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NMR Study of the Phosphate-Binding Loops of *Thermus thermophilus* Elongation Factor Tu†

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ABSTRACT: The phosphoryl-binding loops in the guanosine diphosphate binding domain of elongation factor Tu were studied by ^{15}N heteronuclear proton-observe NMR methods. Five proton resonances were found below 10.5 ppm. One of these was assigned to the amide group of Lys 24, which is a conserved residue in the phosphoryl-binding concensus loop of purine nucleotide binding proteins. The uncharacteristic downfield proton shift is attributed to a strong hydrogen bond with a phosphate oxygen. The amide protons from the homologous lysines in N-ras p21 [Redfield, A. G., & Papastavros, M. Z. (1990) *Biochemistry 29*, 3509-3514] and the catalytic domain of *Escherichia coli* elongation factor Tu [Lowry, D. F., Cool, R. H., Redfield, A. G., & Parmeggiani, A. (1991) *Biochemistry 30*, 10872-10877] also resonate downfield in similar positions. We propose that the downfield shift of this lysine amide proton is a spectral marker for this class of proteins. We also have studied the temperature dependence of the downfield resonances and find a possible conformation change at 40 °C.

There is a large class of proteins that bind and hydrolyze purine nucleoside triphosphates, whose structures change when they hydrolyze purine nucleoside triphosphate to purine nucleoside diphosphate. The energy of hydrolysis is coupled to other processes via the conformational change. For example, myosin hydrolyzes the ATP during muscle contraction, and adenylate kinase hydrolyzes ATP as it phosphorylates AMP. Elongation factor Tu (EFTu)[1] hydrolyzes GTP to GDP after it positions the aminoacyl transfer ribonucleic acid on the ribosomal A site. These proteins bind purine nucleoside triphosphate in similar ways, even though their global structures bear no resemblence to each other. There is ample evidence from X-ray crystallographic studies that the α- and β-phosphate oxygens fit into an anion hole. The hole is formed by the backbone amides of the well known eight-residue consensus loop: Gly,X,X,X,X,Gly,Lys,(Ser or Thr) (henceforth loop 1) (Dreusike & Schulz, 1988; la Cour et al., 1985; Jurnak, 1985; Clark et al., 1990; Wooley & Clark, 1989; Jurnak et al., 1990; Pai et al., 1989, 1990; Milburn et al., 1990: Schlichting et al., 1990, Bourne et al., 1990, 1991).

Saraste et al. (1990) have compared the sequences of loop 1 among several such proteins from different origins, and they found that the lysine residue is the only absolutely conserved residue in this loop, which suggests that it has a conserved structural role. In H-ras p21, adenylate kinase, and EFTu crystal models, the lysine is at the amino terminus of an α-helix, and its amide group points toward the phosphate oxygens. The lysine side chain spans the loop, and the ϵ-amino group hydrogen bonds to the carbonyl oxygen of the first glycine. Thus, both the side-chain amino group and the

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[1] Abbreviations: HMQC, heteronuclear multiple-quantum correlation spectroscopy; EFTu, elongation factor Tu; HSMQC, heteronuclear single-multiple-quantum correlation spectroscopy; MSL, maleimido-PROXY spin-label; G-domain, catalytic domain of *E. coli* EFTu.

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main-chain amide group of this lysine are structurally important.

The subclass of GDP/GTP-binding proteins has another phosphoryl-binding concensus loop (Dever et al., 1989). The Asp,X,X,Gly loop (henceforth loop 2) may be responsible for a regulatory protein conformation change upon hydrolysis of GTP. In EFTu and H-ras p21 crystal models, this loop contacts the β-phosphate oxygens. The γ-phosphate can only be accommodated if the loop moves. Such a movement might strain other regions of the protein.

The orientations of these consensus loops have been determined by crystallography. The models are sometimes ambiguous because hydrogen bonds are indirectly determined by the distance and angle between heavy atom centers. For instance, the amides in loop 1 are pointing toward the phosphate oxygens, but the major hydrogen-bond donors cannot be distinguished in currently available EFTu models. The NMR chemical shift can provide evidence for hydrogen-bond formation. The electron density at a nucleus manifests itself in NMR by shifting the frequency of the nuclear spin resonance. It is well documented that proton resonances shift to a higher frequency (downfield) when they are hydrogen bonded (Pimentel & McClellan, 1960; Robillard & Schulman, 1972; Wagner et al., 1983). In this way, NMR can help determine the major hydrogen-bond donors.

