



## 糖苷水解酶——生物转化制备活性糖苷与苷元的有效工具

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**摘要:** 糖苷广泛存在于自然界, 具有多种药理活性, 是人类发现与生产药物的重要来源。糖苷中糖链部分的组成与其药理活性密切相关, 改变糖苷分子中的糖链结构能改变糖苷的药理活性, 为开发药物提供更多的化合物资源。糖苷水解酶修饰糖链具有效率高、成本低、污染小等优点, 被广泛应用于活性糖苷与苷元的制备。本文系统地总结了糖苷水解酶转化制备活性糖苷与苷元的研究进展, 为研究糖苷酶生物转化制备活性化合物提供数据资源, 为相关的研究和生产提供有用的文献资料。

**关键词:** 糖苷, 糖苷水解酶, 生物转化

糖苷是糖或糖衍生物的半缩醛羟基与另一非糖物质缩合而成的化合物。天然糖苷主要来自植物的次生代谢产物。出于自身的防御需要, 植物合成大量的糖苷<sup>[1]</sup>, 为人类提供了研究、开发和生产药物候选化合物的丰富资源。糖苷类化合物具有祛风湿、抑菌、抗炎、抗肿瘤、免疫调节、改善呼吸道及消化道等多种重要的药理活性<sup>[2-4]</sup>, 目前市场销售的药物约有 70%以上与糖苷类化合物相关。随着糖工程的兴起<sup>[5]</sup>, 以及分离纯化和鉴定技术的发展, 对于植物中糖苷类有效成分的研究将更为深入, 应用将更加广泛。根据结构不同, 糖苷有多种分类方式。按苷原子的不同可分为氧糖苷、硫糖苷、碳糖苷和氮糖苷, 其中氧糖苷最为常见。糖苷结构多样性产生多种药理活性<sup>[6]</sup>。糖苷

的药理活性不仅与苷元有关, 与糖链部分也密切相关。糖苷中糖链的单糖组成、糖苷键构型、糖基连接方式等都影响糖苷的活性及代谢途径<sup>[7]</sup>, 有些糖苷通过水解生成苷元, 产生更好的药理活性, 如槲皮素、京尼平等。修饰糖苷分子中的糖链部分, 研究其构效关系, 对发现新型的糖苷药物具有重要意义。利用酸或碱催化等化学法能够水解糖链, 也可以采用微生物以及酶催化等生物学方法水解糖链。化学法水解糖苷有时会产生较多的副产物, 同时容易引起环境污染, 而生物学方法可能克服这些问题。因此, 糖苷水解酶被认为是制备活性糖苷与苷元潜在的有效工具。本文系统地总结了糖苷水解酶转化制备活性糖苷与苷元的研究进展。

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## 1 糖苷水解酶概述

糖苷水解酶是真正意义上的水解酶,不需要任何辅酶和辅因子<sup>[8]</sup>。糖苷水解酶数量众多,广泛存在于细菌、真菌、植物种子和动物器官中,根据氨基酸残基序列和结构的相似性可划分为不同家族,目前已有 145 个糖苷水解酶家族被报道<sup>[9]</sup>。

糖苷水解酶的性质和功能一直是糖生物学领域研究的热点。我国对糖苷水解酶的研究始于 20 世纪 50 年代末,张树政院士等分析比较了酒精工业中不同曲霉的淀粉酶系的组成,在国内首先用纸电泳法分离测定了淀粉酶。1966 年从 150 株根霉中筛选出 3 株产高活力淀粉葡萄糖苷水解酶的根霉,并对其酶活性质进行初步探究<sup>[10]</sup>。80 年代开始,张树政院士开展了多种糖苷酶的基础和应用研究,大力倡导糖生物学和糖工程前沿计划。作为糖生物学的奠基人之一,张树政院士长期致力于微生物生物化学和糖生物学的研究,在糖苷酶的结构与功能、糖生物学和糖生物工程学中取得了显著的成就,为我国的酶制剂工业及酶学的发展做出了奠基性的贡献。

随着糖类化合物在生物学领域的重要性越发凸显,有关糖苷水解酶的研究与应用也越来越受到关注。目前,糖苷水解酶主要是通过分离纯化和分子克隆技术从微生物或动植物体中获得的。于巍等<sup>[11]</sup>从土壤微生物中筛选得到 *Enterobacter cloacae* YW2112 菌株,从中分离纯化的糖苷酶能特异性水解神经节苷脂中连接神经酰胺和寡糖链之间的糖苷键,是研究神经节苷脂结构与功能的重要工具。张树政等<sup>[12]</sup>从巨大芽孢杆菌的全基因组 DNA 文库中重组构建了  $\beta$ -淀粉酶,经氨基酸序列比较分析发现该酶依次由信号肽域、糖基水解酶催化功能域和淀粉结合域 3 个功能结构域组成。

