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NMR Study of the Phosphate-Binding Elements of *Escherichia coli* Elongation Factor Tu Catalytic Domain†

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ABSTRACT: The phosphoryl-binding elements in the GDP-binding domain of elongation factor Tu were studied by heteronuclear proton observe methods. Five proton resonances were found below 10.5 ppm. Two of these were assigned to the amide groups of Lys 24 and Gly 83. These are conserved residues in each of the consensus sequences. Their uncharacteristic downfield proton shifts are attributed to strong hydrogen bonds to phosphate oxygens as for resonances in N-ras-p21 [Redfield, A. G., & Papastavros, M. Z. (1990) *Biochemistry 29*, 3509–3514]. The Lys 24 of the EF-Tu G-domain has nearly the same proton and nitrogen shifts as the corresponding Lys 16 in p21. These results suggest that this conserved lysine has a similar structural role in proteins in this class. The tentative Gly 83 resonance has no spectral analogue in p21. A mutant protein with His 84 changed to glycine was fully ^{15}N-labeled and the proton resonance assigned to Gly 83 shifted downfield by 0.3 ppm, thereby supporting the assignment.

We are interested in the large class of purine nucleoside binding proteins that couple the binding and hydrolysis of purine triphosphate to some other useful function. Proteins of this class give rise to diverse processes such as polypeptide chain elongation and initiation, control of cell proliferation, muscle contraction, neurotransmission, and hormone action. These proteins appear similar in the way they bind purine triphosphate. They have one concensus element (henceforth element 1) Gly-X-X-X-X-Gly-Lys-(Ser or Thr), where X indicates a variable residue and parentheses indicate the possible residues of a single position that is not strictly conserved. Structural studies have shown that some of the amide protons in this element are pointing toward phosphate oxygens. The lysine side chain spans the element and the ε-amino group hydrogen bonds to the carbonyl group of the first glycine in

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the element. The subclass of guanosine triphosphate binding proteins have two additional consensus elements. One is Asp-X-X-Gly (henceforth element 2), which is close to the magnesium ion and the β-phosphate oxygens of bound GDP.[1] In fact, the γ-phosphate can only be accomodated if element 2 moves. Element 2 may be involved in a regulatory change in protein conformation upon hydrolysis of GTP to GDP (La Cour et al., 1985). Another concensus element (not addressed in this work) has been shown to interact with the base and confer specificity to the GTP-binding proteins (Bourne et al., 1990, 1991; Dever et al., 1989; Saraste et al., 1990).

We report the NMR study of the amino-terminal guanosine triphosphate binding domain of elongation factor Tu (henceforth G domain and EF-Tu). EF-Tu is a ubiquitous prokaryotic GTP-binding protein necessary for the accurate translation of mRNA. During an elongation cycle, EF-Tu interacts with GTP, GDP, aatRNA, ribosome, and EF-Ts. A Mg^{2+} ion and monovalent cations are required for these interactions. EF-Tu·GTP binds any aatRNA except for the initiator fMet-$tRNA_f$ and carries it to the programmed ribosome. This complex facilitates hydrolysis of EF-Tu·GTP to EF-Tu·GDP and P_i. As a consequence, the EF-Tu changes specificity and dissociates from the ribosome complex to permit the next step of elongation. The interaction between the ternary complex EF-Tu·GTP·aa-tRNA and the programmed ribosome and the associated GTP hydrolysis plays an essential role in the fidelity of translation [recently reviewed in Thompson (1988)].

X-ray crystallographic studies have shown that EF-Tu has three structural domains (Jurnak, 1985; la Cour et al., 1985). In order to study the structure–function relationships of this multifunctional protein, Parmeggiani et al. (1987) have isolated the N-terminal domain of EF-Tu (G domain) by site-directed mutagenesis of one of its encoding genes, *tuf A*. The truncated protein conserves the basic activities of EF-Tu, i.e., the stoichiometric binding of GDP and GTP and the hydrolysis of GTP, with similar ionic constraints (Jensen et al., 1989). A series of substitutions in the G domain of EF-Tu and in the intact molecule induce functional alterations with striking similarities to the effects of the corresponding substitutions in homologous Ha-ras p21 (Jacquet & Parmeggiani, 1988, 1989; Gümüsel et al., 1990; Cool & Parmeggiani, 1991; Cool et al., 1990).

