} , ADP, formate	80	12.1 (0.7)	2.3 (0.2)	2.8 (0.3)	–
M^{2+} , ADP, formate, H_4 folate	80	12.1 (0.7)	2.6 (0.2)	3.3 (0.4)	–
Tetramer, M^{2+} , ADP, formate	40	10.7 (0.7)	0.90 (0.07)	0.98 (0.09)	1.7 (0.2)
Tetramer, M^{2+} , ADP, formate, H_4 folate	80	10.1 (0.6)	4.52 (0.33)	8.18 (1.5)	27.0 (5.0)
Monomer, M^{2+} , ADP, formate	20	13.0 (1.2)	2.91 (0.25)	3.75 (0.51)	2.42 (0.33)
Monomer, M^{2+} , ADP, formate	20	10.0 (1.0)	2.5 (0.17)	3.33 (0.41)	2.06 (0.25)
Monomer, M^{2+} , ADP, formate	80	9.83 (0.54)	0.71 (0.06)	0.77 (0.07)	2.05 (0.19)
Monomer, M^{2+} , ADP, formate, H_4 folate	80	9.83 (0.54)	0.87 (0.08)	0.95 (0.1)	2.53 (0.27)

from the observed relaxation rate of the paramagnetic Mn^{2+} complex. Thus, pT_{1p} is directly related to the distance between the paramagnetic cation and the observed nucleus. The distance was calculated assuming two limiting conditions, that τ_c is frequency independent and that τ_c is maximally frequency dependent [4]. Under the experimental conditions employed, τ_c is dominated by the electron spin relaxation time (τ_s) of Mn^{2+} in the complex.

In some experiments the enzyme concentration used was such that the MnADP complex was present in sufficient concentration to contribute to the observed T_{1p} . In these cases, T_{1M} for the monomer· MnADP ·formate complex was determined by solving simultaneous equations of the type shown in eqn. 4.

$$T_{1p}^{-1} = p_{MN} \cdot T_{1M(MN)}^{-1} + p_{EMN} \cdot T_{1M(EMN)}^{-1} \quad (4)$$

This equation relates the observed T_{1p}^{-1} to the sum of the T_{1M}^{-1} values for the enzyme· MnADP ·formate (EMN) and MnADP ·formate (MN) complexes multiplied by their respective mol fractions. Data from two experiments were used to solve the simultaneous equations. The resulting T_{1M} value was then used in calculating the formate-carbon to Mn^{2+} distance in the monomer complex.

H.p.l.c.

The molecular mass of the enzyme in the enzyme–ligand solutions was monitored by h.p.l.c. (Beckman model 334) equipped with a 7.5 mm $\times$ 30 cm TSK-250 3000 SW size-exclusion column (Bio-Rad, Richmond, CA, U.S.A.). The temperature of the column was maintained at 5 °C. The mobile phase of the column contained 20 mM-sodium phosphate, pH 6.8, and 10 mM-2-mercaptoethanol.

H_4 Folate binding

The binding of [^3H](6S)- H_4 folate was measured by using a micropartition system (Amicon MPS-1 with YMT membrane). The enzyme–substrate solution containing [^3H](6RS)- H_4 folate was centrifuged in the MPS-1 apparatus at 2500 rev./min for 2 min. The radioactivity of the solution centrifuged through the membrane, representing the concentration of unbound (6S)- H_4 folate

and total (6R)- H_4 folate which does not bind, was used to calculate free and bound concentrations of (6S)- H_4 folate and a K_a value.

RESULTS AND DISCUSSION

Interactions of formate with the monomer and tetramer

Equilibrium binding studies have demonstrated that the catalytically inactive monomer retains its binding site for the nucleotide but exhibits little binding of H_4 folate [5]. Previously nothing was known about the interaction of formate with the monomer. Equilibrium methods are not practicable because of the apparent low association constant (assuming that the K_d value is similar to the K_m value of about 5 mM). However, binding of formate to the enzyme· Mn ·nucleotide complex could be tested by measuring the relaxation rate of a formate nucleus. Studies with the tetramer using Mn^{2+} in the form of an enzyme· MnADP complex have demonstrated the binding of formate to the tetramer, and distances of the formate hydrogen and carbon to the Mn^{2+} , have been calculated [4]. We have used the same approach in examining binding of formate to the monomer.

