Structural Analysis of *Thermus thermophilus* Elongation Factor G

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Elongation Factor G (EF-G) is one of the soluble protein factors participating in translation; the biosynthesis of proteins which takes place on ribosomes. Specifically, EF-G catalyses the translocation step in the elongation cycle of protein synthesis: movement of mRNA and tRNA carrying the growing peptide from the A-site to the P-site of the ribosome. As a result, the ribosome moves along the mRNA, one codon at a time.

EF-G with bound GTP is in its active conformation, the "ON state", and binds to the ribosome .The protein goes through conformational changes as it interacts with the ribosome and GTP is hydrolysed. EF-G leaves the ribosome in the "OFF state" with bound GDP (1). The hydrolysis of GTP changes the conformation of EF-G but is not needed to actually drive the translocation. Related proteins that participate in different steps of translation are initiation factor 2 (IF-2) and elongation factor Tu (EF-Tu). EF-G and these related factors, as well as many distantly related GTP-binding proteins, all share the ability to switch between different conformational states upon GTP hydrolysis. The GTP-binding domain in EF-G, comprising roughly 1/3 of the protein, has the conserved sequence elements found in many GTP-binding proteins (2). It was predicted to be very similar to its counterpart in EF-Tu (3), and the G-protein p21 (4) since those structures have similar 3-dimensional structures despite their remote overall sequence similarity (3).

We have studied the structure of EF-G from *Thermus thermophilus* which has a length of 691 residues (77000 Da). Crystallization of the protein was done without adding any nucleotide cofactor (5). The phases were obtained using six derivatives which were judged using the program NORMAN (6). Refinement of heavy atom parameters and calculation of phases was done with the program MLPHARE (7). Phase modification, using the program SQUASH (8), was applied using isomorphous phases to 3.2 Å with phase extension to 2.8Å. In order to locate the GTP-binding domain we used the model for the homologous GTP-binding domain from the EF-Tu:GDP structure from *E.coli* (3). This domain could not be easily identified in the initial map but we assumed that one known mercury position was at the only cysteine residue in the whole protein, found in the GTP-binding domain. The building of the model is currently in progress using the interactive graphic program O (9). A partial model (30 % of the structure) was refined with XPLOR (10), phases combined with SIGMAA and new improved phase calculated with SQUASH.

Structure of the GTP-binding domain of EF-G.This is a preliminary model and regions in this domain still not incorporated in the model are residues 1-11, 39-69 and 156-204.

The domain-structure of EF-G compared to underlying sketch of the structure of EF-Tu:GDP when the GTP-binding domains are superimposed. Domain B contains α-helices packed against a β-sheet. Domain C is probably a β-type structure. Helices have also been identified in domains D and E.

Comparison of the GTP-binding domains of EF-G(yellow) and EF-Tu (white) . Shown in red is the region which is unique to EF-G.

Our goal is to analyse EF-G in different conformational states, *i.e.* solve the structure of the protein in complex with different ligands like nucleotides and antibiotics. Of great interest is to identify the regions involved in interaction with the ribosome and study how this interaction may result in the activation of the GTP hydrolysis by the protein. We have obtained crystals of the protein in several different forms. Below are the cell parameters for the 3 different forms analysed until now. All 3 forms crystallize in space group P212121. The isomorphous difference (ΔF/F) between form 2 with and without GDP is 0.4 - 0.5.

EF-G without nucleotide, form 1: 75.6 Å x 106.0 Å x 116.4 Å
EF-G without nucleotide, form 2: 79.2 Å x 92.2 Å x 118.8 Å
EF-G:GDP , form 2: 77.9 Å x 92.2 Å x 117.6 Å

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