NMR and crystallographic studies on H-ras p21 verified that p21 and domain I of EF-Tu are similar as was initially predicted by McCormick et al. (1985) and Jurnak (1985). Consensus elements 1 and 2 are placed in the same relative orientation in both proteins, and hydrogen bonds from peptide NH groups to phosphate oxygens are suggested by the crystal models (la Cour et al., 1985; Jurnak, 1985; Yamasaki et al., 1989; Clark et al., 1990; Jurnak et al., 1990; Woolley & Clark, 1990; Pai et al., 1989, 1990; Milburn et al., 1990; Tong et al., 1991; Schlichting et al., 1990). Our NMR approach is slightly different from traditional NMR studies. We observe resonances from labile protons in large biomolecules by labeling specific groups with ^{15}N, as suggested by prior biochemical evidence. Here, as with a previous study from our laboratory, we present NMR evidence for strong hydrogen bonds from amide protons in the phosphoryl-binding site of the G domain. Isotope-aided NMR studies of N-ras p21 complexed to GDP showed anomolously downfield-shifted amide proton resonances that could be assigned to amide protons from concensus element 1. These downfield shifts were presumed to be due to strong hydrogen bonds to phosphate oxygens as indicated by the X-ray structures (Campbell-Burk et al., 1988; Campbell-Burk, 1989; Redfield & Papastavros, 1990). The interesting downfield proton resonances were successfully assigned in p21. Thus, it was of interest to us to look for downfield amide proton resonances in the structurally homologous G domain. Concensus elements 1 and 2 in the G domain are at positions 18–24, Gly-His-Val-Asp-His-Gly-Lys, and at positions 80–83, Asp-Cys-Pro-Gly, respectively. We show that the G domain has at least two downfield proton resonances that we assign to amides in elements 1 and 2. One downfield resonance was assigned to the conserved lysine from element 1 in the G domain, and it has nearly the same chemical shift in both proton and nitrogen dimensions as the conserved lysine from element 1 in p21 [D. Lowry, reported in Redfield and Papastavros (1990)]. We propose that the downfield proton resonance from lysine in the Gly-X-X-X-X-Gly-Lys element may be a universal spectroscopic marker for this class of proteins. We also find a downfield-shifted glycine resonance which we assign to Gly 83 in element 2. We study a mutant protein in which His 84 is replaced by glycine and find that the proton resonance assigned to Gly 83 shifts downfield by 0.3 ppm, which is consistent with its assignment. This conserved glycine is proposed to play an important structural role in EF-Tu, but it should not be taken to be universal among GTP-binding proteins (as for the role of the lysine in element 1) because a hydrogen bond from the homologous glycine in p21 is not suggested by the NMR data (Campbell-Burke, 1989).

Materials and Methods

The G domain is encoded by the runaway replication vector pCP40*tuf A*(Δ610–1179), which contains a λ-pL promoter (Parmeggiani et al., 1987). The promoter is regulated by a temperature-sensitive cI repressor encoded by a second plasmid, pcI857. A shift in temperature from 28 to 42 °C induces runaway replication of pCP40 and expression of the G domain (Remaut et al., 1983). [^{15}N]Glycine-labeled G domain was produced from the original RM435 strain, whereas [^{15}N]-lysine- and [^{15}N]glycine/[^{15}N]lysine-labeled G domain were produced from a Lys A^- strain, ET505, obtained from the *Escherichia coli* Genetic Stock Center (Yale). Fully ^{15}N-labeled G domain was produced from expression vectors transformed into a JM101 strain. The G domain was purified from cells grown in either minimal or defined media as described in Muchmore et al. (1989). For the defined medium, only half of the specified unlabeled amino acids were used, and 0.5 g/L of each ^{15}N-labeled amino acid(s) was used. The cell culture was grown to late log phase at 28 °C. The cells were then centrifuged and resuspended in fresh media containing ^{15}N and immediately induced by rapidly shifting the culture from 28 to 42 °C for 2 h. This was accomplished for large cultures by immersion of the incubation flask in a tub of 60 °C water until the culture reached 42 °C, and then placing it in a shaking incubator for 2 h.

All purification buffers were the same as were used previously (Parmeggiani et al., 1987). Three to six grams of cells were sonicated in 50 mL of sonication buffer. The lysate was centrifugated at 100000*g* for 3 h at 2 °C. The supernatant was dialyzed against the starting buffer and then loaded onto a 15 cm × 7 cm^2 QA-52 ion exchange column (Pharmacia).

