EVIDENCE FOR CONFORMATIONAL CHANGES IN ELONGATION FACTOR TU INDUCED BY GTP AND GDP

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SUMMARY

A comparative study of the rates of tritium-hydrogen exchange in three liganded states of the protein elongation factor Tu (EFTu) reveals a substantial conformational difference between the free (EFTu) or GTP-bound (EFTu·GTP) forms and when GDP is present (EFTu·GDP). This conformational difference is accentuated with short time tritiations. There are 25-35% more very slow hydrogens in EFTu·GDP than in EFTu·GTP, indicating that GDP induces a tighter conformation in EFTu than does GTP. Thus, a rationale is provided for the difference in reactivity of EFTu·GTP and EFTu·GDP for AA-tRNA and a conformational role in regulating protein biosynthesis may be proposed for GTP and GDP. Finally, we demonstrate that nucleoside polyphosphates may cause sizeable conformational changes in proteins.

INTRODUCTION

Elongation factor Tu (EFTu), a protein required for protein biosynthesis, promotes the binding of aminoacyl tRNA to ribosomes in the presence of m-RNA and GTP (1). The presumed intermediate in the process, the ternary complex AA-tRNA EFTu·GTP, forms readily from AA-tRNA and EFTu·GTP. When the ternary complex interacts with ribosomes plus mRNA, AA-tRNA is bound to ribosomes, GTP is hydrolyzed and EFTu·GDP and P_i are released. Although the EFTu·GDP complex is very stable, another protein, EFT_s, catalyzes the exchange of EFTu·GDP with free GTP, thereby regenerating EFTu·GTP (2,3). The complex of EFTu with GTP interacts strongly with AA-tRNA ($K_{diss} < 10^{-8}$ M) (4); however, EFTu·GDP does not interact with AA-tRNA to a measurable extent ($K_{diss} > 10^{-5}$ M) (4).

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To determine why the interaction of EFTu with AA-tRNA is absolutely dependent upon GTP instead of GDP, we have searched for conformational differences among EFTu, EFTu·GTP, and EFTu·GDP as may be revealed by differing rates of hydrogen exchange between the tritiated, liganded protein and the solvent. The results of our study provide a rationale for the regulatory role of GTP and GDP and may be relevant to the more general question of how nucleoside phosphates cause structural changes in biological systems.

MATERIALS AND METHODS

Guanylyl methylene diphosphonate (GDPcP) was obtained from Miles Laboratories, Inc. Tritiated water (1 Ci/gm) was obtained from Schwarz Bioresearch. Elongation factor Tu (as EFTu·GDP) was a homogeneous, recrystallized preparation previously described (5).

The readily dissociable EFTu·GDPcP complex was used in the tritiation procedure described below because the three EFTu species to be studies could be prepared from it by simple manipulations. Furthermore, it is stable toward hydrolysis, an advantage in long tritiation experiments. EFTu·GDPcP was prepared by passing 10 mg of EFTu-GDP through a 1.5 x 80 cm column of Biogel P-4 equilibrated with GDPcP (1 mM) in Tris-HCl (pH 8, 50 mM), EDTA (1 mM). It was found that 80-90% of the GDP could be replaced by GDPcP by this procedure. To the combined fractions containing EFTu·GDPcP, $MgCl_2$ (10 mM) was added, and the complex was precipitated with $(NH_4)_2\ SO_4$ (2 M), and was stored frozen in liquid nitrogen.

The technique of tritium-hydrogen exchange has been described (6), and essentially identical methods were used for this study. The exchange measurements were made with an automated sample handling system to be described elsewhere (7), and duplicate experiments were run on several occasions to check system reproducibility and the consistency of the results with different protein preparations.

