GEORGIAN MEDICAL NEWS

ISSN 1512-0112

NO 7-8 (352-353) Июль-Август 2024

ТБИЛИСИ - NEW YORK

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press. Published since 1994. Distributed in NIS, EU and USA.

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

GEORGIAN MEDICAL NEWS
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Содержание:

GEORGIAN MEDICAL NEWS
No 7-8 (352-353) 2024

ISOLATION AND PURIFICATION OF TRANSGLUTAMINASE 1 USING BIOCHEMICAL TECHNIQUES

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Abstract.

Transglutaminase 1 catalyzes the creation of covalent bonds between proteins, play an essential role in various biological processes and industrial applications. The study aims to isolate and purify transglutaminase 1 from the blood serum of healthy individuals using numerous biochemical techniques. TGMs 1 are isolated and purified from the blood serum of healthy volunteers samples who were not smokers and had not taken any medications at the time of the sample collection. The results show that these techniques included precipitation with 65% ammonium sulfate, dialysis, and negative ion exchange chromatography, successfully separating a single prominent band with high activity using DEAE-cellulose. The enzyme activity recovery was estimated at approximately 33.01%. Subsequently, gel filtration using Sephadex G-100 revealed a single fraction with high TGM 1 activity. This fraction exhibited a purification factor of 9.09, with an estimated recovery of enzyme activity of around 29.6%. The isolated and purified TGM 1's approximate molecular weight was around 73,115 Daltons, as assessed through gel filtration chromatography with Sephadex G-100. The study indicated that the optimal conditions for the isolated and partially purified TGM 1 enzyme were a pH of 6.4 and a temperature of 37°C, using a concentration of 0.5 mmol/L of the substrate tetramethylbenzidine. The results indicated that purified TGM1 may be an alternative to other sources.

Key words. Transglutaminase, biological processes, ion exchange chromatography, DEAE-cellulose.

Introduction.

Tumors, commonly referred to as cancer, are a broad category of disorders marked by the body's aberrant cells growing and spreading out of control [1]. The human body usually carefully controls cell division, development, and death to preserve the integrity of tissues and organs. On the other hand, this regulatory system malfunctions in cancer, resulting in the development of a tumour, a mass of tissue [2]. Every year, the number of cancer cases rises due to pollution in the land, water, and air and the introduction of canned goods without proper quality control [3]. Both tumors and malignancies require appropriate management and treatment [4]. Insufficient cultural awareness in this field may contribute to increased infections and highlight the importance of genetic susceptibility. Early detection, prompt diagnosis, and regular medical check-ups and screenings can enhance the early identification of the illness [5]. Cancer symptoms may include loss of weight without cause, exhaustion, pain, modifications to urinary or bowel habits, a chronic cough, throat clearing, pain or discomfort in the abdomen, trouble swallowing, and a chronic fever or illnesses [6,7-10].

Tumors can arise from various types of brain cells and may be classified as either benign (non-cancerous) or malignant (cancerous) [11]. Brain tumors can originate from different brain cells or tissues and can be categorized according to their location, size, and features [12]. The following are typical signs of brain tumours: seizures [13], persistent headaches [14], vomiting and nausea [15], vision changes [16], issues in speech [17], and changes in hormones and appetite [18, 19]. Cancer development is impacted by many factors [19], including genetic factors [20], environmental exposures [21], lifestyle [22], and, occasionally, random mutations and hormonal factors [23].

Transglutaminases are a class of enzymes that help to create covalent links between proteins, and this process is known as transamidation. The body has a variety of transglutaminases, including those in the brain [24]. According to related studies, transglutaminases have been linked to cancer development, particularly brain tumours [25]. An enzyme known as transglutaminase 1 (TGM 1) is essential to the keratinization process, creating the skin's protective outer layer, called the epidermis. This enzyme is especially crucial for forming the epidermal barrier, which shields the body from contaminants, dehydration, and infections from the outside world [26] (Figure 1A).

TGM 1 facilitates protein cross-linking through a well-coordinated enzymatic mechanism, which is crucial for many biological activities, including blood coagulation, wound healing, and tissue stability [28] (Figure 1B).