[1] Abbreviations: aa-tRNA, amino-acylated transfer ribonucleic acid; DTT, dithiothreitol; ET-Ts, elongation factor Ts; EF-Tu, elongation factor Tu from *E. coli*; G-domain, isolated amino-terminal domain of elongation factor Tu; GDP, guanosine 5′-diphosphate; GTP, guanosine 5′-triphosphate; HMQC, heteronuclear multiple-quantum correlation; NOE, nuclear Overhauser effect; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

The G domain was eluted with a linear 60–220 mM KCl gradient. Fractions containing the G domain were identified by SDS–PAGE. They were pooled and concentrated to 5 mL in a 62-mm stirred ultrafiltration cell with a YM-10 filter (Amicon) and then loaded onto a 50 cm × 1.8 cm^2 Sephadex G-75 column (Sigma). The G domain fractions were pooled and concentrated to 2 mL in a 43-mm stirred ultrafiltration cell with a YM-10 filter at 15 psi. Unless indicated otherwise, the sample was then dialyzed against NMR buffer consisting of 25 mM Tris, 5 mM $MgCl_2$, 150 mM KCl, 0.004% NaN_3, 1 mM DTT, 20 μM GDP, pH 7.6, at 4 °C in 10% D_2O. After dialysis, the sample was concentrated to give 1–2 mM protein using a Centricon-10 cartridge spun at 1000*g*. Protein concentration was determined by a Bradford assay (Bio-Rad) using bovine serum albumin as a standard. Protein integrity was judged by the quality of the NMR spectra. The protein spectra were of varying quality, but the overall quality seemed to improve when 20 μM GDP was used in all column buffers.

[^{15}N]Glycine-labeled protein was spin labeled with 3-maleimido-PROXYL as described in Hata-Tanaka et al. (1989). The protein sample was diluted to 0.5 mM in NMR buffer without DTT, and dialyzed against the same buffer with 1 mM spin label. The sample was incubated for 3 h at 24 °C, then dialyzed overnight at 4 °C in NMR buffer (without DTT) to get rid of excess spin label.

We used three NMR techniques to study the G domain. Two-dimensional heteronuclear multiple-quantum correlation spectroscopy (HMQC) was used to correlate chemical shifts of resonances from ^{15}N–^{1}H spin pairs (Bax et al., 1983). It is a sensitive technique, but we were not able to observe all the resonances. One-dimensional difference decoupling is a more sensitive isotope-editing technique, in which an observe proton pulse is followed by ^{15}N-decoupling during the free induction decay. Two spectra are acquired with the decoupler frequency set on and off the nitrogen resonance. When these two spectra are subtracted, the unlabeled resonances cancel, but the labeled resonances do not because they are, respectively, a singlet and a 90 Hz doublet in each of the spectra. One-dimensional difference decoupling is more sensitive than two-dimensional HMQC or its one-dimensional equivalent, because decoupling requires no preparation time during which the signal would decay (Griffey & Redfield, 1987). The third technique we used is called isotope-filtered NOE (Griffey & Redfield, 1987). It is a one-dimensional NOE experiment that is edited to display only NOEs from one labeled proton. The ^{1}H and ^{15}N frequencies of a particular spin pair are determined from a previously recorded HMQC spectrum. Selective irradiation is set at the ^{1}H frequency with ^{15}N decoupling on and off the ^{15}N resonance of the spin pair. When the decoupling is on resonance, the proton resonance is a singlet and is saturated by the selective irradiation. When the decoupling is off resonance, the proton resonance is split by 100 Hz and is not significantly affected by the irradiation. NOEs from all but the one spin pair are cancelled out in the difference spectrum. It is also easy to determine if the destination resonance is labeled by leaving the decoupler off during the FID and looking for the appearance of a 90 Hz splitting in the destination resonance (Redfield & Papastavros, 1990). All samples were approximately 2 mM in protein concentration. Chemical shifts were measured relative to DSS for proton spins and NH_3 for nitrogen spins.