RESULTS

In two experiments using a 26 hour tritiation period there was only a

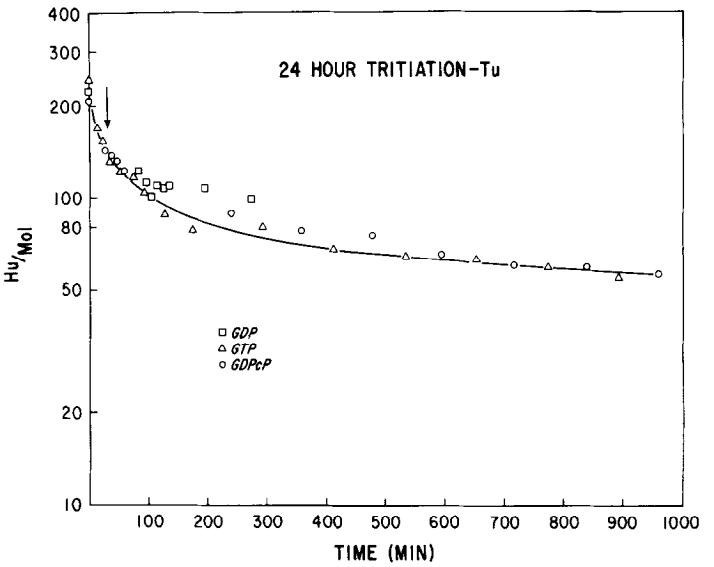

Figure 1. Back exchange curves for liganded EFTu protein after 24 hours of tritiation. Solutions were prepared immediately before using by dissolving 3 mg of precipitated protein in 200 μl TMK buffer (10 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM KCl, pH 7.0 at 25°) which also contained 1 mM GDPcP. Tritiated water was added, 2-3 mCi tritium/300 μl final solution, and the sample was incubated at 0-5° for 24-26 hours. After tritiation, the unbound tritium was removed by passing the solution through a 7 cm G-25 Sephadex column containing the TMK buffer plus 5 mM DTT. The eluant from the first column, here referred to as the exchanging pool, contained THO-free tritiated protein. Exchange with water was allowed to proceed at 0°, and at timed intervals 40 μl aliquots were automatically passed through a second Sephadex column containing only TMK buffer. The eluant from the second column was analyzed for protein by UV absorbance at 225 nm and for tritium by scintillation counting. The number of unexchanged tritiums per mole of protein (Hu/Mol) was calculated using $E_{225\ nm} = 3.4 \times 10^{-3}$.

slight indication of a difference between the exchange rates of EFTu·GDP and EFTu·GTP (Figure 1); however, the data did show that about 30% of the 260 hydrogens per molecule, observable by this procedure, exchange very slowly ($t1/2 \geq 24$ hrs) in either EFTu species. A peptide or amide hydrogen freely accessible to water and not involved in secondary or tertiary structure should exchange completely within a few seconds at pH 7 (8,9). Thus, the only hydrogens we observe are those whose accessibility to water is limited by the protein structure. Those hydrogens which exchange very slowly must comprise the "core" of the molecule, or the region of lowest "breathing" rate (8,10). Approximately 30 per cent of the peptide backbone is located in these tightly structured internal regions of the protein.

