Protein purification methods and structure functions biology essay

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Protein purification is the method by which particular protein of interest is being separated from a complex mixture. This is a very important process in order to understand the structure, function and interactions of particular protein. First of all separation of protein from the matrix containing it and then its separation from non protein parts and finally separating desired protein from other proteins. There are three general analytical purification methods on the basis of protein properties. Firstly on the basis of isoelectric point, then size or molecular mass and lastly on the basis of hydrophobicity or polarity. Purification of protein is generally dependent upon its properties which are determined by its amino acid sequence. (http://en. wikipedia. org/wiki/Protein_purification). Molecular parameters of human TNF alpha such as molecular weight and isoelectric point was studied by using the postparam program of ExPASY web tool. The molecular weight was found to be 58675. 5 and theoretical pI was 5. 06.

Purification

The recombinant human TNF alpha is synthesized by the transformed K. lactis and was secreted out into the nitrogen free minimal medium because of Î±-MF signal sequence present in the expression vector pkLAC2. Purification of human TNF alpha was done in few steps procedure.

The first step in the purification of particular protein is its recovery from the medium and in this case TNF alpha is released outside of the cell in extra cellular medium. This is done by the help of centrifugation at 5000 rpm for 20 minutes to separate cells and the culture media. The desired protein is present in the supernatant from where it is collected and subjected to further purification.

Affinity Chromatography

The human TNF alpha protein has been tagged with the hemagglutinin (HA) peptide. In order to purify the protein, Pierce HA Tag IP/Co-IP Kit (Thermo Fisher Scientific, 2010) was used that provide affinity resin and other reagent to perform immunoprecipitation or co-immunoprecipitation reactions by using HA tagged protein as bait. This kit contains the prepared agarose affinity resin, buffers, microcentrifuge spin columns, a positive control and easy-to-follow instructions. Highly specific anti- HA anitibody is covalently immobilized on the beaded agarose in the kit. There is precipitation of HA tagged protein on the agarose after incubation. This specific interaction complex is being easily eluted from the resis by using simple washing steps and then is supplied with SDS-PAGE sample loading buffer for further analysis.

SDS-PAGE

After isolating and extraction of the desired protein, its purity is checked by using a technique known as SDS-PAGE (Sodium dodycyl sulphate polyacrylamide gel). SDS-PAGE is a technique used for the characterization and separation of peptides and proteins in a complex mixture. The characterization of SDS-PAGE depends upon the molecular weight of a particular protein and its electrophoretic mobility. The structure of protein also effects its migration of protein through the gel. Sodium dodycyl sulphate (SDS) is anionic surfactant produce a negative charge coat on the proteins. These charged particles move towards their opposite charge electrode under the influence of electric fluid during electrophoresis. The electrophoresis of the protein through the gel is majorly affected by the ionic composition, pH

and temperature of electrophoretic medium. In electrophretic migration, the pour size of the gel is also one of the major factors that affect electrophoresis. (Creighton, 1998)

After loading the sample, the gel was run at constant current for 2-3 hours. Uniform bands were observed on the gel indicating the purity of protein.

Mass spectrometry

Mass spectrometry is a technique which can be used to determine the purity of protein. As molecular weight of a protein is an important characteristic, that can indicate its purity. Mass spectrometer is a technique that detects mass to charge ratio of ions. The procedure involved is first loading of the sample for ionization in mass spectrometer. The molecules can be manipulated easily when they are ionized. These ionized molecules are separated on the basis of their mass to charge ratio in analyzer region. Afterwards, these separated ions are detected analyzed by the detector. Mass spectrometer works in a high vacuum, so that the traveling of the ions is not hindered by the air molecule during their passage from one end to the other.

The mass obtain in the result is analyzed and compared with the known mass of TNF-alpha. In case of similarity in the masses, it is confirmed that the protein is completely pure. Any dissimilarity indicates that the protein is not completely purified. (Creighton, 1998)

Biological Activity of TNF-alpha

The biological activity of TNF-alpha can be determined by Human TNF-alpha Elisa kit (Signosis, Inc 2010). The principle of this assay is based upon solid phase ELISA. The mouse anti human TNF-alpha antibody is used in this assay for immobilization of TNF-alpha protein. For detection purpose, goat anti human TNF-alpha antibodies, which are conjugated to horseradish peroxidase (HRP) with streptavidin are used. The two antibodies and the sample are incubated for allowing the reaction to take place. It leads to a sandwich formation of TNF-alpha protein in between the two antibodies. The wells are then washed after incubation, so that the unbound enzyme-linked antibodies are removed. Tetramethylbenzidin (TMB) is added in the well which is a HRP substrate. It develops a blue color while reacting with streptravidin-HRP conjugate. A stop solution is then added which changes the blue color into yellow. It is then analyzed spectrophotometrically. Absorbance is measured at 450 nm. (Signosis, Inc 2010)

Component of Human TNF-alpha Elisa Kit

Mouse anti-human TNF-alpha antibody coated 96 well microplate.

Goat anti-human TNF-alpha antibodies, biotin labeled.

Streptravidin-HRP conjugate.

Substrate.

Standard recombinant TNF-alpha (290ng/ml)

Assay wash buffer.

Blocking buffer.

Stop solution.