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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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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.

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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.

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SUBSTRATE SPECIFICITY OF B-GLUCOSIDASE FROM *YUCCA GLORIOSA* LEAVES

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Abstract.

Yucca gloriosa leaves contain a considerable number of steroid glycosides. In the plant's intact leaves, the biosynthesis of furostanol glycosides occurs, which are then converted into spirostanol glycosides by the action of β-glucosidase. Two forms of β-glucosidase are found in *Yucca gloriosa* leaves. Form I (molecular weight 32,000) hydrolyzes both oligofurostanosides, converting them into the corresponding oligospirostanosides, as well as the synthetic substrate 4-nitrophenyl-β-D-glucopyranoside. Form II (molecular weight 68,000) hydrolyzes only 4-nitrophenyl-β-D-glucopyranoside and does not cleave oligofurostanosides. Both enzymes have an optimum temperature of 37°C and an optimum pH of 6.3-6.5. Glucono-1,5-lactone inhibited the activity of both enzymes. The β-glucosidase of Form I shows higher affinity for its natural substrates than for the synthetic ones. The Km value for the β-glucosidase of Form I is 7.7 mM in relation to the total oligofurostanosides of the leaves of *Yucca gloriosa*, and 18.3 mM in relation to the synthetic substrate. The affinity for the natural substrates is higher than for the synthetic ones. The data received allow us to conclude that the affinity of Form I β-glucosidase from *Yucca gloriosa* leaves does not depend on either the structure of the oligosaccharide fragment linked to the nucleus or the structure of the aglycone (of steroid origin).

Keywords. β-glucosidase, *Yucca gloriosa*, oligofurostanosides, oligospirostanosides, substrate specificity.

Introduction.

The specificity of plant β-glucosidases is of great interest for both fundamental science and practical applications, as the problem of hydrolysis by endogenous glucosidases is closely related to the technological processes involved in the processing of important types of plant-based raw materials. Additionally, studying the metabolism of physiologically active glycosides can help us understand their role in plants [1-7].

Oligofurostanosides, which are water-soluble glycosides, are mostly concentrated in *Yucca gloriosa* leaves. When glucose is removed from the C26 atom of the steroid nucleus, a furan ring is simultaneously formed, converting oligofurostanosides into oligospirostanosides. β-Glucosidases from various sources, including endogenous β-glucosidases, can catalyze this process [8-11].

Oligofurostanosides are localized in the mesophyll cells of *Yucca gloriosa* leaves, whereas the hydrolyzing enzyme, β-glucosidase, is found in the epidermis. The drying process disrupts cell integrity, allowing the enzyme to react with furostanol, removing its side-chain carbohydrate. As a result, the open core closes, transforming the glycoside into its spiro form [12,13].

The steroidal sapogenin tigogenin was isolated from *Yucca gloriosa* leaves. Tigogenin was recognized by the Ministry of Medical Industry of the former Soviet Union as a cost-effective industrial raw material for the synthesis of 5α-steroidal hormonal drugs [14-17].

In this context, the present paper focuses on the study of the substrate specificity of β-glucosidase from *Yucca gloriosa* leaves, using both a synthetic substrate (4-nitrophenyl-β-D-glucopyranoside) and natural substrates (oligofurostanosides) isolated from various plants. These natural substrates differ from each other in the structure of the aglycone and the oligosaccharide part linked to the aglycone at the C3 atom.

Materials and Methods.

The middle-tier leaves of *Yucca gloriosa* plants growing in open ground in Tbilisi, Georgia, were used for this study. Oligofurostanosides from steam-fixed leaves of this plant were isolated from aqueous extracts using a previously proposed method, based on the coprecipitation of furostanol glycosides with proteins during salting-out with ammonium sulfate [18]. Oligospirostanosides from wild onion inflorescences were obtained using the same method. Deltoside, protodioscin, and protodeltofolin were isolated from the rhizomes and leaves of D. deltoidea [19]. Tomatoside, capsicoside, and melangoside preparations were also used as substrates.

