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Isolation And Purification Of Lysyl Oxidase 4 From The Serum Of Glaucoma Patients

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ABSTRACT
The protein's isolation and purification were part of this thesis. protein from serum in the blood of an individual with gluacoma disease, the investigation of various elements influencing its efficacy, along with figuring out its molecular weight. In which Ammonium sulfate deposition produced a protein leachate, which was treated with the DEAD-Cellulose ion exchanger. (0-65%) following dialysis membrane sorting.The enzyme's high efficacy was demonstrated by the package, which was also used to determine the enzyme's molecular weight using SDS-PAGE and electrical migration technology, which was roughly equivalent to 66,000 kilodalton. As for the concentration of the protein, the findings indicated that an increased protein concentration would result in an increase in enzyme activity.

Keyword: Isolation, purification, lysyl oxidase 4, glaucoma.

1. INTRODUCTION

An irreversible blindness is caused by the neurodegenerative disease glaucoma. Loss of the eye's retinal ganglion cells (RGCs) and the optic nerve's axons is its primary characteristic. It is thought regarded as the second most prevalent cause of permanent blindness and primarily affects older adults (Jacobi & Van Zyl, 2020). Primary open-angle glaucoma (POAG) and primary angle-closure glaucoma (PACG) are the two forms of glaucoma (Wang et al., 2024). Among glaucoma, POAG, or primary open-angle glaucoma, is the most prevalent. An opening or closure of the anterior chamber angle, which consequently stops fluid elevated intraocular pressure and outflow , is a characteristic of angle-closure glaucoma, a rare form of the condition. According to Krzyzanowska et al. (2022), some of the symptoms include vision impairment and excruciating headache pain. The following risk factors have been linked to an increased risk of developing glaucoma: age, genetics, ethnicity, hypertension, diabetes mellitus, cardiovascular diseases, and elevated intraocular pressure (IOP) (Fujita et al., 2023). According to Qureshi et al. (2021), the primary treatments for gluacoma disease are pharmaceutical therapy, laser therapy, and surgical interventions. Protein lysine 6 oxidase, is an amine oxidase that requires copper to function. a crucial role in the biogenesis of connective tissue matrices by crosslinking collagen, elastin, and other extracellular matrix proteins. (NikhalaShree et al., 2023) . Several lysyle oxidases have been demonstrated to act as copper-dependent amine oxidases, igniting the formation of lysine-derived cross-links in extracellular matrix proteins. The newest member of this family is lysyl oxidase-like 4 (LOXL4) (Tan et al., 2021). Along with the typical LOX family domains, LOXL4 also has four Cysteine-rich domains of scavenger receptors (Wang et al., 2023). Maintaining the mechanical and structural integrity of connective tissues throughout the body depends on lysyl oxidase. When lysine residues in collagen and elastin undergo oxidative deamination, volatile aldehydes are produced, which then spontaneously condense to form mature cross-links (Rodriguez-Pascual et al., 2022).

2. AN AIM OF THIS STUDY

Using the ion exchange technique, separate the protein from a serum containing glaucoma. Then, use electrical migration technology to estimate the protein's molecular weight. Maintain the ideal circumstances for the protein.

3. SUBSTANCES AND METHODS

(1.3). Protein purification and isolation

(.1.3.3). Assessment of the protein's effectiveness

Using the methodology the researchers employed, the protein's efficacy was calculated (Shastry & Rao, 1975).

3.1. Serum preparation

Following the patient's moral clearance and compliance with the Nineveh Health Department's controls, a 25 ml blood sample was obtained from a 37-year-old donor with gluacoma disease who was chosen to serve as the study model in the hospital. Following separation and serum collection, the protein concentration and efficacy were assessed using the modified Laury method. The serum was then stored in a freezer to subsequently go through a number of purification procedures required to isolate and examine the protein's characteristics.