Two extremely downfield-shifted proton resonances were found in N-ras p21, and both were assigned to residues in concensus loop 1 (Campbell-Burke et al., 1988; Campbell-Burke, 1989; Redfield & Papastavros, 1990). One of the resonances was from the ubiquitous lysine, and the other was assigned to a glycine that is not conserved in the loop and not present in EFTu. It was suggested that these amides were donating hydrogen bonds to one or more phosphate oxygens. The successful studies of N-ras p21 prompted us to study the catalytic domain of EFTu from *Escherichia coli*. Five resonances were found below 10.5 ppm. Two of the resonances were assigned to amides in loops 1 and 2. One resonance was assigned to the conserved lysine in loop 1, and the other resonance was assigned to the glycine that is conserved in loop 2 [D. Lowry, reported in Redfield and Papastavros (1990) and Lowry et al. (1991)]. Two resonances were assigned to histidine imidazole NH groups because of their upfield nitrogen chemical shifts (Bachovchin, 1986).

We report here a similar study of intact EFTu cloned from the *tufl* gene of the thermophilic bacterium *Thermus thermophilus* (Seidler et al., 1987). The 45-kDa protein is large for a proton NMR study, but it is stable at 60 °C, so that isotope-edited NMR data were obtained in a reasonable amount of time by raising the temperature, which narrowed the resonances. The most important result from this work is that aspects of the spectra of the catalytic domain of EFTu from *E. coli* (henceforth G-domain) taken at 17 °C are similar to those of spectra of EFTu from *T. thermophilus* taken at 60 °C. We find three downfield amide proton resonances with nearly the same proton and nitrogen chemical shifts (referenced to water) as the ones in the G-domain. We conclude, on the basis of selective labeling with amino acids, spin-labeling with a nitroxide group, and replacement of Mg^{2+} with Mn^{2+}, that all three resonances are from amides in the phosphoryl-binding region. One resonance is assigned to the ubiquitous lysine from loop 1. The homologous lysines from loop 1 in N-ras p21, *E. coli* EFTu, and *T. thermophilus* EFTu are also extremely downfield-shifted, so that such a resonance would probably be present in spectra of other purine-binding proteins. Another downfield EFTu resonance is either from Gly 18 in loop 1, Gly 84 in loop 2, or possibly a glycine in the long loop (residues 40–60) that is absent from the crystal structure due to trypsin modification. It is probable that it is Gly 84, by comparison with the G-domain. If so, then the role of Gly 84 (homologous to Gly 83 in the G-domain) appears to be conserved among EF's Tu. The resonance from the homologous glycine in N-ras p21 does not have a significant downfield shift, and therefore may not have a significant role as a strong hydrogen-bond donor.

We also study the temperature dependence of the intensities and chemical shifts of the downfield resonances. We find that the intensities of some resonances decrease smoothly with decreasing temperature. The intensities of other resonances disappear abruptly at 40 °C, which may indicate a cooperative conformation change.