Results

General Spectral Features. Figure 1 shows a ^{15}N–^{1}H HMQC spectrum of [^{15}N]glycine-labeled G domain. There are 16 glycine residues in the G domain, but only 13 glycine

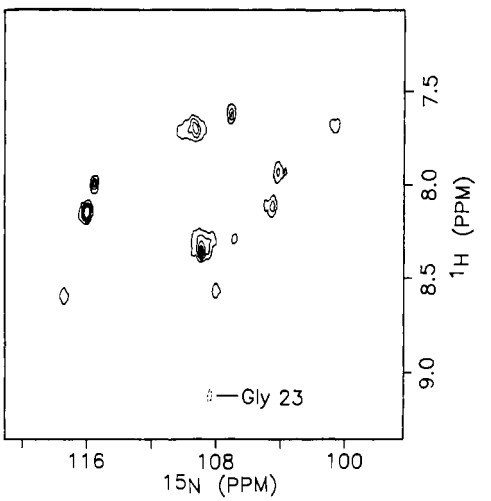

FIGURE 1: HMQC spectrum of G domain labeled with [^{15}N]glycine at 17 °C. There are 13 out of 16 glycines present in the spectrum, one of which can only be seen at the noise level, below the level of this plot. The position of this peak, identified as glycine 23, is indicated in the spectrum. Resolutions and spectral widths were 10 Hz/point, 8 kHz (proton) and 16.67 Hz/point, 4 kHz (nitrogen). The recycle time was 0.8 s and one complete 2-D cycle was acquired.

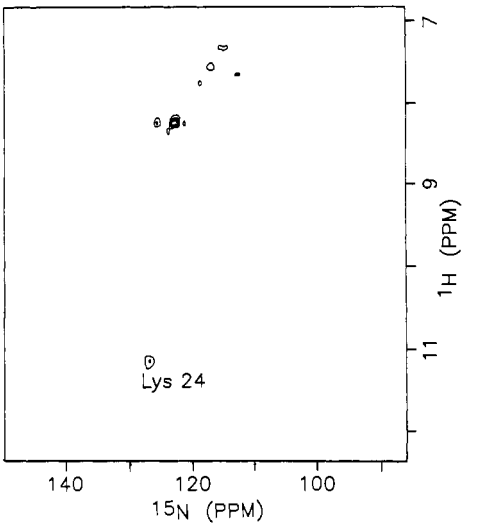

FIGURE 2: HMQC spectrum of G domain labeled with ^{15}N at the α position of lysine. The downfield lysine is visible in this spectrum, unlike the downfield glycine in Figure 1. Resolutions and spectral widths are 10 Hz/point, 8 kHz (proton) and 16.67 Hz/point, 4 kHz (nitrogen). The recycle time as 0.6 s. A total of 16 complete 2-D cycles were acquired. The sample temperature was 24 °C.

resonances are discernable in the HMQC spectrum. Two more glycine resonances are apparent in the difference-decoupled spectrum. They both resonate at frequencies uncharacteristic for amide protons, one at 11.3 ppm, the other at 6.8 ppm (Figure 5a). They are broad and are usually absent in our HMQC spectra. The broadness of these resonances suggests that there is internal motion at or near these spins on the time scale of their T_2's, or about 10 ms.

Figure 2 shows an HMQC spectrum of [$^{15}N_\alpha$]lysine-labeled protein. There are 10 lysines in the G domain. Nine lysine resonances are observable in the HMQC spectrum of this sample. One lysine amide proton has an uncharacteristic chemical shift of 11.1 ppm. Another lysine resonance is extremely narrow and intense compared with the other resonances, and this is most likely due to a lysine in a disordered or flexible part of the protein.

The HMQC spectrum of fully ^{15}N-labeled G domain had the same distinctive downfield shifted amides (glycine in-

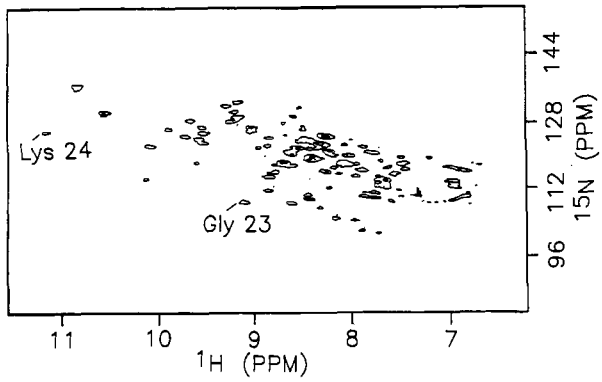

FIGURE 3: HMQC spectrum of fully ^{15}N-labeled G domain taken at 18 °C. Current assignments are indicated in the figure. The histidine imidazole protons are not visible because they are folded in and have negative intensity. Resolutions and spectra widths were 10 Hz/point, 8 kHz (proton) and 31.25 Hz/point, 4 kHz (nitrogen). The recycle time was 0.8 s. Seven complete 2-D cycles were acquired.

