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ISOLATION AND PURIFICATION OF PENTRAXIN -3 FROM HUMAN SERUM

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ABSTRACT

Pentraxin 3 (PTX3) is the prototype member of the long pentraxin subfamily and a key soluble pattern recognition molecule of the humoral innate immune system, particularly in neurons and neurotransmitters. The present study aimed to isolate and partially purify PTX3 protein from the serum using various biotechnological processes, as well as determine the molecular weight of PTX3. The research involved using serum from a healthy person for partial purification of PTX3 using three sequential steps: Saline displacement precipitation, dialysis, and size exclusion chromatography were used with Sephadex gel number G-75. The results showed an increase in the specific concentration and the number of isolations of PTX3(5.1) (1.15), respectively ،following first step in precipitation and the recovery was 86.9 of PTX3. Also, the specific concentration increased to (5.7) in dialysis. The recovery amount of the total PTX3level reached 84.9%. In addition, two protein peaks (A and B) were detected, with PTX3corresponding to peak B, showing a purification fold of 33.2 compared with the crude serum after the gel filtration process, with a 60.2 recovery rate, and its molecular weight was determined by gel filtration technique, revealing an approximate molecular weight of 41000 Da.

KEYWORDS: Pentraxin 3, Separation, Gel filtration, Sephadex G-75, Dialysis, Molecular weight.

INTRODUCTION

Osmand et al. (1977) coined the term "pentraxin," derived from the Greek words for "five" (penta) and "berry" (ragos). It's a family of soluble immunoglobulins. Under an electron microscope, these proteins exhibit a distinctive appearance, with their subunits arranged as ring-like discs (Massimino *et al*., 2023).

Pentraxin 3 (PTX3) is a complex glycoprotein; its basic sequence spans 381 amino acids and consists of two main chains: the amino-terminal domain (N-terminal). This enables it to engage with a variety of ligands outside of traditional immune responses. This domain extends for 178 amino acids and is structurally unorganized, giving it high flexibility to bind to diverse molecules and facilitating its biological interactions (Shah *et al*., 2025). The second terminal domain, the carboxyl terminal (C-terminal), contains the conserved structural signature of the pentraxin family (pentraxin signature). It spans 203 amino acids and folds into a beta-barrel-like structure (beta -jelly roll topology), a characteristic of the pentraxin family (Liu et al., 2025; Massimino rt al., 2023).

Its molecular weight is approximately 42 kDa (Koussih et al., 2021). It undergoes post-translational modifications, such as glycosidic linkage, particularly at the Asn220 site, where sugar chains with varying compositions depending on the cell type that produced the protein are attached. This affects the protein's stability and regulates its interactions with components of the immune system, such as complement proteins. The protein also contains several cysteine residues that contribute to the formation of disulfide bonds, which are essential for stabilizing the protein's three-dimensional structure (Noone et al. 2022). This structure is stabilized by disulfide bonds between cysteine molecules at specific sites, such as Cys103, Cys77 in the amino acid segment and Cys318, Cys317 in the carboxyl group (Guo et al., 2025).

PTX3 facilitates microbial recognition and immune response modulation by being stored as a monomer in neutrophil granules and aggregating upon activation, the tetravalent state of PTX3 is functional and can support the formation of extracellular matrix and recognize fibroblast growth factor 2 (FGF2) (Scuderi *et al*., 2024). Notably, PTX3's conversion from an octavalent to a tetravalent form has been linked to better sepsis survival rates, underscoring the critical role that PTX3 aggregation dynamics play in the course of the disease and its potential for treatment (Noone et al., 2022).

MATERIALS AND METHODS

Separation of Pentraxin 3 from serum

With the assistance of the Mosul Blood Bank, fresh serum was collected from a healthy donor (35 mL). He is 36 years old and was used in subsequent purification steps. Purification stages were conducted later using various techniques, (Thikra and Jehan, 2014) as described below:

1. Precipitation and Separation of Protein Using Ammonium Sulfate

Precipitation with (NH4)2SO4, in which serum proteins precipitate based on the saturation of the serum. At 75% saturation rate, a gradual addition of (NH4)2SO4 to blood serum was conducted at 4°C, and an electric stirrer was continuously operated to stir for 60 min. After that, to complete the sedimentation process, the solution was subjected to magnetic stirring in the refrigerator at 4°C for 24 hours (Robyt & White, 1987). (NH4)2SO4 competes with proteins for interaction with water molecules. For this reason, solvent molecules are unable to interact with proteins, resulting in protein molecules aggregating together at high concentrations due to protein-protein interactions. The precipitate is then ready for cold centrifugation (Robyt and White, 1987).