Materials and Methods

Protein Purification. EFTu from the *tufl* gene of *T. thermophilus* was previously cloned into JM101 cells. Protein expression is controlled by the *taq* promoter, so overexpression is induced by addition of 1 mM IPTG to the cell culture. EFTu was specifically labeled with either [^{15}N]glycine or [$^{15}N_a$]lysine/[^{15}N]glycine using defined media as described in Lowry et al. (1991). Fully ^{15}N-labeled EFTu was produced by growing cells in an M9 minimal medium containing $^{15}NH_3Cl$. Cell cultures were grown overnight to stationary phase in 2 L of unlabeled media. The cells were harvested, resuspended at A_{600} = 1, and induced for 5.5 h in fresh media containing the appropriate ^{15}N-labeled compound(s). The cells were sonicated (6 g/50 mL) in a buffer (buffer A) consisting of 50 mM Tris-HCl pH 7.5, at 4 °C, 10 mM $MgCl_2$, 1 mM DTT, 20 μM GDP, 20 μM PMSF, and 5% (v/v) glycerol. The lysate was centrifuged at 100000*g* at 4 °C for 2 h to pellet cell debris. The supernatant was dialyzed against 2 × 500 mL of buffer A and loaded onto a 75-mL Q-Sepharose FF (Pharmacia) column. The EFTu fraction was eluted with a 0–500 mM KCl 1-L gradient of buffer A without PMSF. The elution of *T. thermophilus* EFTu was monitored by SDS–PAGE, and it elutes in the same fractions as does EFTu from *E. coli*. The two proteins were separated by heat denaturation of the *E. coli* EFTu at 60 °C for 15 min. The precipitated *E. coli* protein was pelleted at 13000*g*, at 24 °C. The supernatant was concentrated to 5 mL and either immediately concentrated to give a 1 mM protein NMR sample or first loaded onto a Sephacryl S200 HR (Pharmacia) gel filtration column (65 cm × 1.8 cm^2) and eluted with buffer A + 50 mM KCl without PMSF.

NMR Spectroscopy. We used isotope-aided NMR experiments with semiselective jump–return pulses (Plateau & Gueron, 1982). The ^{15}N isotopes are used to edit spectra such that only resonances from protons attached directly to ^{15}N are observed. Most of the two-dimensional data were obtained using heteronuclear multiple-quantum correlation (HMQC) (Bax et al., 1983). The spectrum in Figure 1 was obtained by a high-resolution variant of this method called HSMQC (Zuiderweg, 1990). Each resonance corresponds to a protein ^{15}N–^{1}H group, and its coordinates in the spectrum give proton and nitrogen chemical shifts. Good HMQC spectra of 45-kDa EFTu at 60 °C were obtained in 8 h. One-dimensional difference decoupling was also used. It is more sensitive than HMQC, because there is no preparation time as in HMQC (Griffey & Redfield, 1987). About 5 min are required for us to obtain usable one-dimensional ^{15}N difference-decoupled proton-observed spectra. The experiment is useful for observing isolated proton resonances or for quickly determining the integrity of the protein sample. It is also helpful for

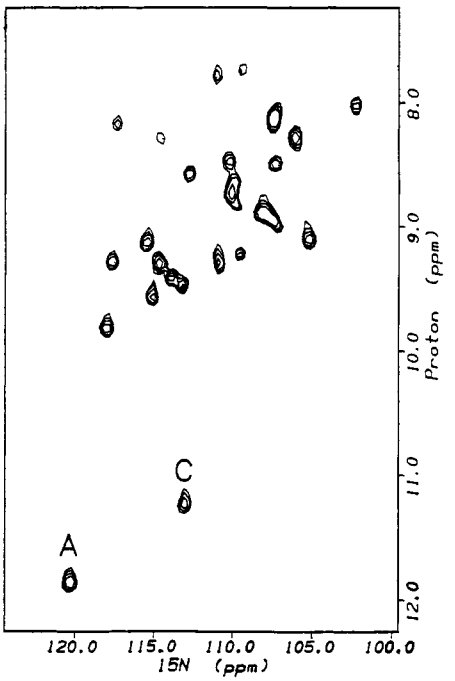

FIGURE 1: Nitrogen–proton HSMQC spectrum of [^{15}N]glycine-labeled EFTu obtained at 60 °C. The spectral widths were 10 240 Hz proton and 4000 Hz nitrogen. The resolution was 20 Hz per point in the proton dimension and 15.6 Hz per point in the nitrogen dimension. A total of 99 200 transients were recorded with a 0.8-s recycle time. The contour plot was executed by software kindly provided by Hare Research.

observing a signal from dilute samples or for confirming weak, questionable HMQC resonances. Proton shifts are referenced to H_2O, assumed to be at 4.8 ppm, and ^{15}N shifts are referenced to ^{15}N ammonia.