cluded). It also had two imidazole NH resonances from histidine, as determined by their ^{15}N shift (Bachovchin, 1986). This spectrum showed less than 100 distinct amide resonances (Figure 3). A stack plot reveals that the central region of this spectrum has many sharp intense features and a severe lack of chemical shift dispersion, which suggests that the protein sample is partially disordered. However, the downfield resonances, which are the focus of this paper, were reproducible in samples prepared from at least eight different cell growths and purifications. The protein spectra varied in quality in the sense that the intensity of resonances in the central region of the HMQC spectra varied relative to resonances in the downfield region. Often the samples would precipitate in the NMR tube, but this problem was overcome by keeping the protein concentration below 1 mM. A good sample would last a few days at 17 °C, after which the downfield resonances would disappear even though the protein remained soluble. It seemed that spectra with the most chemical shift dispersion were obtained with protein purified in buffers containing GDP, but even these spectra still had a severe lack of resonances, indicating slow internal motions of the protein and/or partial disorder.

Assignment of Glycine 23 and Lysine 24. The last two residues (glycine and lysine) of element 1 were identified by one-dimensional isotope-filtered NOE experiments as shown in Figure 4 (Griffey & Redfield, 1987; Redfield & Papastavros, 1990). In the sample specifically labeled with lysine, isotope-filtered NOE from the lysine (at 11.07 ppm ^1H, 126.8 ppm ^{15}N) gave an NOE to a resonance at 9.07 ppm. In the sample specifically labeled with glycine, isotope-filtered NOE from the glycine (at 9.07 ppm ^1H, 108.7 ppm ^{15}N) gave an NOE to a resonance at 11.07 ppm. In the sample doubly labeled with glycine and lysine, isotope-filtered NOE from the ^{15}N–^1H frequency of lysine was observed with and without decoupling at the glycine ^{15}N resonance frequency; the ^{15}N splitting was observed in the latter experiment (data not shown). A similar set of NOEs is seen in N-ras p21 between Gly 15 and Lys 16 (Redfield & Papastavros, 1990). In the G domain, Gly 23–Lys 24 is the only Gly–Lys pair closer than 5 Å in the crystal model of La Cour et al. (1985).

Assignment of Glycine 18 and 83. Both Gly 18 and Gly 83 are conserved residues in elements 1 and 2, respectively. These two glycines are near Cys 81, and we tried to assign these glycines by spin labeling Cys 81 with maleimido-PROXYL. A spin-label has an unpaired electron that gives a strong random magnetic field at the positions of nearby nuclei. On the basis of other studies with NMR of spin-labeled proteins, the random field is expected to broaden nuclear spin resonances that are within about 10 Å (Kosen, 1989). Spin labels often contain bulky groups that may perturb the protein structure. Such a perturbation would cause resonances to shift from their ordinary position. The spin label was therefore expected to broaden and possibly shift nearby resonances. We spin labeled a [^{15}N]glycine-labeled sample as described above. The difference-decoupled spectrum clearly showed the disappearance of the most downfield resonance and a significant attenuation of the most upfield resonance (Figure 5a,b). No other glycine resonances were significantly affected by the spin label as judged by HMQC (Figure 5c).

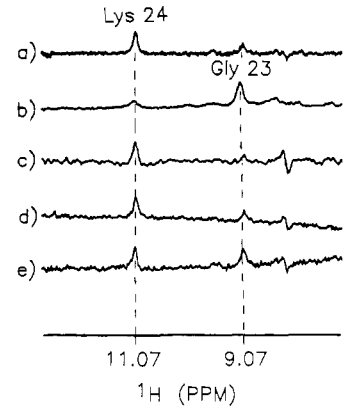

FIGURE 4: (a) Isotope-filtered NOE from a lysine resonance to a possible glycine resonance in a lysine-labeled sample at 15 °C. The resolution and spectral width was 5 Hz/point, 4 kHz. A 200-ms preirradiation was followed by a 1-ms homogeneity spoil, a 3-ms recovery time, and a semiselective observe pulse. The recycle time was 0.6 s. A total of 13792 FID's were accumulated. (b) Same experiment as in spectrum a, but with irradiation on the glycine resonance in a glycine-labeled sample at 18 °C. (c–e) Buildup of NOE from the lysine resonance to the glycine resonance in a lysine-labeled sample. The mixing times were 20, 40, and 80 ms, respectively. The numbers of accumulated FID's were 19776, 13088, and 8320, respectively. All three experiments used a 0.6-s recycle time, a 1-ms homogeneity spoil, a 3-ms spoil recovery delay, and a semiselective J–R observe pulse. The resonance from the Tris protons was weakly irradiated to saturated it during the recycle time. The transfer rate from the lysine amide to the glycine amide is approximately 12 s^{-1}. This rate is probably not useful for distance determination because of possible two-step magnetization transfer (spin diffusion) through the C_α proton.