3.2. Isolation and deposition of the protein using ammonium sulfate:

Ammonium sulfate was gradually added while solid (Robyt F.J. & White J. B., 1987). The mixture is stirred with a magnetic motor and kept at a low temperature (4°C) for 60 minutes until it reaches serum and saturation (65%). Following a 24-hour cooling period, the precipitate was separated from the leachate using a cooled centrifuge for 10 minutes at a speed of xg 4000. The precipitate was then dissolved using the least amount of regulated phosphate solution (0.02) at a pH of 6.8, where the amount of protein and the enzyme's efficacy were estimated. The precipitate solution was then stored at -20° C until it was needed again. In 2008, Muhaisen et al.

3.3. Membrane sorting

To eliminate ammonium sulfate, the solution from the previous step was membrane sorted using the Robyt F.J. & White J. B. (1987). where (15 ml) of the solution is put inside the bag, which is then closed from the top edge and regrettably tightly seals the lipoxygenase enzyme from patients with allergic reactions (Ahmed and Hilali, 201 0) .The solution is submerged in phosphate regulate solution at a concentration of (mm 20) and an acic function (pH=6.8) while being stirred with a permanent magnet motor for a duration of 24 hours and at a temperature of 4 °C (considering the replacement of the regulated solution every 4 hours until the final quantity of the resulting solution is finished) (Kamal & Hasan, 2019)

3.4. An exchange of ions chromotograph

An ion chromotograph is made by adding a solution of controlled phosphate with a pH of 6.8 (0.02M) to a weight (30 g) of negative ion exchange (DEAE-Cellulose) that has been activated with acid and base and rinsed with water multiple times until pH = 7. The controlled solution passed for at least an hour before passing the models if the resin was removed and placed in the separation column, which is2.5 cm in diameter and 40 cm in length , and slanted to prevent the formation of air bubbles (Plummer, T.D., 1978).

3.5. Statistical analysis

In order to compare more than two variables and determine the distinction at the p-level of probability 0.05, the data were statistically analyzed using the Duncan test and the ANOVA test, the statistical program 18SPSS (Burton, 1994).

4. RESULTS AND DISCUSSION

4.1. Study of Protein separation and purification from blood serum

protein that has been partially purified by saline displacement (ammonium sulfate deposition). evaluated against the overall efficacy of the raw Protein was isolated from a glaucoma patient's serum using various life techniques. The first method used to purify the enzyme was sedimentation ammonium sulfate salt (neutral salt), which is preferable to be used in the first step of the purification process of enzymes because it is a salt made up of di-

charged ions (So4-3) and (NH43), which gives it a high ionic strength and greater efficiency in protein deposition than the other neutral single-charge salts, like sodium chloride, because the ionic force is directly proportional to the charge square of salt ions (Al-Fayyad & Al-lehebe, 2021). In contrast to the total effectiveness of the raw, the protein's overall effectiveness recovered by 73.09 percent, as indicated by the results in Table (1).

Table (1): Steps for purifying protein from serum of glaucoma patients

Purificationsteps	The size (m1)	Protein (pg/ml)	Total protein (pg)	Recovery%
Raw serum	23	235.21	5409.83	100
Precipitation by Ammonium Sulphate (before dialysis)	19.41	203.72	3954.20	73.09
Sorting of membrane (after dialysis)	25.21	185.52	4676.95	86.45
Ion exchange Package	27	175.46	4737.42	87.57

Enzymatic unit (U): It travels to the amount of enzyme that releases one micromole from the base substance per minute.
Specific efficacy (U/mg): The number of enzyme units found in one mg of protein.