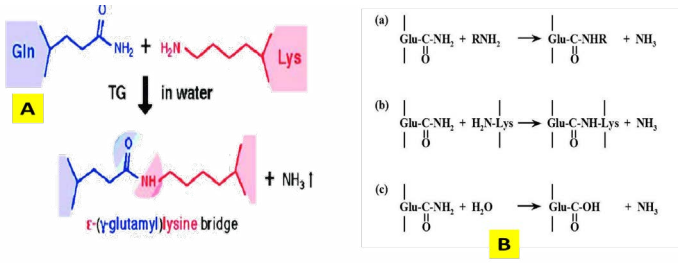


Figure 1. *(A)Transglutaminase mechanism [27]. (B) TGM 1 enzyme reactions [29].*

The study aims to isolate and purify TGM 1 from the blood serum of healthy individuals via different biological techniques. The molecular weight of the isolated enzyme from human blood serum was also found. TGMs 1 are enzymes that facilitate the formation of covalent bonds between proteins. They are essential in various biological processes and have numerous industrial applications. Additionally, TGM 1 has made significant contributions to biotechnological research, particularly in the field of antibody-drug conjugates, which holds great promise for future development.

Materials and Methods.

Isolation and Purification of TGM 1 from Blood Serum: In this section of the investigation, TGM 1 was isolated and purified from blood serum samples obtained from five healthy

volunteers, ages 25 to 27, who were not smokers and had not taken any medications at the time of the sample collection. Later, purification procedures were carried out utilizing several biotechnologies; they will be explained in the following manner:

Salt Displacement Sedimentation Using Ammonium Sulphate: Ammonium sulphate (AS) was gradually added in solid form to blood serum at a saturation level of 65%, while continuously stirring with a stirrer for 1 hour at 4°C (Figure 2). The solution was then left to sit at 4°C in the refrigerator for 24 hours to complete the sedimentation process. The precipitation process using ammonium sulfate is an initial stage of purification in which proteins in blood serum are deposited based on the serum's saturation level with AS [30]. AS competes with proteins for solubility and interaction with water molecules, leading to protein aggregation at high concentrations. This aggregation occurs because strong protein-protein interactions surpass the interactions between proteins and the solvent [31].

The precipitate was extracted using ammonium sulfate, and the supernatant—a transparent liquid—was separated. A cooling centrifuge was used for forty-five minutes. Next, a small amount of distilled water was used to dissolve the silt. The modified Lowry technique was used to determine the protein content after determining the volumes of the protein precipitate solution and the supernatant [33]. The enzyme activity was measured in the protein precipitate solution and the clear liquid. Both solutions were preserved at -20 °C for use in subsequent steps.

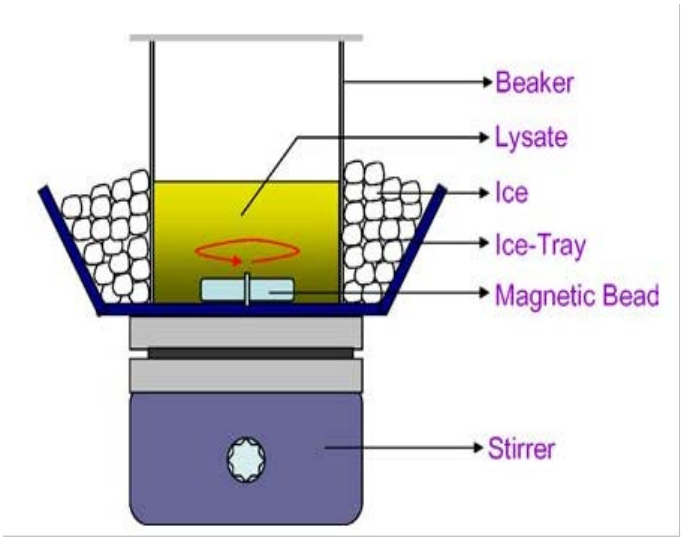


Figure 2. *Protein precipitation using ammonium sulphate [32].*

Dialysis: The protein solution was prepared by placing it in washed cellophane tubes and tightly tying them. After that, the tube was cleaned with distilled water and immersed in a volumetric container filled with 2.5 liters of an ammonium bicarbonate (NH4HCO3) solution at a 0.1 M concentration [35]. The Donnan effect may impact the cellophane membrane, so this was done to prevent it. Ammonium bicarbonate is the only salt that is completely removed by lyophilization, as shown in the following equation.