Assignment Strategy. Labeling with specific [^{15}N]amino acids allows us to assign resonances by amino acid type. Further progress toward assignments is made by spin-labeling the protein. The spin-label's unpaired electron will broaden and often obliterate nearby nuclear resonances (Kosen, 1989). The chemically reactive group on the spin-label is often highly specific for certain amino acid side chains. In this case, the spin-label is maleimido-PROXYL (MSL), which is specific for cysteine sulfhydryl groups. Proteins from thermophilic bacteria have few cysteines. EFTu from *T. thermophilus* has only one cysteine at position 82 in concensus loop 1. We use the same procedure to spin-label EFTu that we used to spin-label G-domain (Lowry et al., 1991). Another technique for obtaining assignments is to replace the active site Mg^{2+} with Mn^{2+}. The paramagnetic Mn^{2+} ion will broaden nearby resonances. The Mg^{2+} was removed by dialysis of the NMR sample against 1 L of NMR buffer containing 5 mM $MnCl_2$, followed by dialysis against 1 μM $MnCl_2$ to remove excess ions. These experiments are successful in effecting spectral changes that, in conjunction with specific labeling by amino acid type and reference to the crystal model, suggest sequence-specific assignments of resonances to Gly 84, Gly 18, and Lys 24. The crystal derived distances are taken from the Brookhaven Protein Data Bank file PDBETU.ENT, deposited by la Cour et al. (1985). These assignment techniques are not as rigorous as assignment techniques based on *J* coupling or NOE, but they are useful when the latter techniques fail.

Results

[^{15}N]Glycine-Labeled EFTu. Figure 1 shows an HSMQC spectrum (Zuiderweg, 1990) of [^{15}N]glycine-labeled EFTu from *T. thermophilus*. Each resonance is from a glycine amide group in the protein. There are 40 glycines in EFTu, but there are only 31 resolved resonances in the spectrum. The lack of some resonances may be explained by the existence of chemical exchange. Amide protons that exchange with water at a rate somewhat faster than the line width of the 31 resolved resonances (~30 Hz) would not have observable resonances in the HMQC and difference-decoupled spectra. Slow exchange (10–1000 s^{-1}) between different amide conformations or chemical environments would also cause resonances to broaden.

Table I: Amide Shifts of Homologous Downfield Residues in N-ras p21, G-Domain, and EFTu

residue[a]	p21[b]		G-domain[c]		EFTu[d]	
	^{1}H	^{15}N	^{1}H	^{15}N	^{1}H	^{15}N
Lys 16/24/24	10.6	126.5	11.07	126.8	11.12	126
Gly 60/83/84	8.31	108.7	11.34	114.3	11.27	113.1

[a] Residue numbers for p21/G-domain/EFTu. [b] From Redfield and Papastavros (1990) and Campbell-Burk (1989). [c] From D. Lowry et al. (1991). [d] This research.

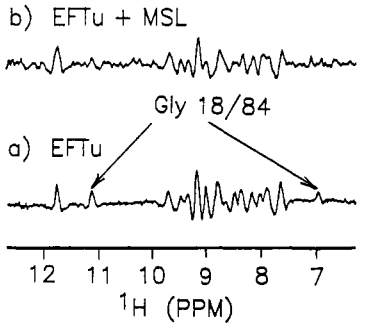

FIGURE 2: (a) One-dimensional difference-decoupled spectrum of the same sample as in Figure 1. (b) Difference-decoupled spectrum of [^{15}N]glycine-labeled EFTu, spin-labeled at Cys 82 with MSL. One downfield resonance attenuates, and the most upfield resonance disappears in the spin-labeled protein. This pattern of attenuation is similar to that seen with spin-labeled G-domain and suggests the indicated assignments.