Table 1 contains the results of these experiments and compares them with results obtained with the tetramer. In the absence of enzyme, MnADP apparently interacts with formate as can be seen by the effect of replacing Mg^{2+} with Mn^{2+} . In the presence of enzyme the effect of Mn^{2+} is enhanced. The pT_{1p} value for the formate carbon in the tetramer· MnADP ·formate complex is similar to that reported previously [4]. When H_4 folate is present with the complex a large increase in pT_{1p} occurs which is consistent with previous findings [4]. This effect is attributed to the trapping of formate at the active site, resulting in a decrease in the $k_{\text{off}}(\tau_m^{-1})$ value of formate by a factor of 600 [4]. The difference in the spin-lattice relaxation times of formate carbon in the presence of the monomer when Mn^{2+} replaces Mg^{2+} demonstrates that formate also binds near the divalent cation in the monomer. The pT_{1p} value is very close to that of the tetramer complex. However, the addition of H_4 folate to this complex caused little or no change in the pT_{1p} value (compare the value of 2.53×10^{-4} s with the range of

Table 2. [^{13}C]Trimethylammonium ion relaxation times of various solutions

Component concentrations were: 25 mM-Tris/HCl, pH 8.0, 50 mM-2-mercaptoethanol, 1 mM-ADP; 40 μM - M^{2+} ; 0.1 M-sodium formate; 2 mM- H_4folate ; 0.8 mM enzyme; 0.1 M- $(\text{CH}_3)_3\text{NH}^+$. Measurements were done at 20 MHz and 5 °C. Numbers in parentheses each represent one S.D.

Components	$T_1(\text{Mg}^{2+})$ (s)	$T_1(\text{Mn}^{2+})$ (s)
M^{2+} , ADP, formate, $(\text{CH}_3)_3\text{NH}^+$	4.70 (0.51)	4.73 (0.63)
M^{2+} , ADP, formate, $(\text{CH}_3)_3\text{NH}^+$, H_4folate	4.65 (0.59)	4.36 (0.23)
Enzyme, M^{2+} , ADP, formate, $(\text{CH}_3)_3\text{NH}^+$	4.45 (0.52)	4.00 (0.32)
Enzyme, M^{2+} , ADP, formate, $(\text{CH}_3)_3\text{NH}^+$, H_4folate	5.20 (0.44)	5.12 (0.69)

Table 3. Effect of NH_4^+ on the observed relaxation rates of [^{13}C]methylammonium ion in tetramer-substrate complexes

The concentrations of components were: 0.2–0.9 mM enzyme; 0.1 M-sodium formate; 0.1 M- CH_3NH_3^+ ; 0.1 M- NH_4Cl ; 1 mM-ADP; 2 mM- H_4folate ; 40 μM - M^{2+} ; 25 mM-Tris/HCl, pH 8.0; 50 mM-2-mercaptoethanol. Measurements were done at 20 MHz and 5 °C. p (expected) values are based on pT_{1p} values of the corresponding complex in solutions lacking NH_4Cl . p (calculated) values are from the expected pT_{1p} and the observed T_{1p} values.

Components	T_{1p} (expected) (s)	T_{1p} (observed) (s)	p (expected)	p (calculated)
Tetramer, M^{2+} , ADP, CH_3NH_3^+ , NH_4^+	2.87	12.9	0.009	0.0020
Tetramer, M^{2+} , ADP, formate, CH_3NH_3^+ , NH_4^+	2.83	17.9	0.008	0.0013
Tetramer, M^{2+} , ADP, H_4folate , CH_3NH_3^+ , NH_4^+	2.81	10.9	0.009	0.0023
Tetramer, M^{2+} , ADP, H_4folate , formate, CH_3NH_3^+ , NH_4^+	6.63	35.0	0.008	0.0015

(2.05–2.42) $\times 10^{-4}$ s found in the absence of H_4folate), in contrast with the results found with the tetramer complex, but consistent with previous findings [5] that the coenzyme does not bind (or binds much less efficiently) to the monomer.