$$NH_4HCO_3 \rightarrow NH_3\uparrow + H_2O + CO_2\uparrow$$

It is noted that (both parts of the salt are gas) can be disposed of. The dialysis process was conducted at 4°C with a magnetic stirrer for 24 hours, replacing the solution thrice. The next day, ions from sedimentation salt and proteins with MW < 10 kDa were removed. The process separated large molecules from ions and compounds with small MW using a semi-osmotic membrane. Dialysis involves using cellophane tubes cut according to sample size, with a molecular weight cut off, allowing compounds with < 10 kDa to pass. These semi-osmotic membranes prevent molecules over 10 kDa from passing. The final solution volume is calculated, the protein amount is estimated using the Modified Lowry method, and enzyme activity is measured. The solution is preserved at -20°C until used in the next ion exchange process.

Ion Exchange Chromatography (IEC):

IE is a chromatographic analysis method based on partition chromatography. It involves using a stationary phase resin, which can be a cation or anion exchanger carrying acidic or basic groups (-SO3H or -COOH). IE is used for isolating and purifying compounds of varying types and electrical charges [30]. Various biological compounds, like proteins, are isolated using separation columns with appropriate resins, such as anion exchangers. Samples are placed on the resin, and buffer solutions are passed through. The process involves two stages: the adsorption of charged materials to be purified on the resin and the separation and removal of charged compounds using pH and ionic strength-specific solutions [35].

The 50 g of diethylaminoethyl-cellulose anion exchanger resin was weighed, and phosphate buffer (NaH2PO4-Na2HPO4) was added at 10 mmol/L and pH 7.1. A glass separator column was filled with DEAE-Cellulose to prevent air bubble formation and compact the resin. The excess buffer solution was withdrawn, and DEAE-Cellulose was added until the resin reached 120mm height. The column was left at 4°C for 12 hours for stability, and a new suspension was added when the level decreased due to compactness. The column was kept in a cold room during use to preserve enzyme activity during purification [30]. The protein solution from the dialysis process was injected into a separator column containing negative ion exchanger type DEAE-cellulose. The solution was suspended for 60 minutes before passing through a buffer solution. Filtrate portions were collected in test tubes, and the absorbance of the filtered protein fractions was measured using a spectrophotometer. The protein top with high TMG 1 activity was incidental by measuring activity of enzyme in the protein fractions collected after dialysis injection.

Gel Filtration Chromatography (GFC):

GFC is a method for separating compounds based on molecular size and weight differences. It is also used to estimate protein weight [31]. The process involves preparing a column for GFCT. A 4x14.5mm column containing Sephadex G-100 gel is used to separate proteins with a fractionation range of 3000-130000 Dalton. The gel is placed at a height of 50cm, preventing air bubbles and settling to withdraw excess water. The column is washed with a buffered phosphate solution at pH 7 [31]. The sample is passed through the column, and the internal volume (Vi) is calculated using tryptophan (Trp), while the outer volume (V°) is determined using blue dextran [31].

Results and Discussions.

Separation of Transglutaminase 1 From Human Serum:

Five healthy individuals' blood serum was used to purify TGM 1. To perform purification on the sample, the immediate blood sample is collected and then kept for a 15-minute centrifugation at 3000 rpm to produce blood serum, which is then utilized.

Several biotechnologies have been used for the separation process, like dialysis, salting out, and ion exchange utilizing a DEAE-cellulose anion exchanger. After that, GFC using Sephadex G-100 completed the separation process. Sephadex G-100, the highest peak obtained from the gel filtration procedure, was then used to stabilize the ideal conditions for the isolated enzyme and determine its molecular weight.

Salting out:

In this process, by adding ammonium sulfate, the protein precipitates [36]. At 4 °C, the precipitation process has been carried out with a saturation ratio of 65%. The resultant precipitate was collected using centrifugation (8000 rpm, for 45 min at 4°C). The precipitate portion of the mixture has a higher specific activity than the filtrate portion (27.614 U/mg), and it has undergone 1.19 times as many purification cycles. So, the purification process is completed using the precipitate in the dialysis, and the filtrate is ignored (Table 1).

