ELONGATION FACTOR – TU

Introduction:

At the end of initiation phase of translation the initiating aminoacyl-tRNA is bound to P site on the ribosome. For elongation to occur ribosome need to add amino acids one at a time to the initiating amino acid. Elongation starts with the binding of second aminoacyl-tRNA to A site on ribosome. This process requires an elongation factor EF-TU and energy provided by GTP. EF-TU, a monomeric protein with 393 amino acid and molecular mass 43 KD, is one of the most abundant proteins in the cell and exhibits multifunctional activities.

Functions of EF-TU:

Elongation factor TU is a member of the GTPase family and functions like a switch, i.e. GTP bound active state and GDP bound inactive state. GTPases enzymes play a major central role in catalyzing every single stage of protein synthesis on ribosomes.

EF-TU facilitates the binding of aa-tRNA within a stable ternary complex, EF-TU.GTP.aa-tRNA to the A site of the ribosome. At the initial stage of binding codon and anticodon recognition at A site takes place which stabilizes ribosome anticodon EF-TU.aa-tRNA complex and also activates the GTPase activity of EF-TU which then carries out the GTP hydrolysis and dissociation of Pi(so ribosome acts as GTPase activator protein- GAP) which causes the conformational rearrangement of EF-TU from the GTP form to the GDP and releases aa-tRNA. Because EF-TU.GDP conformation has less affinity for aa-tRNA. As a result, the aminoacyl end of the aa-tRNA is released from EF-TU.GDP, accommodates in the 50S A site and takes part in the peptidyltransferase reaction while EF-TU.GDP dissociates from the ribosome.

EF-TU has several chaperone properties like it interacts with unfolded and denatured proteins and involved in protein folding and protein renaturation after stress. For example EF-TU promotes the functional folding of citrate synthase and α-glucosidase

after urea denaturation. EF-TU increases the refolding of unfolded proteins, protects proteins against thermal denaturation and forms coplexes with unfolded proteins. It has been found that EF-TU-GDP complex is much more active than EF-TU-GTP complex in stimulating protein renaturation. EF-TU is also involved in signal transmission whose mechanism is not known yet.

Crystal structure of EF-TU:

Crystal structure of EF-TU is obtained by hanging drop method and it crystallizes in trimeric form . Each monomer has two chain, one is EF-TU protein and other is t-RNA. EF-TU.GDPNP.enacycloxin IIa. Phe-tRNA complex is refined at 30-3.1 A resolution. A total of 198 water molecules identified at hydrogen bonding positions and the final R-factor and free R-factor are 0.284 and 0.296, respectively at 3.1 A resolution. The average B-factor is 68.3. All the figures are prepared using PyMol.

EF-TU has three domain, D1(an α/β structure), D2 and D3 are made up of β sheet only. The GNP binding pocket is composed of four loops connecting α helices and β strands.

Enacycloxin IIa binding site on EF_TU:

Enacycloxin IIa an antibiotics active against gram +ve and gram –ve bacteria, slightly active against fungi but inactive against yeast. It stabilizes the EF-TU.GTP complex and enables EF-TU.GDP complex to interact with aa-tRNA and ribosomes. Enacycloxin IIa binds at the back of interface of domain D1 and D3. As a result of antibiotic insertion the interface is widened and the domain D1 is pushed upward and tilted forwards. The antibiotic also reorient the side chain of several interface residues and breaking hydrogen bonds between Gln124-Phe374 and Tyr160-Glu315. This confirm that antibiotic binds by an induced- fit mechanism. Ena. IIa form several contact with EF-TU via hydrogen bonds with Arg116, Tyr160, Arg123, Gln124, Arg373, Ala96 and extensive van der waals and hydrophobic interactions. A salt bridge links the carboxyl group of the hexane ring with the side chain of Lys313.

Kirromycin binding site on EF-TU:

Kirromycin also bind at the same site on EF-Tu i.e. at the interface of domain D1 and D3. But the bulkier kirromycin widens the domain D1 and D3 interface of EF-TU slightly more than enacycloxin IIa. Due to binding of kirromycin, helix B is pulled along its axis and helix C is pushed up. Kirromycin forms hydrogen with Arg117, Tyr161, Arg124, Gln125, Arg385. Kirromycin doesn't form any salt bridge. But it has several van der waals and hydrophobic interactions.

Divergences in binding of Kirromycin and Enacycloxun IIa:

The "Head" of enacycloxin touches the tip of the loop formed by domain D3 residues 131-316, whereas head of kirromycin points the other way wrapping around Leu121 and Tyr161 and displacing the Glu118 side chain. Furthermore, kirromycin's "Tail" fills the hydrophobic pocket between Gln124 and Ile92, whereas enacycloxin IIa passes along the outside of the cavity. This reason explains why the binding affinity of enacycloxin IIa for EF-TU is much lower than that of kirromycin. Tha domain D3 residues contacting both antibiotics form a more or less flat surface with bumps that are in both cases tightly complementary to the antibiotic.

Overall changes in EF-TU.GDPNP.EnaIIa.Phe-tRNA complex:

In the enacycloxin complex the C terminus of helix C is pushed up, whereas helix B is pulled along its axis towards the tail of antibiotic. As a result the effector region (residues 41-65) in EF-TU.GDPNP contacts this helix is destabilized and changes conformation. Arg58 and Thr61 are two conserved residue important for GTPase activity and interaction with ribosome, are moved away by ~15 A towards the vacant binding pocket for aminoacyl residue of tRNA. The acceptor stem and part of the CCA end of tRNA are tugged by effector region to follow the displacement of domain D1 induced by the antibiotic binding, whereas the remaining tRNA binds the domain D2 and D3. As a result a unique conformation intermediary between free tRNA and tRNA in ternary complex is formed. The side chain of His84, a residue important for GTPase activity, points towards the phosphate groups of GDP. Divalent cations are critical in influencing

the GTPase activity. There absence abolishes this activity. The most effective divalent cation is mg2+ followed by Mn2+ and Ca2+.

Gly83 at the N terminus and Gly94 at the C terminus Of EF-TU are also very important residue for GTPase activity. If Gly83 and Gly94 are replaced by alanine, the Gly83Ala mutation slows down the association of the ternary complex with the ribosome and abolishes the GTPase activity of EF-TU, whereas Gly94Ala mutation impairs the conformational change of EF-TU from GTP to GDP bound form and decelerates the dissociation of EF-TU.GDP from the ribosome.

Discussion:

The crystal structure of the EF-Tu.enacyloxin IIa complex explains the antibiotic binding site on EF-TU which is on the interface of domain D1 and D3 , overlaps that of kirromycin. Both the antibiotics inhibit protein synthesis by inducing a unique GTP like conformation of EF-TU.GDP.

Overall structures of both the antibiotics and the binding site of two share marked similarities. However there are some differences like the less contact of enacyloxin complex and short tail of enacyloxin that borders an empty hydrophobic pocket, whereas the longer tail of kirromycin fits into this pocket. This observation hint at new approaches for a structure guided antibiotic design. The hybridization of the head moiety of enacyloxin IIa with the tail moiety of kirromycin should result in a compound with increased binding affinity on EF-TU. The binding efficiency of an antibiotic can affect the degree of the antibacterial effect and reduce the onset of resistance by microorganisms.