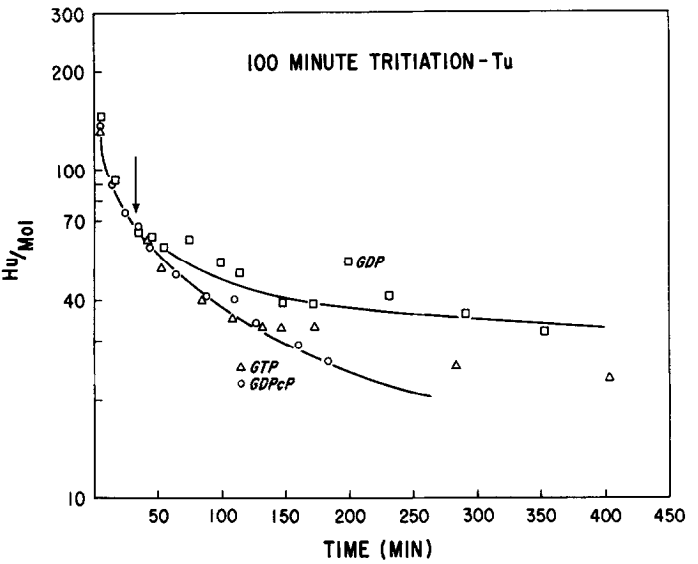

Figure 2. Back exchange curves for liganded EFTu protein after 100 minute tritiation. Samples were prepared as described in the text and experiments conducted as discussed in Figure 1. In all experiments 2 x 10^{-4} M GDPcP was added to the first column buffer. Sufficient GTP and GDP was added, at the time denoted by the arrow, to the pool to give a 2 x 10^{-4} M ligand solution. The affinity of GTP and GDP for EFTu compared with GDPcP insured that we were observing the appropriate liganded protein.

In contrast to the results obtained from the 26-hour tritiation period, the exchange profiles obtained from a 100 minute tritiation period (Fig. 2), showed a clear difference between the rates of exchange of EFTu·GDP and EFTu·GTP. In each of these experiments the exchange rate of EFTu·GDPcP was measured for thirty minutes. At this time we added GTP or GDP, which rapidly formed its complex with EFTu. After this point the exchange curve of EFTu·GDP began to diverge from that of EFTu·GTP. EFTu·GDP was observed to contain about 25-35% more slowly exchanging hydrogens (about 13-20 in number) than EFTu·GTP. In an additional experiment with no added nucleotide, it was found that the exchange rate of EFTu·GDPcP was indistinguishable from that of EFTu·GTP.

The observation that EFTu·GDP has a markedly reduced exchange profile was confirmed by comparing the total profiles of the separately prepared complexes. The nucleotides were added to the EFTu·GDPcP tritiation solution five minutes prior to injection into the first column, as outlined in Figure 3.

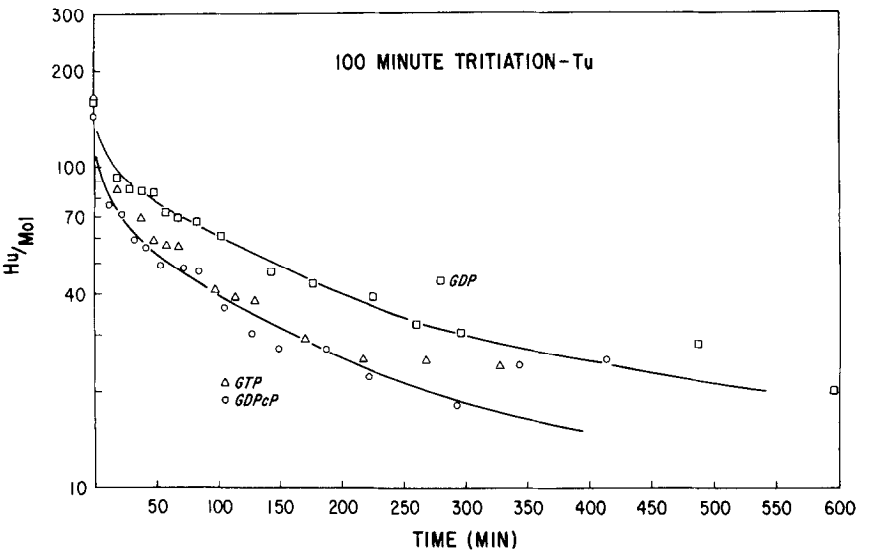

Figure 3. Back exchange curves for liganded EFTu protein after 100 minute tritiation with ligand added during tritiation. The experimental procedure was same as in Figure 1. The EFTu·GDPcP protein was tritiated for 95 minutes, at which time sufficient GTP or GDP was added to make the tritiation mixture 2 x 10^{-4} M in ligand. The sample was incubated for 5 additional minutes and the experiment started. No GDPcP was added to the first column buffer.