Dialysis:

The separated enzyme's specific activity and amount related to the total protein increase throughout the precipitate's dialysis process, which results after salting out. According to the cellophane used in this study, the enzyme's specific activity is 66.057 enzyme (units/mg) of protein, and the number of purification times is 2.86 times greater than when the separation of the enzyme first started. The compounds of protein with small MW (MW< 10,000 Dalton) are leaving the enzyme. Dialysis distinguishes small molecules from big molecules by allowing the passage of only tiny molecules through selectively permeable membranes (cellulose membranes with hole diameters intended to exclude molecules below a certain MW) [36]. This process aims to enhance the specific activity of the isolated enzyme in the dialysis solution while also removing small particles and the ammonium sulfate salt used during the previous protein precipitation step (Table 1).

DEAE-Cellulose Ion Exchange Chromatography:

For thorough and quick purification, selective adsorption and elution of proteins from the polydextran derivatives anion exchange diethylaminoethyl-cellulose (DEAE-cellulose) have been proven to be very effective [37]. The ion exchange results are displayed in Table (1), and Figure (3), where Transglutaminase

Table 1. *Steps of Transglutaminase 1 purification from human blood serum.*

Purification steps	Vol. (mL)	Protein conc. mg/ml	Total protein (mg)	Activity of enzyme (*unit/mL) (ng/L)	Total activity	Specific activity (unit/mg protein)	No. times purification	Recovery %
Blood serum	10	54	650	1499.3	14993	23.066	1	100
Precipitation with ammonium sulfate 65%	9	21	486	1491.2	13420.8	27.614	1.19	63.9
Dialysis of precipitate	9	18	189	1387.2	12484.8	66.057	2.86	61.1
The resulting peak from DEAE-cellulose ion-exchange separation column	5	6	90	1294.7	6473.5	71.927	3.11	33.01
The resulting peak from gel-filtration Sephadex G-100	5	54	30	1259.2	6296	209.866	9.09	29.6

**Enzyme unit: Under the given measurement circumstances, the quantity of enzyme required to convert one micromole of tetramethyl benzidine (TMB) into a product every min or ml.*

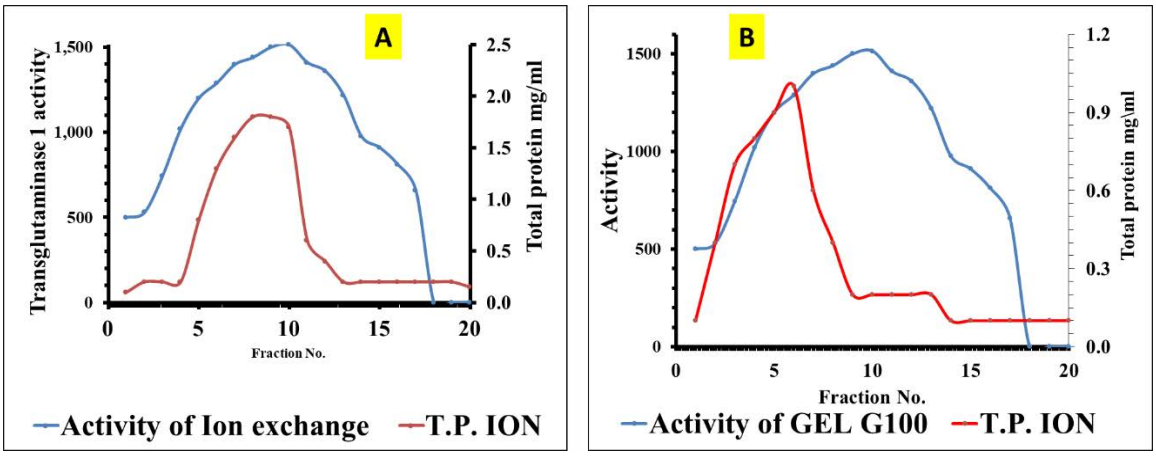


Figure 3. *(A) Elution profile of TGM 1proteinous peak. (B) Elution profile of TGM 1 proteinous peak.*

1's specific activity is measured at 71.927 enzyme units/mg and a single high-activity protein peak has emerged. Table (1) also shows that the enzyme has undergone 3.11 purification cycles.