[^{15}N]Glycine-Labeled Protein Modified with Spin-Label. The downfield glycine resonances in Figure 1 are particularly interesting because, on the basis of experience with G-domain and N-ras p21, they may be donating strong hydrogen bonds to phosphate oxygens. The resonance (C in Figure 1) (at 11.27 ppm proton, 113.1 ppm nitrogen) has almost the same chemical shifts as a resonance in G-domain assigned to the conserved Gly 83 in loop 2 (Table I). The glycine resonance (A) (at 11.88 ppm proton, 120.6 ppm nitrogen) has no analogue in G-domain. It can still be seen in one-dimensional spectra of apoprotein, so its anomolous chemical shift cannot be due to interaction with the GDP ligand (Limmer et al., 1992). When we spin-label the only cysteine in EFTu (Cys 82) with MSL, the glycine proton resonance (C) at 11.27 ppm is strongly attenuated, and the most upfield glycine proton resonance at 7.14 ppm disappears into the noise (Figure 2). This was almost the same pattern observed when G-domain was spin-labeled at the homologous cysteine. In the latter case, the downfield glycine resonance disappeared, and the upfield glycine resonance was strongly attenuated (Lowry et al., 1991). The position of the unpaired electron is not well known and is likely to be spatially delocalized, because the long cysteine side-chain spin-label may be moving among several positions. The reverse pattern of attenuation between G-domain and EFTu indicates, assuming these resonances do correspond with each other in the two proteins, that the protein geometry is somewhat different and that possibly the range of conformations available to the spin-label is different. Gly 84 (Gly 83 in EFTu from *E. coli*) and Gly 18 are both near Cys 82 (Cys

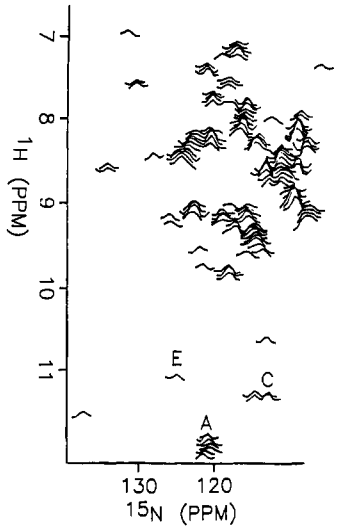

FIGURE 3: An HMQC spectrum of [^{15}N]glycine/[^{15}N$_\alpha$]lysine-labeled EFTu obtained at 50 °C. Another downfield resonance E besides the two glycine resonances appears in this spectrum, and therefore it is from a lysine amide group. Although this resonance appears weak in this figure, we have confirmed its reproducible existence in several one- and two-dimensional runs. In this and Figure 4, we use a threshold stack-plot base on our own software, which provides a better representation of a two-dimensional spectrum having a low signal-to-noise ratio than does a contour plot.

81 in G-domain) in the crystal structure. It is possible that other glycines from the trypsin-modified loop (consisting of residues 40–60 and missing in the crystal structure) are also near Cys 82. In G-domain, a similar upfield resonance was assigned to Gly 18, and the similar downfield resonance was assigned to Gly 83 (Lowry et al., 1991).

[^{15}N]Glycine/[$^{15}N_\alpha$]Lysine-Labeled Protein. Figure 3 shows the HMQC spectrum of [^{15}N]glycine/[^{15}N$_\alpha$]lysine double-labeled EFTu. Unfortunately, the yield from the protein preparation was low, so the NMR protein signal was weak. There is only one extra downfield resonance (at 11.12 ppm proton, 126 ppm nitrogen) as compared with the [^{15}N]-glycine-labeled sample, so it must be from a lysine amide group. The ^{15}N–^{1}H shifts are similar to a lysine resonance in G-domain that was assigned to Lys 24 in the long consensus loop 1 (Table I).