2. Cooling Centrifuge Separation

The precipitate was then exposed to a cold centrifugation process at 10000 xg for 35 minutes. After isolating the supernatant, the precipitate resulting from this process was dissolved in a small amount of distilled water. The protein and pentraxin 3 concentrations in the solution were determined (Rana and Thikra, 2021).

3. Dialysis

Cellophane tubes are used as a semipermeable membrane for this process (Robitt and White, 1987). The dialysis process was performed by placing the precipitate solution in a cellophane tube, which was then placed in a 0.1 M ammonium bicarbonate (NH_4HCO_3) solution (Plummer, 1978). The temperature was maintained at 4°C for 24 hours.

4. Size exclusion chromatography (Gel Filtration Chromatography)

It is separate compounds based on their molecular weights and sizes. It is also called molecular sieve chromatography. Sephadex G-75 was used, and protein peaks were identified by measuring the absorption at 280 nm (Thikra and Jehan, 2014). This technique can also be used to determine the molecular weight of protein peaks (Andrews, 1965).

The steps involved are as follows:

Preparation of Sephadex G-75 Gel

Distilled water was added to the powder of Sephadex gel (G-75), then allowed to swell for 72 hours until complete swelling occurred (Robyt and White, 1987).

Column packing

A separation column with dimensions of (95 x 1.5) cm was used, which contains a gel material of type (Sephadex G-75) that separates compounds with molecular weights of approximately 80,000 Daltons, where the gel material was placed at a height of (82) cm.

Addition of standard proteins

Standard proteins with molecular weights ranging from 2000,000 to 204 Daltons were used to evaluate the approximate molecular weight of the separated protein peaks. Tryptophan (Trp) and blue dextrin were used to determine the column size, along with standard proteins: egg albumin, trypsin, bovine serum albumin (BSA), α-amylase, and pepsin.

Adding samples

The proteinous sample (2mL) was put on top of Sephadex G-75, followed by (2mL) of distilled water. Collect the displaced liquid in 3 minutes with 60 mL/h. Proteins were detected by measuring absorbance at 280 nm, also PTX3 was detected by determining its concentration. The proteinous peak in which PTX3 was found (peak B) was collected, lyophilized, and kept in the freezer in a tight sample tube for further experiments.

Freeze-Drying Technique (Lyophilization) Lyophilizer

The protein fractions obtained from gel filtration chromatography were freeze-dried using a lyophilizer to achieve concentration (Al-Barhawi & Al-Wash, 2022).

Estimation of Total Protein Using the Modified Lowry Method

The assay by Schacterle and Pollack (1973) is used to determine protein concentration. This is a modification of the Folin-Lowry method.

Estimation of PTX3Concentration

Human PTX3 concentration was evaluated by using a kit from Sunlong Company (China), dependent on an Enzyme-Linked Immunosorbent Assay (ELISA).

RESULTS AND DISCUSSION

Table (1) shows that the use of ammonium sulfate 75% increased the specific concentration (5.1) and the number of isolations (1.15) of PTX3. The recovery rate also reached 86.9 %, indicating that ammonium sulfate salt is

a good precipitator for proteins in general (Thikra and Noori, 2023) and for PTX3 in particular.

Table 1: Partial purification of PTX3 from the serum.

Purification Steps	Total Volume (ml)	Total Protein (mg)	Total Conc. ofPTX3 (pg)	Specific Conc.	Times Number of Purification	Recovery %
Serum	35	242	1068	4.4	1	100
Proteinous precipitate solution	29	180	928	5.1	1.15	86.9
Dialysis	27	158	907	5.7	1.3	84.9
Gel filtration/Sephadex G-75 (peak B) after Lyophilizer	4	4.4	643	146	33.2	60.2

Table (1) also shows that dialysis increases the specific concentration (5.7) while decreasing the recovery rate (84.9%) of PTX3, highlighting the importance of dialysis for removing interfering substances (Thikra and Liqaa, 2019).

Figure (1) shows the appearance of peaks A and B, as well as their respective elution volumes (74 and 149 mL). The highest specific concentration of PTX3 was found in the second peak (B); therefore, this peak was considered the isolated PTX3, as indicated in Table (1), where the number of isolations reached 33.2.