There are two cysteines in the G domain, Cys 81 and Cys 137. Cys 81 has two glycines within 10 Å of its sulfhydryl group, and Cys 137 has one glycine within 10 Å of its sulfydryl group. This suggests that the glycine resonances (at 11.34 ppm ^1H, 114.3 ppm ^{15}N and 6.95 ppm ^1H, 106.1 ppm ^{15}N) may be from Gly 18, Gly 83, or Gly 172, the only glycines within 10 Å of the reactive sulfhydryl group on Cys 81 or Cys 137, respectively.

It is probably not possible to clarify the resonance assignments on the basis of the relative attenuation or broadening of these two glycine resonances, because the precise location of the nitroxide group on the spin label is unknown. Because of the length of the cysteine–spin label side chain, the electron spin could be moving among several positions. Thus, the results of the spin label experiment indicate only that the downfield glycine resonance could be from one of three glycine residues.

In order to verify the assignment of Gly 83, we studied a fully ^{15}N-labeled mutant G domain, in which His 84 was changed to glycine. This mutant protein retains wild-type binding activity of GTP and GDP, but the hydrolysis activity is greatly decreased (Cool & Parmeggiani, 1991). The mutation was expected to change the resonance of the neighboring

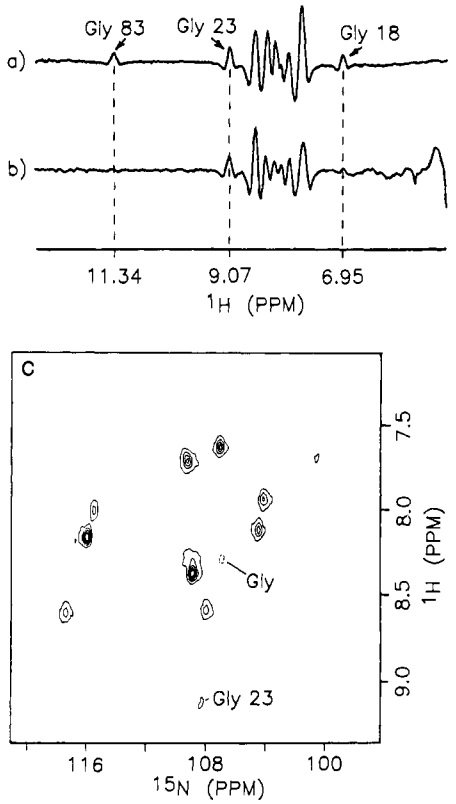

FIGURE 5: (a) Difference-decoupled spectrum of [^{15}N]glycine-labeled G domain at 17 °C. (b) Difference-decoupled spectrum of the same sample as in spectrum a, but treated with maleimido-PROXYL. Note the disapperance of the most downfield resonance and attenuation of the most upfield resonance. Both 1-D spectra had a 10 Hz/point resolution and an 8K spectral width with a 0.8-s recycle time. The other glycine resonances were not significantly perturbed by the spin label as determined by HMQC. (c) HMQC of the spin-labeled sample, obtained as in Figure 1, with which it should be compared.

Gly 83 amide proton. We did find that the proton shift of the most downfield glycine resonance shifted even further downfield, by 0.3 ppm, and this confirms its assignment to Gly 83. The other downfield resonances were unchanged. All resonances from other glycines are resolved in the spectra of fully labeled EF-Tu, and none of them was shifted by more than 0.1 ppm in the ^{1}H dimension (Table I). In other NMR studies of mutated proteins, such chemical shift perturbations have been found localized around the site of the mutation, as might be expected (Redfield & Papastavros, 1990; McIntosh et al., 1987).