Gel Filtration Chromatography:

A separation column (4 x 14.5 mm) containing G-100 separates the proteinous chemicals generated from the ion exchange process. After passing the ion exchange-derived protein peak, a single protein peak appears in the gel filtration findings. After monitoring TGM 1 activity, it is discovered that the peak corresponds to a high specific activity of the enzyme, reaching 209.866 enzyme units/mg, and that the number of purification cycles reaches 9.09 times, as shown in Table (1).

Determination of the approximate molecular weight of TGM 1 isolated from healthy human blood serum by gel filtration technique:

The molecular weight of the protein band concentrated in a single peak from the Sephadex G-100 separation column has been estimated. This peak exhibits the highest specific enzyme activity, measuring 209.866 enzyme units per mg of protein, with a purity factor of 9.09. Therefore, it has been used to estimate the enzyme's molecular weight by passing several compounds with known molecular weights, ranging from 204 to 2,000,000 Daltons, through a separation column. This process determined the column's properties, including the internal volume (Vi) of 360 mL and the void volume (Vo) of 55 mL. Table 4 lists the standard substances that passed through the Sephadex G-100 separation column and their molecular weights and elution volumes. A straight line was observed when plotting each standard substance's elution volume against its molecular weight's logarithm, as shown in Figure 4. This line indicates that the approximate molecular weight of TGM1 is 73,115 Dalton. However, researchers report that the molecular weight of TGM 1 ranges from 85,000 to 110,000 Dalton.

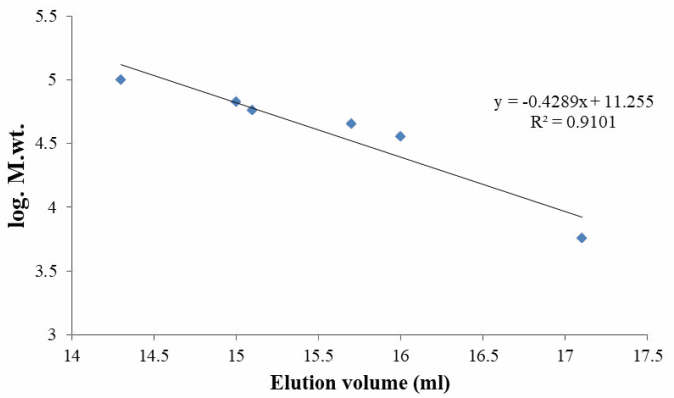


Figure 4. *Standard curve for determining the approximate MW of TGM 1.*

The molecular weight of TGM 1, determined through gel filtration chromatography on a Sephadex G-100 column, is approximately 73.1 kDa. This result aligns with studies characterizing transglutaminases from various sources, including bovine liver, which reported similar molecular weights [38].

TGMs 1 are enzymes that catalyze the formation of covalent bonds between proteins, playing important roles in various biological processes and industrial applications [39]. While the molecular weight may vary slightly depending on the source and purification methods, approximately 73 kDa is a reliable estimate for TGM 1 [38,39] (Table 2).

Table 2. *MW and Velution (elution volumes) of the standard substances used in the gel filtration Sephadex G-100 to determine the molecular weight of TGM 1 purification from blood serum.*

Standard substances	**MW (Dalton)**	**$V_{elution}$ (mL)**
Blue dextran (Void volume (Vo))	2000000	4.5
Hexokinase	100000	14.3
Bovine serum albumin (BSA)	67000	15
α–amylase	58000	15.1
Egg albumin	45000	15.7
Pepsin	36000	16
Insulin	5750	17.1
Tryptophan (Internal volume (Vi))	204	20
The unknown (Peak of enzyme)	73115	14.9*

** This value is derived from Figure 3, which displays the elution volume of the enzyme separated from human blood serum using gel filtration with Sephadex G-200.*

Study of optimum conditions for TGM 1 activity partially purified from blood serum

pH impact on the TGM 1 Activity:

The pH of an enzymatic reaction significantly influences its velocity. Variations in pH can provide insights into the ionizable groups of the acids in the enzyme's active site involved in catalysis [38]. Figure 5A illustrates the relationship between TGM 1 activity, measured in U/mL, and pH levels. The activity of TGM1 increases with rising pH from 2.4 to 6.4, reaching an optimum pH of 6.4. Beyond this point, the reaction rate begins to decline.