Spectral Sensitivity to Mn^{2+}. All of the downfield resonances mentioned above are resolved in the HMQC spectra of fully ^{15}N-labeled EFTu (Figure 4C,D). When Mg^{2+} was replaced by Mn^{2+} in such a sample, the downfield lysine resonance disappeared. This is consistent with its assignment to Lys 24, which is 6.38 Å away from Mg^{2+} in the crystal structure. In order to confirm these changes, they were also observed by difference decoupling. A nonselective decoupling comparison indicated that one or more resonances at about 11.2 ppm were being attenuated by ligation of Mn^{2+} compared to Mg^{+2} (Figure 5). Selective difference decoupling (Figure 6) confirmed that resonances D and E were attenuated by Mn^{2+}, while resonances A and C were not. This result is inconsistent with the crystal structure and the assignment of one of them to Gly 84, because Gly 84 is only 5.55 Å away from Mg^{2+} in the crystal model. However, a small increase in the distance from the proton to the magnetic ion could make a large difference in the nuclear spin relaxation, which varies as the inverse sixth power of the distance, and thus in the intensity. The unidentified resonance D (at 11.21 ppm proton, 137.1 ppm nitrogen) is strongly attenuated. Tryptophan indole protons resonate near this frequency, but the crystal structure places no tryptophans near the Mg^{2+}-binding site. If we assume that this resonance is from an amide group and that it is hydrogen bonded to a phosphate oxygen, then Asp 21 is a likely candidate since the resonance from the homologous Gly 13 in N-ras p21 also has an extreme downfield shift.

Lack of Spectral Sensitivity to Aluminum Fluoride. Aluminum fluoride is known to activate the G-protein $G_o\alpha$. The AlF_4^- complex resembles inorganic phosphate. It binds $G_o\alpha$·GDP as a transition-state analogue of the γ-phosphate. It activates the α-subunit and causes the $\beta\gamma$-subunits to dissociate (Gilman et al., 1987). $G_o\alpha$ also has a concensus loop 1, so we tested whether aluminum fluoride would be a transition-state analogue for EFTu. Fully ^{15}N-labeled EFTu was dialyzed against NMR buffer containing 10 mM NaF and 10 μM $AlCl_2$. No change is observed in the HMQC spectrum, and so aluminum fluoride is probably not a good transition-state analogue for EFTu (data not shown). The same conclusion was also reached by Hazlett et al. (1991). They could not detect changes upon addition of EFTu in either fluorescence or ^{19}F NMR of aluminum fluoride.

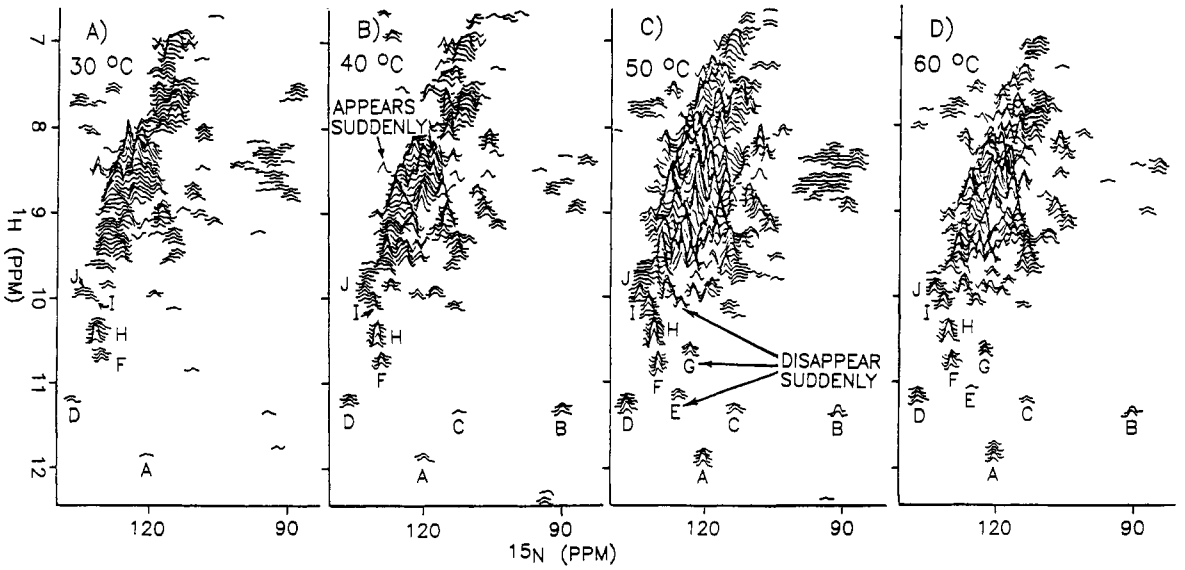

FIGURE 4: HMQC spectrum of fully ^{15}N-labeled EFTu at (A) 30, (B) 40, (C) 50, and (D) 60 °C. Most resonances lose intensity slowly with temperature, but other resonances (indicated in the figure) disappear suddenly as the temperature is decreased below 50 °C. At least one new resonance appears at 40 °C and increases in intensity with decreasing temperature.