随着分子生物学技术的不断发展,随机突变和定向进化在改良糖苷水解酶中应用广泛。中国科学院微生物研究所唐双焱等<sup>[13]</sup>通过 DNA 重组技术提高了芽孢杆菌糖化酶的热稳定性,并预测出突变酶热稳定性提高的机理。目前,糖苷水解酶的制备和工业化应用已取得显著成效。糖苷水解酶易通过发酵生产获得,随着基因工程和蛋白质工程的快速发展,重组糖苷水解酶由于具有表达量高、易纯化等特点被广泛使用。酶法转化过程条件温和、专一性好、产量高、绿色环保,因此糖苷水解酶成为转化制备活性糖苷与苷元的有效工具。

## 2 氧糖苷的生物转化

目前,糖苷水解酶被应用于多种活性糖苷与苷元制备的研究。其中,氧糖苷中的皂苷和黄酮苷的酶法制备研究最为广泛。经过科研工作者们多年的努力,糖苷水解酶制备活性糖苷与苷元取得了诸多可喜的成果<sup>[14-16]</sup>。

### 2.1 皂苷的生物转化

皂苷是苷元为三萜或甾烷类化合物的一类糖苷,是人参、甘草和薯蓣等许多中草药的有效成分之一(主要皂苷结构见图 1),具有抗氧化、抗肿瘤、抗衰老、增强免疫力等多种功效。文献中对人参皂苷生物转化研究报道较多。目前,分离鉴定出的 150 多种人参皂苷,在人参中含量不同,人参皂苷 Rb1、Rb2、Rc、Rd、Re 和 Rg1 等含量高达 80%,而人参皂苷 Rg3、Rh2、F2 和 Compound K (C-K)等稀有皂苷含量很少甚至没有<sup>[17]</sup>。研究表明,有些稀有皂苷具有较好的药理学活性<sup>[18]</sup>。但由于含量低,制备和生产受到限制。同一类型的人参皂苷具有相同的苷元,只是糖链不同。稀有人参皂苷与含量较高的同种类型的皂苷往往只差

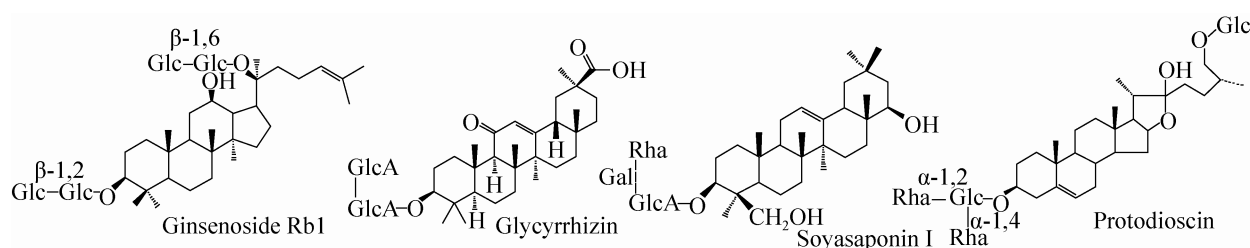


图 1. 主要皂苷结构示意图

Figure 1. Structure of main saponins.

别 2–3 个糖基。因此, 可以酶催化水解高含量皂苷转化制备相同类型的活性稀有皂苷。

不同的糖苷水解酶具有不同的选择性, 水解人参皂苷的路径也不相同。如表 1 所示, 利用不同的糖苷水解酶可以制备不同的稀有人参皂苷。通过水解人参皂苷 Rb1、Rb2、Rb3 和 Rc 的 C-20 位外侧糖基可以制备人参皂苷 Rd。从中国白玉蜗牛 (China white jade snail)<sup>[19]</sup>和 *Thermus caldophilus*<sup>[20]</sup>中分离纯化的  $\beta$ -葡萄糖苷水解酶能够实现人参皂苷 Rb1 到 Rd 的转化, Kim 等<sup>[21]</sup>利用分子克隆技术从土壤微生物中获得能够将人参皂苷 Rb1 转化为 Rd 的重组糖苷水解酶。随后, 科研工作者从 *Thermotoga thermarum*<sup>[22]</sup>和 *Bifidobacterium longum* H-1<sup>[23]</sup>中克隆得到葡萄糖苷酶, 提高了转化制备人参皂苷 Rd 的效率。利用重组技术从 *Flavobacterium johnsoniae*<sup>[24]</sup>和 *Thermus thermophilus*<sup>[25]</sup>中获得的葡萄糖苷酶不仅可以将人参皂苷 Rb1 转化成 Rd, 同样可以水解绞股蓝皂苷 XVII (G17) 的 C-20 位糖链, 生成人参皂苷 F2。除了葡萄糖苷酶外, 从人参根<sup>[26]</sup>和 *Leuconostoc* sp.<sup>[27]</sup>中获得了能转化人参皂苷 Rc 为 Rd 的  $\alpha$ -L-阿拉伯呋喃糖苷水解酶。从 *Bifidobacterium breve*<sup>[28]</sup>和 *Bifidobacterium longum*<sup>[29]</sup>中得到  $\alpha$ -L-阿拉伯呋喃糖苷水解酶和  $\alpha$ -L-阿拉伯吡喃糖苷水解酶, 能够转化人参皂苷 Rc 和 Rb2 成为 Rd。有文献报道, *Caldicellulosiruptor saccharolyticus*<sup>[30]</sup>

