^{31}P NMR STUDY ON THE GUANINE NUCLEOTIDE BINDING OF ELONGATION FACTOR Tu FROM *THERMUS THERMOPHILUS*

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1. Introduction

In the process of protein biosynthesis, the important role of polypeptide chain elongation factor Tu (EF-Tu) is to promote the GTP-dependent binding of aminoacyl-tRNA to ribosomes [1,2]. Ef-Tu has at least two binding sites, one for GDP or GTP and the other for aminoacyl-tRNA. The conformation around the aminoacyl-tRNA binding site is altered allosterically on the ligand change from GDP to GTP, and only EF-Tu · GTP can interact with aminoacyl-tRNA [2]. On the nature of aminoacyl-tRNA binding site, various spectroscopic studies have been carried out for EF-Tu from *Escherichia coli* [3–7]. Since *E. coli* EF-Tu is unstable, we have made ^{1}H NMR studies [8,9] on EF-Tu from an extreme thermophile, *Thermus thermophilus* HB8 [10–12], and found that a histidine residue is important in the binding of *T. thermophilus* EF-Tu with aminoacyl-tRNA [9]. In this study, we have attempted to analyze the environment of guanine nucleotide binding site of *T. thermophilus* EF-Tu by the use of ^{31}P NMR spectroscopy, and found that β-phosphate resonance of GDP and β and γ-phosphate resonances of GTP are in fact affected markedly by the binding with EF-Tu.

2. Experimental

EF-Tu · GDP was purified from *T. thermophilus* HB8 as in [10]. EF-Tu · GTP was prepared from EF-Tu · GDP by incubation with phosphoenolpyruvate and pyruvate kinase [13]. The $^{2}H_2O$ solution of EF-Tu · GDP or EF-Tu · GTP was prepared as in [8]. The sample solution (in 10 mm tube) contained 2 mM guanine nucleotide, 10 mM $MgCl_2$, 100 mM NaCl and 0.1 mM dithiothreitol. ^{31}P NMR spectra were recorded at 50°C on a Bruker WH270 spectrometer at 109.3 MHz, with proton broad band decoupling. Chemical shifts are given in ppm upfield from external H_3PO_4 (10%). pH (direct meter reading) was also measured at 50°C using Radiometer PHM26 pH meter.

3. Results and discussion

^{31}P NMR spectra of GDP and GTP were obtained at various pH values in the presence and absence of *T. thermophilus* EF-Tu. Fig.1 shows the representative spectra of EF-Tu · GDP and EF-Tu · GTP. α-, β- and γ-phosphate resonances were assigned by the comparison with spectra of free GDP and GTP (see fig.2). In fig.2A,B, the pH titration curves of EF-Tu · GDP and EF-Tu · GTP, respectively, are shown with solid lines while the pH titration curves of free GDP (pK_a = 5.27 ± 0.03) and free GTP (pK_a = 5.14 ± 0.02) are shown with broken lines. These pK_a values are due to the second ionization of the terminal phosphate groups.

Over pH 5.74–9.52 where EF-Tu · GDP is stable and active, the chemical shifts of ^{31}P resonances of EF-Tu-bound GDP are constant, indicating that the phosphate groups are fully ionized in this pH range. Furthermore, there appear to be no ionizable groups which affect the ^{31}P chemical shifts of bound GDP in this pH range. The extremely tight binding of GDP with EF-Tu ($K_d = 1.1 \times 10^{-9}$ M [11]) suggests strong interactions between the phosphate groups of GDP and amino acid residues of EF-Tu, including electro-

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static interaction. The above observation indicates that such amino acid residues do not have pK_a between pH 5.74–9.52. Therefore, basic residues such as arginine and/or lysine residues, rather than histidine residues, are probably important for the binding with GDP. This agrees with our conclusion [9] that histidine residues are not directly involved in the binding with guanine nucleotides, although they are important for the interaction with aminoacyl-tRNA.

Similar results were obtained for EF-Tu · GTP as shown in fig.2B, where the chemical shifts of α-, β- and γ-resonances are unchanged over pH 5.80–8.61. GTP as bound to EF-Tu is also found to be in the fully ionized form and no ionizable amino acid residues affect the ^{31}P chemical shifts of bound GTP in this pH region.

^{31}P chemical shifts are known to be sensitive to small distortions in O–P–O bond angles [14–17] and internal rotation angles [15–17]. ^{31}P chemical shifts of protein-bound nucleotides may also be affected by the ring current of aromatic amino acid residues as well as the electrostatic interaction with basic residues.