The distance from Mn^{2+} to the formate carbon in the monomer enzyme· MnADP ·formate complex was estimated in the following manner. It was assumed that exchange of free and bound formate was rapid, as has been established for the tetrameric enzyme complex [4], and that τ_c for the monomer complex is the same as in the tetramer complex (both are equal to the τ_s of Mn^{2+}). The pT_{1p} values presented in Table 1 yield a value of r in the tetramer complex of 0.57 nm assuming that τ_c is frequency-independent and 0.53 nm assuming frequency-dependence of τ_c . These values are close to those reported earlier [4]. The corresponding values for the monomer complex are 0.59 nm and 0.55 nm, respectively. Therefore, distances found for the monomer complexes are very close to those found for the tetramer complexes, and it is concluded that formate binds both monomer and tetramer enzyme· MnADP complexes in a similar manner.

During the course of these studies some apparent inconsistencies in the results with H_4folate were sometimes found. In some cases, the pT_{1p} values of monomer· MnADP ·formate mixtures were 2–3 times higher than those shown in Table 1 and an increase in the pT_{1p} value was obtained when H_4folate was added. Upon further investigation it was determined that at high

protein concentrations the monomer readily aggregates into a catalytically inactive form(s), which has a molecular mass higher than the tetramer and is eluted in the void volume of a TSK 3000 SW column. This aggregation phenomenon was most pronounced when the monomer was produced by overnight dialysis followed by concentration to achieve a 1–2 mM concentration (60–120 mg/ml). Earlier work showed that the monomer is not stable, and over a period of hours, loses its ability to re-associate to the active tetramer [11]. This instability is undoubtedly related to the aggregation phenomenon. When solutions contained only monomer, as detected by h.p.l.c., low values for pT_{1p} were found and no effect of H_4folate on pT_{1p} was observed (Table 1). The aggregate could be made intentionally by incubating the concentrated monomer at 4 °C. The resulting solution showed a larger value of T_{1p} implying a lower value for p for the monomer· MnADP ·formate complex, and a subsequent increase in T_{1p} when H_4folate was added, indicative of binding of the coenzyme to the aggregate. That H_4folate did bind was shown by a micropartition binding assay. H_4Folate did not bind to protein which was known to be in the monomer form but did bind after the aggregate was produced with an apparent K_d value of 1 mM, or about 30 % of the value reported for the tetramer.

Interaction of monovalent cations with the tetramer

Specific monovalent cations are required by formyl-tetrahydrofolate synthetase for catalytic activity and for maintenance of the quaternary structure [12]. In previous

Table 4. [^{15}N] NH_4^+ relaxation times in enzyme-substrate complexes

The concentrations of components were: 1 mM enzyme active sites; 20 μM - M^{2+} ; 1 mM-ADP; 0.1 M-sodium formate; 2 mM- H_4folate ; 0.1 M- ^{15}N NH_4NO_3 ; 0.1 M-triethanolamine/HCl, pH 8.0; 50 mM-2-mercaptoethanol. Measurements were done at 30 MHz and 20 °C. Numbers in parentheses each represent one s.d.