The following reagents were used in the experiments: 4-nitrophenyl-β-D-glucopyranoside, 4-nitrophenyl-β-D-galactopyranoside, glucono-1,5-lactone, para-chloromercuribenzoate, p-dimethylaminobenzaldehyde, and DEAE-cellulose.

Determining β-glucosidase Activity: The cleavage rates of 4-nitrophenyl-β-D-glucopyranoside and 4-nitrophenyl-β-D-galactopyranoside were analyzed by measuring the amount of 4-nitrophenol released. The cleavage rate of oligofurostanosides was assessed by their reduction in the reaction mixture, using a colorimetric method based on a colour reaction with p-dimethylaminobenzaldehyde [20].

Composition of the incubation mixture: 0.2 ml of 0.05 M phosphate-citrate buffer, pH 6.3, containing 1 mM substrate, was added to 0.2 ml of cell-free extract. The mixture was incubated at 37°C for 10-15 minutes. The reaction was stopped by adding 2-3 ml of 96% ethanol. In the control samples, enzymes were inactivated by boiling for 5 minutes. One unit of β-glucosidase activity was defined as the amount of enzyme catalyzing the cleavage of 1 nmol of substrate per minute. Specific enzyme activity was expressed in nmol/mg of protein. Enzyme activity was measured in triplicate. Protein concentration was determined using a colorimetric method based on protein precipitation with amido black dye [21].

Extraction and Partial Purification of β-Glucosidase from *Yucca gloriosa* Leaves: Yucca leaves were ground in a mortar with 0.05 M phosphate-citrate buffer at a leaf-to-buffer ratio of 1:15. The homogenate was filtered through cloth and centrifuged at 1,000 g for 15 minutes. The supernatant was

treated with ammonium sulfate to achieve 30% salting-out. The solution was then centrifuged, and the resulting sediment was dissolved in the same buffer and dialyzed in distilled water overnight. Ammonium sulfate [$(NH_4)_2SO_4$] was added to the supernatant to achieve 80% salting-out, and the solution was processed as described above.

Chromatography on a DEAE-cellulose column was performed using NaCl in phosphate-citrate buffer (pH 6.3) with increasing ionic strength for elution. Fractions of 3-5 ml were collected. All enzyme isolation and purification steps were conducted at 4°C.

Determination of Km and Vmax Values: The dependence of the enzymatic reaction rate on substrate concentration was studied at optimal pH (6.3) and temperature (37°C) in 0.05 M phosphate-citrate buffer. p-Nitrophenyl-β-D-glucopyranoside was used at concentrations ranging from 0.15 to 6.5 mM, while oligofurostanoside concentrations ranged from 0.32 to 2 or 10 mM. The Vmax and Km values were calculated graphically using the double reciprocal (Lineweaver-Burk) method.

Results and Discussion.

Cell-free extracts from *Yucca gloriosa* leaves were capable of cleaving both p-nitrophenyl-β-D-glucopyranoside and oligofurostanosides. Experiments with these extracts revealed that the conversion of oligofurostanosides into oligospirostanosides occurred at a significantly higher rate than the cleavage of p-nitrophenylglucopyranoside. Notably, when the leaves are ground, oligofurostanosides are instantly cleaved by endogenous β-glucosidase to form oligospirostanosides.

Further analysis of β-glucosidase activity on both synthetic and natural substrates showed that two distinct forms of β-glucosidase are present in the leaves of this plant. Interestingly, these forms could be separated at the initial stage of fractionation using ammonium sulfate salting-out. Form I (30% fractionation with ammonium sulfate), with a molecular weight of 32,000, cleaves both natural substrates—oligofurostanosides—and the synthetic substrate p-nitrophenylglucopyranoside. Form II (30%-80% salting out with ammonium sulfate) cleaves only the synthetic substrate and does not act on oligofurostanosides. This suggests that Form II β-glucosidase may be involved in the metabolism of glycosides of non-steroid origin.