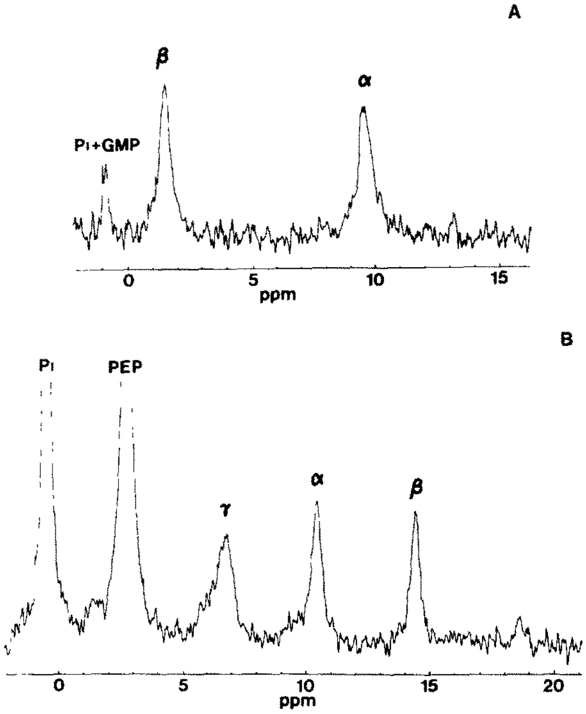
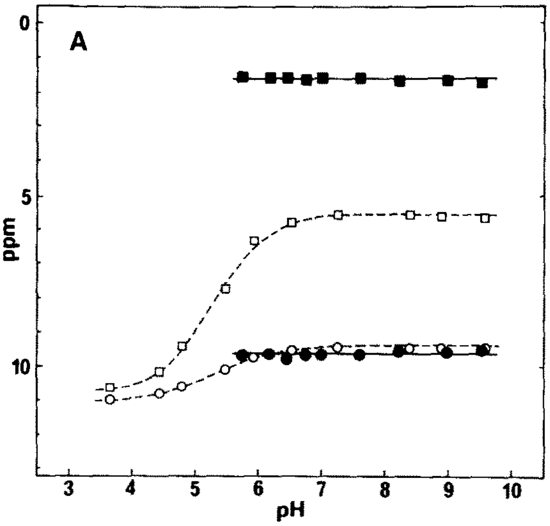


Fig.1. ^{31}P NMR spectra of guanine nucleotides bound to EF-Tu at 50°C. (A) Spectrum of 2 mM EF-Tu · GDP at pH 6.32 (23 000 transients). P_i and GMP arise from the decomposition of GDP. (B) Spectrum of 2 mM EF-Tu · GTP at pH 5.80 (4096 transients). The sample solution was prepared from EF-Tu · GDP, with phosphoenolpyruvate (PEP) and pyruvate kinase. The resonance of P_i is probably due to the degradation of PEP.



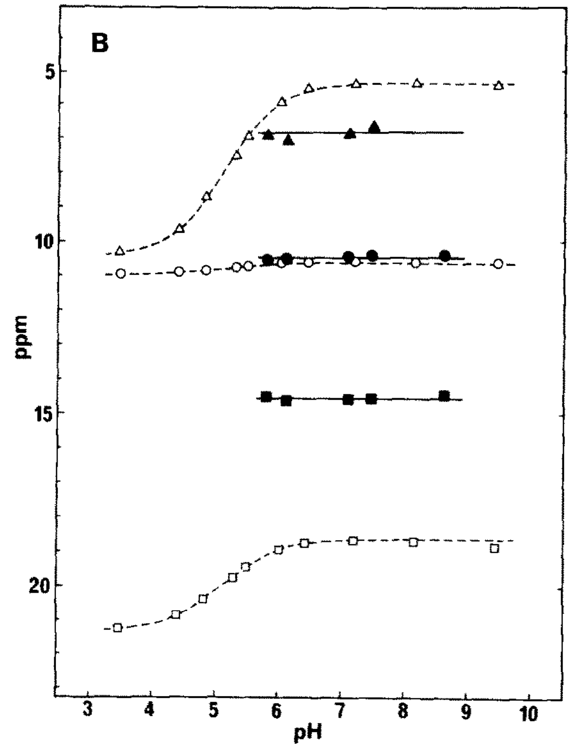


Fig.2. pH dependences of ^{31}P chemical shifts of (A) free GDP (open symbols) and EF-Tu · GDP (filled symbols) and (B) free GTP (open symbols) and EF-Tu · GTP (filled symbols); (○,●) α; (□,■) β; (△,▲) γ.

Therefore, the ^{31}P chemical shifts of bound nucleotides may not readily be correlated quantitatively with the conformation and environment of phosphate moiety in the guanine nucleotide binding site of EF-Tu. Nevertheless, the β-phosphate resonances of GDP and GTP are shifted downfield by ~4 ppm on the binding with EF-Tu, while the γ-phosphate resonance of GTP is shifted upfield by ~ 1.5 ppm (fig.2A,B). On the other hand, the α-phosphate resonances of GDP and GTP are little affected by the binding with EF-Tu. This observation probably indicates that EF-Tu recognizes the β- and γ-phosphate groups of guanine nucleotides rather than the α-phosphate groups. In fact, EF-Tu does not bind 5′-GMP which has the α-phosphate group alone. It may also be noted that the magnitudes of the downfield shifts of β-phosphate resonances on the binding with EF-Tu are nearly the same (~4 ppm) for both GDP and GTP, although EF-Tu binds GDP more tightly [11]. This observation suggests that the β-phosphate moiety of these two guanine nucleotides are bound to EF-Tu in a similar manner. Accordingly the specific interaction of the γ-phosphate group of GTP with the amino acid residues in the nucleotide binding site appears to be important for the allosteric alteration of the conformation around the aminoacyl-tRNA binding site of EF-Tu.

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