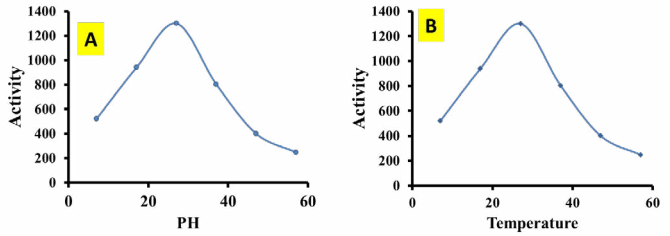


Figure 5. *(A) pH Impact on the activity of the purified TGM 1. (B) Temperature Impact on Activity of the Purified TGM 1.*

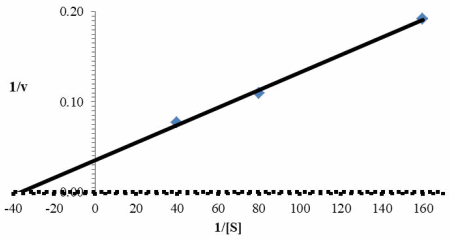
Temperature Impact on the TGM 1 Reaction Rate:

Most chemical reactions occur more rapidly as temperature increases. Higher temperatures give reactant molecules greater kinetic energy, leading to more effective collisions per unit of time. Enzymes are complex protein molecules whose catalytic activity arises from a precise and highly ordered tertiary structure [40]. This structure is shaped by specific amino acid R-groups, which create stereospecific substrate binding sites and a catalytic center. Consequently, a graph of reaction velocity versus temperature typically shows a peak, known as the optimum temperature, where the enzyme maintains consistent activity for at least the duration of the assay [41].

Figure 8 illustrates the impact of temperature on the reaction velocity of transglutaminase 1. The reaction velocity increases with temperature, ranging from 7°C to 57°C, peaking at 37°C. Beyond this point, there is a gradual decline in activity due to denaturation, resulting in a reduced interaction rate. These findings align with a previous study [39], which reported that transglutaminase 1 isolated from rat kidney tissues exhibited maximum activity at 37°C (Figure 5B).

Impact of Different Substrate Concentrations on TGM1 Activity with Determination of Km (Michaelis-Menten Constant) and Vmax (Maximum Velocity)

Figure 6 illustrates the effect of varying concentrations of the substrate 3,3′,5,5′-Tetramethylbenzidine (TMB) on the activity of partially purified TGM 1, identifying the optimum concentration that yields the Vmax. The results indicate that the reaction velocity of TGM 1 increases with substrate concentration until it reaches Vmax at the optimum level. Additionally, the enzyme activity correlates with the Michaelis-Menten equation, demonstrating a hyperbolic curve that reflects a direct relationship between enzyme activity and substrate concentration. The results indicated that enzyme saturation occurs at a substrate concentration of 0.5 mM/L for TMB. Analysis using the Lineweaver-Burk plot revealed that the Vmax value is 28.57 U/mL, and the Km value is 0.02739 mM/L.



pH	Temp. (°C)	Substrate Conc. (mM/L)	K_m (mM/L)	V_{max} (U/mL)
6.4	37	0. 5	0.02739	28.57

Figure 6. *Line Weaver–Burk plot for partially purified TGM 1 from blood serum. The optimum conditions for measuring the TGM activity enzyme partially purified from healthy human serum.*

The Km value is a key constant in enzyme studies. It represents the substrate concentration at which the reaction velocity is half of its maximum (Vmax). It indicates the enzyme's affinity for its substrate; a lower Km value indicates a higher affinity. Additionally, Km provides an estimate of the intracellular substrate concentration, while Vmax reflects the amount of active enzyme present.

Conclusion.

In conclusion, TGM 1 is a protein-modifying enzyme essential for many biological processes, including blood clotting, wound healing, and tissue homeostasis. It achieves this by facilitating protein cross-linking reactions through a coordinated enzymatic mechanism. The current work provides knowledge on developing transglutaminase inhibitors that can be used to treat some human diseases. It also provides insights into the use of this enzyme in industrial and biological applications, the food and pharmaceutical industries, and biotechnology applications. In addition, TGM has made significant contributions to biotechnological research, particularly as an antibody-drug conjugate. This area of research shows great promise for further development.

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