Components	$T_1(\text{Mg}^{2+})$ (s)	$T_1(\text{Mn}^{2+})$ (s)	T_{1p} (s)	$10^3 \times pT_{1p}$ (s)
M^{2+} , ADP, formate, NH_4^+	113.4 (13.3)	36.6 (1.1)	54.0	—
Enzyme, M^{2+} , ADP, formate, NH_4^+	32.4 (2.6)	11.3 (0.54)	17.3	2.53
Enzyme, M^{2+} , ADP, formate, NH_4^+ , H_4folate	—	33.5 (0.34)	—	—

studies we had shown that the relaxation rates of carbon and protons of CH_3NH_3^+ (an active monovalent cation) are influenced by the paramagnetic cation in the enzyme·Mn·nucleotide complexes. However, the distances from Mn^{2+} to the carbon and protons are sufficiently large that a non-specific interaction of the cation with the protein could not be ruled out. We have now done additional experiments to demonstrate that the effect is specific. The relaxation rate of the carbon of $(\text{CH}_3)_3\text{NH}^+$ (an inactive monovalent cation) is not affected by Mn^{2+} in a variety of enzyme complexes. Some of those examined are shown in Table 2. The results demonstrate that $(\text{CH}_3)_3\text{NH}^+$ cannot closely approach enzyme-bound Mn^{2+} .

In another type of experiment the specificity of the CH_3NH_3^+ interaction with the enzyme was examined by determining the effect of the most active cation, NH_4^+ , on the relaxation rate of the CH_3NH_3^+ carbon in the presence of enzyme and MnADP (Table 3). pT_{1p} values for complexes containing CH_3NH_3^+ in the absence of NH_4^+ were determined and then used to calculate expected T_{1p} values of complexes assuming saturation of the enzyme·MnADP complexes with CH_3NH_3^+ and no effect of NH_4^+ . The observed values were higher than expected and could be explained by a reduction in the amount of enzyme-bound CH_3NH_3^+ . According to these calculations, an amount of NH_4^+ equal to the concentration of CH_3NH_3^+ , led to an 80% reduction in the concentration of the enzyme- CH_3NH_3^+ ion complex.

We also made use of [^{15}N] NH_4^+ and measured the effect of enzyme-bound Mn^{2+} , in the form of an enzyme·MnADP·formate complex, on the spin-lattice relaxation of the ^{15}N nucleus. The results in Table 4 show that there is an increased effect of the paramagnetic divalent cation when the enzyme is present, indicating that NH_4^+ binds close to the MnADP complex. Distances were calculated assuming τ_c values of 10.3 ns (frequency-independent) and 38 ns (frequency-dependent) and were found to be 0.6 nm and 0.5 nm, respectively. These compare with the value of 0.8–0.9 nm found for the distance from Mn^{2+} to the carbon of CH_3NH_3^+ [4]. Table 4 also shows the effect of H_4folate on the T_{1p} value. When the coenzyme is present there appears to be little or no paramagnetic contribution. This is similar to the effect of H_4folate on [^1H] H_2O , [^1H]formate, [^{13}C]formate and [^{13}C] CH_3NH_3^+ relaxation rates. Previously, this effect of H_4folate has been interpreted as a great reduction in the ability of the bound ligands to exchange with free ligands. This could be due to a physical block by the coenzyme or a conformational change in the protein.

SUMMARY

The results of n.m.r. spin-lattice relaxation-rate studies demonstrate that formate binds to the monomer form of formyltetrahydrofolate synthetase in such a manner that the carbon atom is the same distance from Mn^{2+} in the monomer·MnADP·formate complex as in the corresponding tetramer complex. Previous equilibrium binding experiments had shown that the metal-nucleotide complexes bind with equal affinity to the monomer and tetramer [5] and that the n.m.r. experiments here confirm the fact that the metal-nucleotide complex does bind to the monomer. On the other hand, H_4folate did not have the effect of decreasing the T_1^{-1} value of the monomer-bound formate as it does with the tetramer-bound formate. This result is consistent with the lack of H_4folate binding to the monomer [5].

Studies with monovalent cation interactions with the tetramer demonstrated that CH_3NH_3^+ and NH_4^+ bind close to the bound Mn^{2+} and that $(\text{CH}_3)_3\text{NH}^+$ does not. The distance from Mn^{2+} to the nitrogen of NH_4^+ is shorter than to the carbon of CH_3NH_3^+ .

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