和 *Rhodanobacter ginsenosidimutans*<sup>[31]</sup>中的  $\alpha$ -L-阿拉伯呋喃糖苷水解酶不仅可以水解人参皂苷 Rc 为 Rd, 同时可以将 Compound Mc<sub>1</sub> (C-Mc<sub>1</sub>) 转化成 F2。Yu 等<sup>[32]</sup>从 *Aspergillus* 中分离纯化的糖苷水解酶则可将人参皂苷 Rb1、Rb2、Rb3 和 Rc 全部转化为 Rd。一些糖苷水解酶能够完全水解二醇型人参皂苷 Rb1、Rb2、Rb3、Rc 和 Rd 等分子中 C-20 位的糖链, 生成人参皂苷 Rg3, 使得 Rg3 能够大规模生产, 被开发成为抗肿瘤的药物。*Paecilomyces bainier*<sup>[33]</sup>和 *Microbacterium esteraromaticum*<sup>[34]</sup>中的葡萄糖苷酶能直接将人参皂苷 Rb1 水解成 Rg3, 而从 *Microbacterium esteraromaticum*<sup>[35]</sup>中分离纯化的葡萄糖苷酶能将人参皂苷 Rb2 水解成 Rg3。利用分子克隆技术从 *Pseudonocardia*<sup>[36]</sup>中克隆得到的重组糖苷水解酶可转化人参皂苷 Rb1、Rb3 和 Rd 制备 Rg3。同样, 水解人参皂苷中 C-3 位的糖基也可制备一系列活性稀有人参皂苷。从 *Sphingomonas*<sup>[37]</sup>和 *Sphingopyxis alaskensis*<sup>[38]</sup>中克隆得到的重组葡萄糖苷酶能水解人参皂苷 Rb1、Rb2、Rc、Rd 和 Rg3 分子中 C-3 位糖链外侧的葡萄糖, 相应地制备 G17、Compound O (C-O)、C-Mc<sub>1</sub>、F2 和 Rh2。有些糖苷酶则能直接水解 C-3 位内侧葡萄糖基, 如来源于 *Terrabacter ginsenosidimutans*<sup>[39]</sup>和 *Esteya vermicola*<sup>[40]</sup>的葡萄糖苷酶能水解人参皂苷 Rb1、Rb2、Rb3、Rc 和 Rd 分子 C-3 位的糖链,