Discussion

Missing Amides. We find that the G domain gives spectra with a 30-fold range of resonance intensities that lack sufficient chemical shift dispersion to resolve as many resonances as would be expected. The poor quality of the central part of the spectrum could be due to a destabilization of structure, since the G domain is a truncated protein. The downfield resonances also seem to decrease in intensity relative to the narrow resonances in the central region as the protein samples age. This disappearance of the downfield resonances is of the same time scale as the loss of GDP binding in protein stored at 4 °C in buffer that does not contain glycerol (Jensen et al., 1989; Harmark et al., 1990). This can be taken as additional evidence that the downfield resonances are from residues involved in substrate binding. We conclude that the intense narrow resonances in the central region are from disordered parts of the protein that have rapid internal motions. Slow internal motion in the intact protein is probably responsible

Table I: Resonance Shifts

	wild-type G domain		His/Gly 84 mutant	
assignment	^{15}N (ppm)	^{1}H (ppm)	^{15}N (ppm)	^{1}H (ppm)
Gly 83 amide	114.3	11.34	114.6	11.57
Gly 23 amide	108.7	9.07	109.1	9.09
Gly amide	118.3	8.62	118.1	8.59
Gly amide	109.0	8.60	109.0	8.57
Gly amide	109.8	8.39	109.9	8.39
Gly amide	107.8	8.33	107.6	8.31
Gly amide	116.9	8.17	116.8	8.21
Gly amide	105.3	8.14	105.3	8.09
Gly amide	116.4	8.03	116.4	8.07
Gly amide	105.0	7.96	104.8	7.91
Gly amide	110.1	7.75	110.0	7.71
Gly amide	101.5	7.72	101.5	7.64
Gly amide	107.9	7.66	108.0	7.61
Gly 18 amide	106.1	6.95	106.1	6.90
Lys 24 amide	126.7	11.05	126.8	11.07
His δ_1 or ϵ_2	169.0	11.01	170 ± 5	10.96
His δ_1 or ϵ_2	173.3	12.44	170 ± 5	12.45

for the relative lack of resonances in spectra obtained with fresh samples. There is a long element of residues in the G domain that aligns with the effector element of p21 (Jurnak et al., 1990). The intense overlapping resonances might be from this element. However, element 1 and 2 resonances are always seen, and their distinct shifts indicate that the protein's GDP-binding site is ordered, even though other parts of the protein are disordered.

Downfield Shifts. The Lys 24 amide proton from element 1 has an uncharacteristic downfield proton shift of 11.1 ppm. The homologous Lys 16 in p21 also has an extreme downfield shift of 10.4 ppm that was attributed to a strong hydrogen bond to a phosphate oxygen (Redfield & Papastavros, 1990). The similarity of the chemical shifts of this lysine between p21 and the G domain suggests that this conserved lysine is important for protein–phosphate interaction and that such a downfield resonance may be a universal spectroscopic marker for this class of proteins. Saraste et al. (1990) have proposed, on the basis of sequence data, that this lysine should indeed be the only residue with a common structural role in this class of purine-binding proteins. Their argument was based on the observation that the lysine is the only absolutely conserved residue in this class of proteins. The side chain may be important for element conformation and rigidity, but we find that the amide group is also structurally important for phosphate binding.

There is one downfield-shifted glycine amide proton resonance which we assign to Gly 83. This glycine is in element 2 which is proposed to move upon binding of GTP (La Cour et al., 1985). The crystal structure of the GDP form of trypsin-modified EF-Tu cannot accommodate a γ-phosphate unless this element moves (Woolley & Clark, 1989). This glycine residue is in the vicinity of the β-phosphate oxygen, but no hydrogen-bonding interaction has been suggested, because it is too far away for a direct interaction. It may be binding a water molecule or binding the phosphate oxygen indirectly through a water molecule (J. Nyborg and M. Kjeldgaard, personal communication). The resonance from Gly 83 moves farther downfield in the His/Gly 84 mutant. Thus, the hydrogen bond is maintained and possibly strengthened in the mutant protein.

Conclusion. We have shown that there are probably at least two strong hydrogen bonds from amide protons to either phosphate oxygens or a water molecule. One donor is Lys 24 in element 1. The other donor is Gly 83 in element 2. The analogous Lys 16 in element 1 of p21 is also a hydrogen-bond donor. We speculate that the amide proton resonance from

the lysine in element 1 of other GTP-binding proteins will be similarly shifted.

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Registry No. Gly, 56-40-6; Lys, 56-87-1; 5'-GTP, 86-01-1; 5'-GDP, 146-91-8.

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