BCH210H Assignment1

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1. Physiological Role of the protein and ligands
   1. According to RCSB, the name of 1MAU protein is tryptophan-tRNA ligase, which contains 328 amino acids and is found in Geobacillus stearothermophilus. Its classification is ligase(enzyme). (Retailleau et al. 2003) Subcellular location is cytoplasm. (Drugbank). Tryptophan-tRNA ligase belongs to aminoacyl-tRNA synthetase family. (Retailleau et al. 2003) Aminoacyl-tRNA synthetase is an enzyme that attaches an amino acid to the 3’ end of its cognate tRNA to form an aminoacyl-tRNA. Aminoacyl-tRNA plays an important role in RNA translation. (Aminoacyl-tRNA… 2020) As one of its twenty family members, the amino acid that Tryptophan-tRNA ligase attached to is L-Tryptophanamide. Therefore, Tryptophan-tRNA ligase catalyzes the tryptophan activation.

(ATP + L-tryptophanamide + tRNA ⇌-> AMP + diphosphate + L-tryptophyl-tRNA)

* 1. There are six ligands that can be seen in the structure:

1. ATP Adenosine-5’-Triphosphate(C10H16N5O13P3).

This is the largest ligand since its molecular weight is the largest. Its role is to provide energy by breaking phosphate bond to create AMP and free phosphate groups.

1. LTN L-Tryptophanamide (C11H13N3O).

This is the second largest ligand that can been seen in tryptophan-tRNA ligase. It is tryptophan derivative and is essential material to form L-tryptophyl-tRNA.

1. CIT Citric Acid (C6H8O7).

Citric acid is an intermediate in the citric acid cycle. The citric acid cycle is “a key of metabolism, producing usable energy in the form of ATP”. (Citric… 2020)

1. GOL Glycerol (C3H8Oc)

Glycerol has three hydroxyl group, which is helpful to stabilize the structure and activity of tryptophan-tRNA ligase. (Gekko & Timasheff 1981)

1. MG Magnesium ion (Mg)

Ionic interaction. It can help maintain protein’s structure and help with PPi exchange.

1. NA Sodium ion (Na)

Sodium helps protein keep fluids in a normal balance.

1. Purification of the protein

Baker’s yeast is used since it is easy to obtain. The temperature is maintained at 3-5 degrees Celsius. The buffer used is potassium phosphate and its PH value is 7.5. Using buffer is to maintain a stable PH environment to better undergo the purification process. Potassium phosphate is chosen because its PH value is closest to the PH required. Tryptophan-tRNA ligase is purified in the following few steps. (Charles & Charter 1987)

* Ammonium sulfate fractionation(precipitation)

Solid ammonium sulfate was added and solved with mechanical stirring, then centrifuge the solution, and remove the precipitate after that. Process is repeated for several times. (Tigerstrom & Tenor 1967) This step can separate proteins by changing their solubility in a high-salt environment.

- Column chromatography on calcium potassium gel(hydroxyapatite)

It is a mix mode chromatography. Calcium potassium gel column was washed potassium phosphate buffer and then with distilled water. (Tigerstrom & Tenor 1967) It can help separate proteins and DNA, thus helps to purify the protein.

- aminohexyl Sepharose chromatography.

It belongs to affinity chromatography. Aminohexyl-Sepharose is a commercially available starting material for low-molecular weight and carboxyl-containing ligands. It contains a spacer arm, so any carboxyl-containing ligand can easily bind to the amino group of the resin. Tag is the amino group of the resin. (Barker & Bailey 1988) Therefore, Tryptophan-tRNA ligase can bind to the tag and be separated from those that can’t bind to the column.

Gel electrophoresis is used to analyze the purified enzyme’s structure and purification. SDS gel can deduce the subunit’s weight by comparing to MW markers. Purification can be calculated based on its weight. In the research, SDS gel electrophoresis shows that the subunit is ‘discrete’, and by comparing with MW markers, the molecular weight of the subunit is 50000. Therefore, the enzyme appears to be a dimer of alpha two (Hossain & Kallenbach 1974)

Diagram

Description automatically generatedA picture containing graphical user interface

Description automatically generated

1. Structure of the protein

PyMoL is used to create the electrostatic representation. According to the figure, the binding site closer to ligands is either very electropositive or very electronegative, which means that the existance of ligands in the protein can alter electronegativity of the protein and effect its properties and functions. There are altogether 328 amino acids in the primary sequence. (Retailleau et al. 2003). The protein structure contains the first amino acid at the N-terminus. Normally, the start codon is AUG, and AUG codes for amino acids Met. Since the first amino acid for the protein has already been Met, the first amino acid is at the N-terminus. The full-length protein was expressed and studied in the graph, since there is only one sequence and both the first and last amino acids can be found. Many folding occurs. Alpha helices and beta strands secondary structures are seen in the protein and form stable 3D tertiary structures together, and two tertiary structures(subunits) form the quaternary structure. Ligands assist in bringing distinct regions together. For example, Mg ion can coordinate with different regions of the protein to keep the structure stable. According to research papers, tryptophan-tRNA ligase structure contains three parts, including a canonical dinucleotide-binding fold, a dimer interface and a helical domain with maximum-entropy methods (Doublie et al. 1994). Proteins fold mainly because of hydrophobic effect and they can be kept stable due to non-covalent interactions.

1. Molecular Interactions seen in the protein

Favorable hydrogen binding form secondary structures (alpha helices and beta strands). Weak and non-covalent forces formed between amino acid side chains for folding to occur. Protein has ionic interaction with magnesium ion, generate metal coordination. Also, ATP has metal coordination with magnesium. With the metal coordination, magnesium ion can help connect amino acids to maintain the protein structure. Cation-Pi interaction exists in the protein, which “is a stabilizing electrostatic interaction of a cation with the polarizable pi electron cloud of an aromatic ring”. (Decatur 2011) Tryptophan’s side chain contains the six-carbon aromatic ring. Therefore, it can be stabilized by the cation-Pi interaction. Protein also non-covalently bind to L-Tryptophan amide to react with L-Tryptophan and make the catalyzation happen. Amino acid 183 non-covalently bind to ATP via amide nitrogen and carbonyl oxygen so that ATP can provide energy for the catalyzation. ATP also binds to magnesium ion since magnesium can catalyze the ATP hydrolysis. Besides, ATP has hydrogen bond with water since water is reactant in ATP hydrolysis.

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