In this context, the properties of Form I β-glucosidase, which actively converts oligofurostanosides into oligospirostanosides, will be discussed further. The use of ammonium sulfate fractionation and DEAE-cellulose chromatography allowed for the purification of Form I β-glucosidase from *Yucca gloriosa* by 60-fold (see Table 1).

A partially purified preparation of β-glucosidase Form I was used for the experiments. The pH optimum for this enzyme is between 6.3 and 6.5, and the optimal temperature is 37°C.

A linear dependence of the accumulation of the reaction product on incubation time was observed: 15 minutes for oligofurostanosides as a substrate and 45 minutes for p-nitrophenyl-β-D-glucopyranoside. Consequently, the enzyme was incubated for 15 minutes during the analysis of the natural substrate and for 45 minutes during the analysis of the synthetic substrate. Notably, no inhibition of β-glucosidase Form I was observed due to excessive substrate or reaction product concentrations in the experiments.

Substrate specificity is one of the most important characteristics for understanding the physiological functions of enzymes. Despite numerous reports on plant glucosidases, the authentic specificity of these enzymes has been verified for only a few [22,23].

To study the specificity of β-glucosidase from *Yucca gloriosa* leaves, which is involved in the cleavage of oligofurostanosides, natural substrates (oligofurostanosides) biosynthesized in

Table 1. *Purification of Form I β-glucosidase from Yucca gloriosa Leaves.*

Purification stage	**Total protein mg**	**Total activity U**	**Specific activity U/mg protein**	**Degree of Purification**	**Enzyme yield %**
Original extract	1230	285216.5	231.8	1	100
30% fractionation by ammonium sulphate precipitation; dialysis	55	200210	3620	15	70
DEAE-cellulose chromatography	13	180120	13855	60	63

Table 2. *Substrate Specificity of Form I β-Glucosidase from Yucca gloriosa Leaves.*

Substrat	***Km*** **(mM)**	***Vmax*** **(μmol/min/mg protein)**
4-Nitrophenyl-β-D-glucopyranoside	18.3	3
Total oligofurostanosides of the leaves of Yucca gloriosa	7.7	390
Total oligofurostanosides of the inflorescence of Allium erubescence	7.9	384
Deltoside	7.6	396
Protodioscin	7.8	384
Protodeltofolin	7.8	384
Tomatoside	7.7	390
Capsicoside	8.0	378
Melangoside	8.2	372

various plants were used. These plants differ in both the structure of the aglycone, and the oligosaccharide part linked at the C3 atom of the steroid nucleus.

As shown in Table 2, the Km values of β-glucosidase Form I for various oligofurostanosides from some saponin-bearing plants were found to be consistent. In contrast, the Km value for the synthetic substrate, which is not biosynthesized in plants, differed significantly from these values.

The affinity of Form I β-glucosidase from *Yucca gloriosa* leaves for natural substrates is higher than for synthetic ones.

In contrast to the oligofurostanoside-specific β-glucosidase from Dioscorea deltoidea, which shows absolute specificity for oligofurostanosides and does not convert synthetic substrates [19], the enzyme from *Yucca gloriosa* leaves exhibits broader specificity. It can convert both oligofurostanoside glycosides and synthetic substrates, although its affinity for natural substrates is much higher.

Literature evidence supports the existence of two types of β-glucosidases with different substrate specificities. One type shows absolute specificity for natural substrates [24], while the other, like the enzyme in our study, demonstrates more flexible specificity [25]. For example, β-glucosidases from Cicer arietinum exhibit high specificity for natural isoflavone-7-glucosides [23]. These enzymes, found in roots, leaves, and epicotyls of the plant, exist in three different molecular forms. The Km values for natural isoflavone-7-glucosides and synthetic aryl-glucosides differ by a factor of a hundred for these enzymes. Despite these differences, the β-glucosidases from Cicer arietinum show minimal variation in substrate specificity.