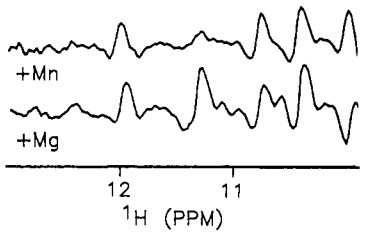

FIGURE 5: Nonselective difference-decoupling experiment on fully ^{15}N-labeled EFTu complexed with either Mn^{2+} or Mg^{2+}, at 50 °C. Note the large attenuation of the feature at 11.2 ppm in the sample with Mn^{2+}.

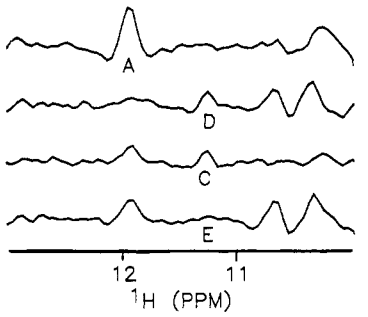

FIGURE 6: Selective ^{15}N difference decoupling of the downfield resonances as indicated, from fully ^{15}N-labeled EFTu complexed with Mn^{2+}. Neither downfield glycine resonance (A and C) is significantly perturbed by the Mn^{2+}. The lysine resonance (E) disappears, and the unassigned resonance (D) is weakened. For comparison, in Figure 4C, peaks A and D are equally strong, whereas peak C is weaker than peak A is, just as it is here.

Temperature Dependence of the HMQC Spectrum. Thermophilic EFTu is stable at 65 °C. We studied the temperature dependence of the downfield amide resonances over a 43 °C temperature range. Four HMQC spectra of fully ^{15}N-labeled protein taken at 30–60 °C are shown in Figure 4. The resonances responded in three different ways. Some resonance intensities decrease slowly with decreasing temperature. Some resonances vanish abruptly between 50 and 40 °C. At least one new resonance appears at 40 °C and becomes more intense with decreasing temperature. Small decreases in resonance intensity with decreasing temperature are probably due to decreased rotational diffusion of the protein. The abrupt changes occur during the same 10 °C interval, between 50 and 40 °C. These changes may indicate a cooperative conformation change.

The proton shifts of Figure 4 are not corrected for the temperature dependence of the H_2O resonance shift. After such correction, these spectra and one-dimensional spectra (not shown) indicate that the resonance assigned to Gly 83 has a relatively strong upfield shift with increasing temperature, possibly due to exposure of the Gly 83 amide group to solvent.

Discussion

We assign four amide resonances to the phosphoryl-binding domain of *T. thermophilus* EFTu. Three of these four resonances have distinct proton shifts below 11 ppm. One downfield resonance is assigned specifically to Lys 24 (peak E), which is strictly conserved in consensus loop 1. This assignment is based on selective labeling and the resonance's diminished intensity due to Mn^{2+}. The downfield proton chemical shift of this lysine amide and the proximity to phosphate oxygens in the crystal model suggest that the amide proton is donating a strong hydrogen bond to a phosphate oxygen. This same conclusion was reached in similar NMR studies of G-domain and N-ras (Redfield & Papastavros, 1990; Lowry et al., 1991). We propose that the hydrogen bond from this lysine amide proton is a strictly conserved feature in this class of proteins.