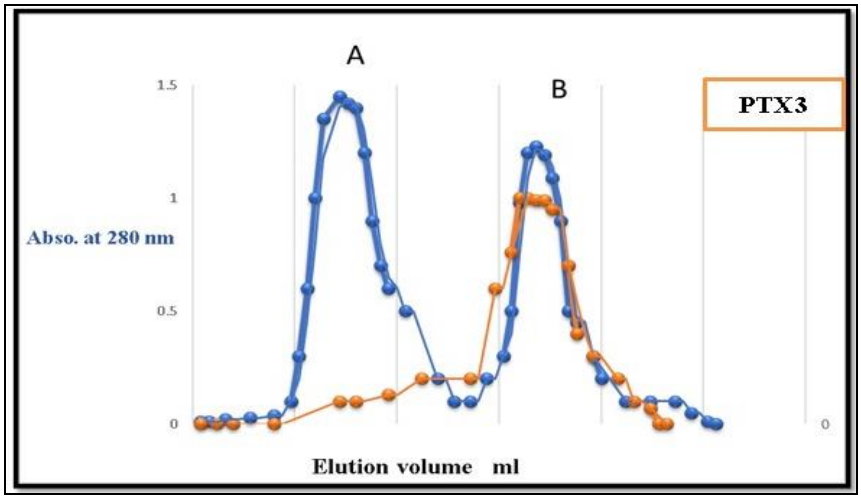

Figure 1: Elution profile of partially purified PTX3 from serum via gel filtration chromatography using a Sephadex G-75 column (95 x 1.5 cm).

Determination of isolated PTX3's (peak B) molecular weight

It was found that the isolated (peak B) PTX3 has an approximate molecular weight of 41000 Da, according to the use of the calibration curve shown in Figure 2 for the gel filtration technique.

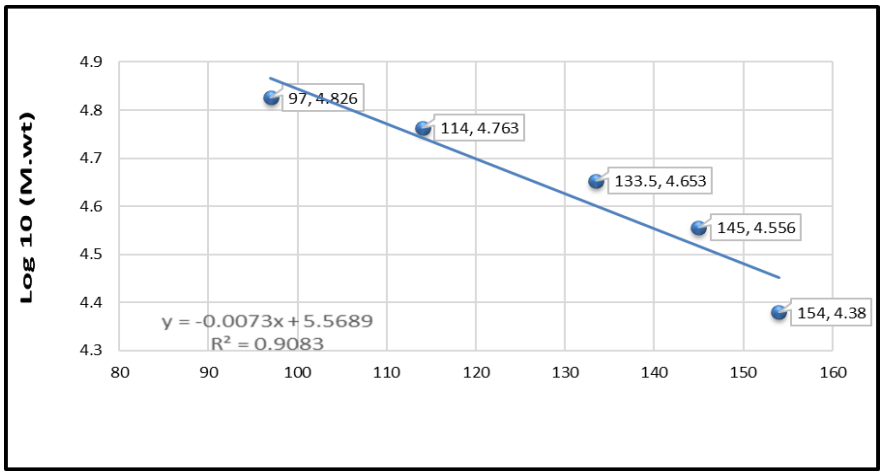

Figure 2: Calibration curve of molecular weight.

The monomeric form of the PTX3 amino acid sequence (381 amino acids) has a predicted molecular mass of 40,165 Da (Liu et al., 2025). In biological systems, PTX3 naturally functions as a homo-octamer (a complex of eight identical monomers) bound together by disulfide links, giving the full active molecule a total molecular weight of approximately 340,000 Da (340 kDa) (Garlanda et al., 2018). In its native physiological state, PTX3 undergoes N-glycosylation at the Asn220 site, which adds roughly 5,000 Da, increasing its apparent molecular weight to 45,000 Da when observed on reducing SDS-PAGE gels (Bottazzi et al., 1997).

CONCLUSION

The study concluded that blood serum is a good source for isolating and purifying PTX3 using ammonium sulfate precipitation, cold centrifugation, dialysis, and size exclusion chromatography. Two protein peaks (A and B) were observed, with PTX3 present at the second peak (B), exhibiting a purity level 33.2 times greater than that of blood serum after filtration. Furthermore, the approximate molecular weight of PTX3, determined using gel filtration, was found to be 41000 Da.

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