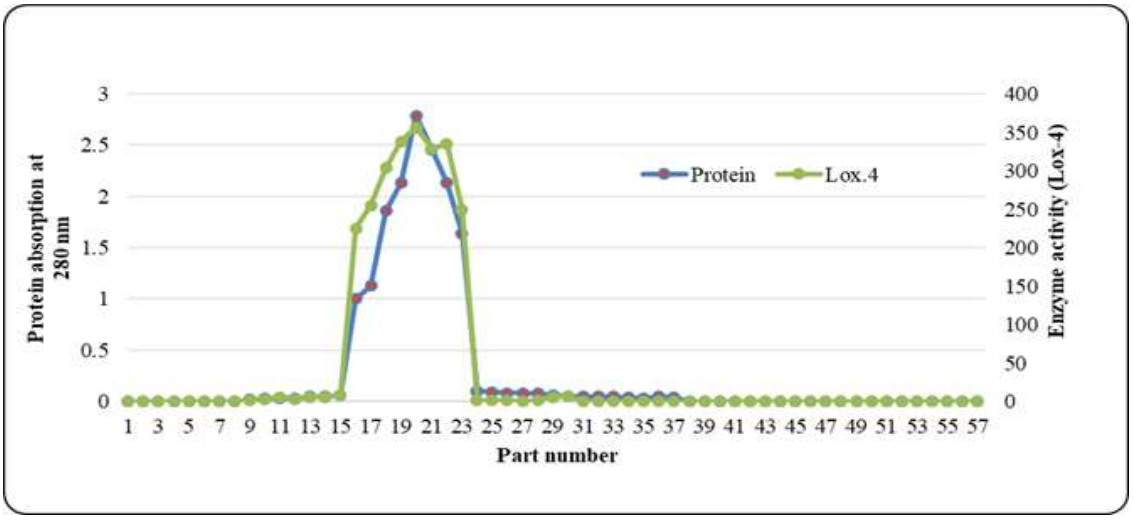

Figure (1): Profile of the protein partially purified from blood serum using ion exchange chromatography technique

4.2. Finding the molecular weight of the protein in the blood serum with the technique Migration (Electrophores):

By injecting the model into the protein solution of the package isolated from the separation process using an ion exchange chromatography technique, the approximate molecular weight of the protein that was separated from the blood serum of glaucoma patients using the electrical migration technique with PAGE-SDS was estimated. As seen in Figure(2), the protein beam was discovered through this procedure at a distance of (4.0) cm from the

origin. This package was used to estimate the approximate molecular weight of the enzyme, which came out to be (66) kilodalton. This finding is in line with earlier research (Dinh et al., 2020; Gilbert et al., 2020).

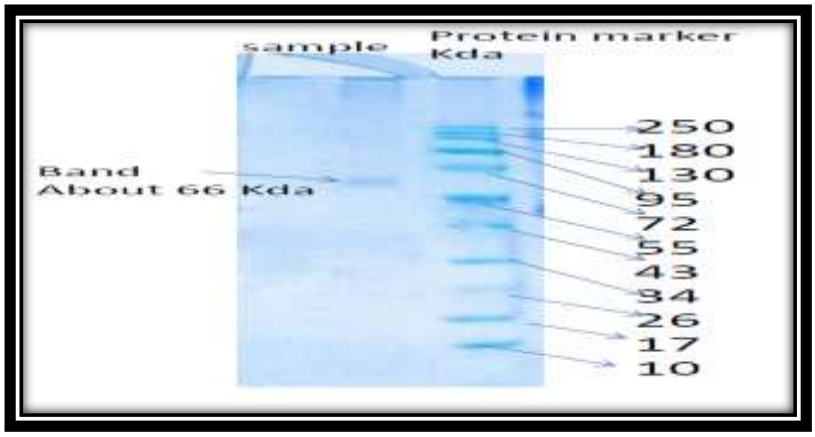

Figure (2) Electrophoresis containing (PAGE-SDS)

CONCLUSION:
In this thesis, the isolation and purification of a serum protein from an individual with glaucoma were completed, and the factors affecting its activity were examined while determining its molecular weight. Ammonium sulfate precipitation yielded a protein leachate, which was further purified using a DEAD-Cellulose ion-exchange column (0–65%) after dialysis. The enzyme's high activity was demonstrated, and its molecular weight was estimated by SDS-PAGE and electrophoretic mobility to be approximately 66,000 daltons. Additionally, the results indicated that higher protein concentrations correlate with increased enzyme activity.

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