Our research is clear evidence that the study of plant glucosidases should not be limited to synthetic substrates. Additionally, the β-glucosidase affinity for its substrate does not depend on the structure of the oligosaccharide fragment linked to the nucleus or on the structure of the aglycone (steroid origin), which is crucial for finding new raw materials for the synthesis of hormonal preparations

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მოკლე მიმოხილვა

იუკა დიდებული ფოთლები შეიცავს სტეროიდული გლიკოზიდების მნიშვნელოვან რაოდენობას. მცენარის ნედლ ფოთლებში მიმდინარეობს ფუროსტანოლური გლიკოზიდების ბიოსინთეზი, რომლებიც β-გლუკოზიდაზას მოქმედებით გადადის სპიროსტანოლურ ფორმაში. იუკა დიდებულის ფოთლებში აღმოჩენილ იქნა β-გლუკოზიდაზას ორი ფორმა. ფორმა I (მოლეკულური წონა 32000) აკატალიზებს როგორც ოლიგოფუროსტანოზიდების (გარდაქმნის მათ შესაბამის ოლიგოსპიროსტანოზიდებად), ასევე სინთეზური სუბსტრატის 4-ნიტროფენილ-β-D-გლუკოპირანოზიდის ჰიდროლიზს. ფორმა II (მოლეკულური წონა 68000) კი შლის მხოლოდ 4-ნიტროფენილ-β-D-გლუკოპირანოზიდს და არ მოქმედებს ოლიგოფუროსტანოზიდებზე. ფერმენტები მაქსიმალურ აქტივობას ამჟღავნებენ 370C, ოპტიმალური pH 6.3-6.5, გლუკონო-1.5-ლაქტონი იწვევს ორივე ფერმენტის ინჰიბირებას. β-გლუკოზიდაზას I ფორმა მეტ სწრაფვას ავლენს ბუნებრივი სუბსტრატების მიმართ, ვიდრე სინთეზურ სუბსტრატთან შედარებით. Km სიდიდე ბუნებრივ სუბსტრატებთან მიმართებაში I ფორმის β-გლუკოზიდაზასთვის არის 7,7mM, ხოლო სინთეზურ სუბსტრატთან მიმართებაში - 18,3mM.

საკვანძო სიტყვები: β-გლუკოზიდაზა, იუკა დიდებული, ოლიგოფუროსტანოზიდები, ოლიგოსპიროსტანოზიდები, სუბსტრატული სპეციფიკურობა.

Краткое изложение

Листья юкки славной содержат значительное количество стероидных гликозидов. В интактных листьях растения происходит биосинтез фуростаноловых гликозидов, которые под действием β-глюкозидазы превращаются в спиростаноловые гликозиды. В листьях юкки славной обнаружены две формы β-глюкозидазы. Форма I (молекулярная масса 32,000) гидролизует как олигофуростанозиды, превращая их в соответствующие олигоспиростанозиды, так и синтетический субстрат 4-нитрофенил-β-D-глюкопиранозид. Форма II (молекулярная масса 68,000) гидролизует только 4-нитрофенил-β-D-глюкопиранозид и не расщепляет олигофуростанозиды. Ферменты имеют оптимальную температуру 37°C и оптимум pH 6.3-6.5. Глюконо-1,5-лактон ингибирует активность обоих ферментов. Форма I β-глюкозидазы проявляет большее сродство к природным субстратам, чем к синтетическим. Значение Km по отношению к природным субстратам для формы I β-глюкозидазы составляет 7.7 мМ, а по отношению к синтетическому субстрату — 18.3 мМ.

Ключевые слова: β-глюкозидаза, юкка славная, олигофуростанозиды, олигоспиростанозиды, субстратная специфичность.