Again EFTu-GDP exchanges markedly more slowly than EFTu·GTP. In an additional experiment tritiated EFTu·GDPcP was passed through column 1 with no added nucleotide. Separate experiments showed that more than 90% of this weakly bound nucleotide dissociates from EFTu during this process, leaving free EFTu. The exchange profile of this species (Fig. 3) resembles EFTu·GTP and is markedly faster than EFTu·GDP.

DISCUSSION

Tritium-hydrogen exchange experiments of proteins at neutral pH measure those hydrogens which, because of the secondary, tertiary or quaternary structures are partially shielded from the solvent. Thus, any conformational change induced by the binding of ligands which alters the tertiary or secondary structure or changes the state of association would be reflected in an increased or decreased exchange rate of some of the peptide hydrogens. The direction of the change would be such as to reflect increased or decreased solvent accessibility to the peptide hydrogens. The most significant aspect

of the exchange study reported here is the finding that a tighter conformation of part of the EFTu molecule results upon substitution of GTP with GDP. The magnitude of this conformational change appears small and it is most readily detectable after short times and limited extents of tritiation. Clearly however, the exchange results reflect a change in "core" hydrogens rather than those readily accessible to the exterior of the molecule. This is apparent from the fact that the exchange curves show maximum deviation at times which reveal the slowest exchanging hydrogens. Thus, upon substitution of GTP by GDP the conformation of the molecule is altered.

It has already been well established that EFTu·GTP will interact with AA-tRNA but EFTu·GDP will not. Out data indicate that this occurs because EFTu·GTP has an altered conformation. More specifically, it suggests that a binding site for AA-tRNA on EFTu is exposed in EFTu·GTP, but masked in the tight conformation induced by GDP. This conformational change has been further characterized by the observation that EFTu·GTP has a binding site for a hydrophobic dye which EFTu·GDP lacks (11). On the other hand, preliminary CD and ORD measurements in the 220-280 nm region by one of us (DLM) revealed no difference between EFTu·GDP and EFTu·GTP.

The function of EFTu depends upon the conformation change which occurs when EFTu·GTP is hydrolyzed to EFTu·GDP during the interaction of the ternary complex with the ribosome. Since EFTu·GTP binds AA-tRNA much more strongly than deacylated tRNA, the protein must interact with the aminoacyl group. This interaction would prevent the aminoacyl group from forming a peptide bond after the ternary complex was bound to the ribosome; however, the hydrolysis of GTP leads to the formation of EFTu·GDP, which allows EFTu to release AA-tRNA, freeing the aminoacyl group for peptide bond formation. It is possible that the sole purpose of GTP hydrolysis is to provide this pathway for the removal of EFTu from the ribosome after AA-tRNA has been bound.

This pathway also introduces another regulatory mechanism for protein synthesis. Since EFTu·GDP must be reconverted to EFTu·GTP by an exchange

reaction catalyzed by EFTs, the rate of AA-tRNA binding is affected by the GTP/GDP ratio, which is determined by the "energy charge" of the organism (12). Furthermore, in principle the reaction may be regulated by other inhibitory nucleotides such as ppGpp, although this has not yet been demonstrated.

It is interesting that free EFTu and EFTu·GTP give similar exchange curves, with possibly similar conformations. Preliminary experiments by one of us (DLM) indicates that free EFTu reacts to some extent with AA-tRNA. These observations suggest that GTP does not greatly affect the protein conformation. Instead, it is GDP that most alters the protein.

There are numerous proteins whose functions are controlled by nucleotide di- and triphosphates, among which are regulatory enzymes and the proteins involved in motility. Nearly all of the proteins are large, multi-subunit aggregates where the precise effect of the nucleotide will be difficult to define. EFTu, a monomer of tractable size, could serve as a useful model for the effect of nucleotides on protein structure.

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