Two of the four resonances from the phosphoryl-binding domain appear to be from Gly 18 and Gly 84, which are conserved residues in loops 1 and 2, respectively. These resonances are assigned solely on the basis of their similarity to assigned resonances in G-domain and N-ras p21 (Table I) and their sensitivity to a spin-label covalently attached to Cys 82. However, the assignment of the downfield resonance (peak C) to Gly 84 is not consistent with its lack of sensitivity to Mn^{2+}, so that the assignment is tentative. It is possible that Gly 84, like Lys 24, is donating a strong hydrogen bond to a phosphate oxygen. A similarly shifted resonance in spectra of G-domain was assigned more rigorously to the homologous Gly 83 from loop 2. The crystal structure of EFTu places Gly 84 in the vicinity of a β-phosphate oxygen, but it is too far away to make direct contact. Another possibility is that Gly 84 is not interacting with the ligand at all but is binding a water molecule or some other acceptor. The chemical shift of the Gly 84 resonance has nearly the same temperature dependence as the water resonance, whereas the other downfield amide resonances do not shift as much. Therefore, Gly 84 is probably more solvent-exposed than the amides corresponding to either of the other glycine resonances (Gly 18 and peak A) or Lys 24.

The latter two amides are likely to be involved in structural hydrogen bonds, since their resonance shifts are less temperature dependent (Kopple et al., 1969). The resonance tentatively assigned to Gly 18 has an unusual upfield proton shift, similar to that of the homologous glycines 18 and 10 in G-domain and N-ras p21, respectively. There is no crystal structure for G-domain, but in the X-ray models of trypsin-modified EFTu and H-ras p21 this glycine has unusual ψ and ϕ angles that are accessible only to glycine residues. The unusual conformation of this residue may be responsible for its extreme upfield proton shift.

Acknowledgments

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Registry No. L-Lysine, 56-87-1; glycine, 56-40-6.

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Retroviral Nucleocapsid Protein Specifically Recognizes the Base and the Ribose of Mononucleotides and Mononucleotide Components†

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ABSTRACT: The interaction of the retroviral nucleocapsid protein (NC) with nucleic acids forms the basis of its varied roles in the replication cycle, which include binding and condensing the viral RNA within the virion, stimulation of the early steps in reverse transcription, and dissociation from RNA in the replication complex. As part of an investigation of the NC binding site and of the forces that drive its interaction with nucleic acids, the relative affinities of NC from avian myeloblastosis virus were determined for a series of mononucleotides and mononucleotide components using a competitive displacement assay utilizing the extrinsic fluorescent probe bis-ANS [Secnik, J., Wang, Q., Chang, C.-M., & Jentoft, J. E. (1990) *Biochemistry 29*, 7991-7997]. The estimated binding affinities were unexpectedly similar for nucleotides, nucleosides, and bases ($K_a > 10^6$ M^{-1}). AMP, UMP, GMP, and CMP bound to NC with essentially equal affinity, indicating that NC does not discriminate between bases. This is consistent with its role in coating, condensing, and packaging the RNA within virions. Nucleosides, bases, riboses, and ribose phosphate bind to NC with 1000-fold higher affinity than inorganic phosphate, indicating that the NC binding site includes elements that recognize nucleotide base and ribose components in addition to phosphate ions. However, the binding affinities of components are not additive, i.e., the K_{app} values for adenine and deoxyribose are very similar to that for deoxyadenosine, indicating that the interaction between the NC subsite and the base and the sugar components is complex. The stoichiometry of the complex between bis-ANS and NC was established to be NC·(bis-ANS)$_3$. The data are consistent with a model in which bis-ANS or nucleotide ligands can compete on a one-for-one basis for binding at three NC subsites, where binding at each site is independent of binding at the other two sites. Taken together, these findings allow us to predict that when NC binds to RNA or DNA, it directly interacts with three nucleotides.

The nucleocapsid (NC) protein is a small basic protein that is an essential, multifunctional component of replication competent retroviruses. During the early stages of viral assembly, sequences within the NC play a role in the specific recognition of genomic viral RNA (Jentoft et al., 1988; Gorelick et al., 1988; Meric & Spahr, 1986). Within viral particles, the 2000 or so copies of this small, basic protein bind to the dimeric genomic RNA (Jentoft et al., 1988; Fleissner, 1971; Davis & Rueckert, 1972) indicating a histone-like role for NC in packaging viral RNA. NC also appears to have a role in the dimerization of the genomic RNA (Prats et al., 1988). NC is generally considered to be a component of the in vivo transcription complex (Fuetterer & Hohn, 1987; Brown

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