表 1. 糖苷水解酶转化人参皂苷

Table 1. Biotransformation of ginsenosides by glycosidase

Product	Substrate	Reaction	Organism	Reference
Rd	Rb1	$\beta$ -Glucosidase	China white jade snail	Luan <sup>[19]</sup>
Rd	Rb1	$\beta$ -Glucosidase	<i>Thermus caldophilus</i>	Son <sup>[20]</sup>
Rd	Rb1	$\beta$ -Glucosidase	Uncultured bacteria	Kim <sup>[21]</sup>
Rd	Rb1	$\beta$ -Glucosidase	<i>Thermotoga thermarum</i>	Zhao <sup>[22]</sup>
Rd	Rb1	$\beta$ -Glucosidase	<i>Bifidobacterium longum</i> H-1	Jung <sup>[23]</sup>
Rd	Rb1	$\beta$ -Glucosidase	<i>Flavobacterium johnsoniae</i>	Hong <sup>[24]</sup>
Rd	Rb1	$\beta$ -Glucosidase	<i>Thermus thermophilus</i>	Shin <sup>[25]</sup>
Rd	Rb1	$\beta$ -Glucosidase	<i>Penicillium oxalicum</i>	Gao <sup>[47]</sup>
Rd	Rb1	$\beta$ -Glucosidase	<i>Cladosporium fulvum</i>	Gao <sup>[48]</sup>
Rd	Rc	$\alpha$ -L-Arabinofuranosidase	<i>Panax ginseng</i>	Zhang <sup>[26]</sup>
Rd	Rc	$\alpha$ -L-Arabinofuranosidase	<i>Leuconostoc</i>	Liu <sup>[27]</sup>
Rd	Rc	$\alpha$ -L-Arabinofuranosidase	<i>Bifidobacterium breve</i>	Shin <sup>[28]</sup>
Rd	Rc	$\alpha$ -L-Arabinofuranosidase	<i>Bifidobacterium longum</i>	Lee <sup>[29]</sup>
Rd	Rc	$\alpha$ -L-Arabinofuranosidase	<i>Caldicellulosiruptor saccharolyticus</i>	Shin <sup>[30]</sup>
Rd	Rc	$\alpha$ -L-Arabinofuranosidase	<i>Rhodanobacter ginsenosidimutans</i>	An <sup>[31]</sup>
Rd	Rb2	$\alpha$ -L-Arabinopyranosidase	<i>Bifidobacterium breve</i>	Shin <sup>[28]</sup>
Rd	Rb2	$\alpha$ -L-Arabinopyranosidase	<i>Bifidobacterium longum</i>	Lee <sup>[29]</sup>
Rd	Rb1/Rb2/Rb3/Rc	Glycosidase	<i>Aspergillus</i>	Yu <sup>[32]</sup>
Rg3	Rb1	$\beta$ -Glucosidase	<i>Paecilomyces bainier</i>	Yan <sup>[33]</sup>
Rg3	Rb1	$\beta$ -Glucosidase	<i>Microbacterium esteraromaticum</i>	Quan <sup>[34]</sup>
Rg3	Rb2	$\beta$ -Glucosidase	<i>Microbacterium esteraromaticum</i>	Quan <sup>[35]</sup>
Rg3	Rb1/Rb3/Rd	$\beta$ -Glucosidase	<i>Pseudonocardia</i>	Du <sup>[36]</sup>
G17	Rb1	$\beta$ -Glucosidase	<i>Sphingomonas</i>	Wang <sup>[37]</sup>
G17	Rb1	$\beta$ -Glucosidase	<i>Sphingopyxis alaskensis</i>	Shin <sup>[38]</sup>
G17	Rb1	$\beta$ -Glucosidase	<i>Cellulosimicrobium cellulans</i>	Yuan <sup>[49]</sup>
G75	Rb1	$\beta$ -Glucosidase	<i>Terrabacter ginsenosidimutans</i>	An <sup>[39]</sup>
G75	Rb1	$\beta$ -Glucosidase	<i>Esteya vermicola</i>	Hou <sup>[40]</sup>
F2	G17	$\beta$ -Glucosidase	<i>Flavobacterium johnsoniae</i>	Hong <sup>[24]</sup>
F2	G17	$\beta$ -Glucosidase	<i>Thermus thermophilus</i>	Shin <sup>[25]</sup>
F2	C-Mc <sub>1</sub>	$\alpha$ -L-Arabinofuranosidase	<i>Caldicellulosiruptor saccharolyticus</i>	Shin <sup>[30]</sup>
F2	C-Mc <sub>1</sub>	$\alpha$ -L-Arabinofuranosidase	<i>Rhodanobacter ginsenosidimutans</i>	An <sup>[31]</sup>
F2	Rd	$\beta$ -Glucosidase	<i>Cellulosimicrobium cellulans</i>	Yuan <sup>[49]</sup>
F2	Rb1/Rb2/Rc	$\beta$ -Glucosidase	<i>Arthrobacter chlorophenolicus</i>	Park <sup>[41]</sup>
Rh2	Rg3	$\beta$ -Glucosidase	<i>Sphingopyxis alaskensis</i>	Shin <sup>[38]</sup>
CK	Rd	$\beta$ -Glucosidase	<i>Terrabacter ginsenosidimutans</i>	An <sup>[39]</sup>
CK	Rd	$\beta$ -Glucosidase	<i>Esteya vermicola</i>	Hou <sup>[40]</sup>
CK	Rb1	$\beta$ -Glucosidase	<i>Fusobacterium</i> K-60	Park <sup>[42]</sup>
CK	Rb1	$\beta$ -Glucosidase	endophytic fungi GE 17-18	Fu <sup>[43]</sup>
CK	Rb1/Rb2	$\beta$ -Glucosidase	<i>Sulfolobus acidocaldarius</i>	Noh <sup>[44]</sup>
CK	Rb1/Rb2/Rb3/Rc	$\beta$ -Glucosidase	<i>Aspergillus niger</i>	Liu <sup>[45]</sup>
CK	Rb1/Rb2	$\beta$ -Glucosidase	<i>Microbacterium esteraromaticum</i>	Quan <sup>[46]</sup>
C-O	Rb2	$\beta$ -Glucosidase	<i>Cellulosimicrobium cellulans</i>	Yuan <sup>[49]</sup>

(待续)

(续表 1)

C-Y	Rb2	$\beta$ -Glucosidase	<i>Terrabacter ginsenosidimitans</i>	An <sup>[39]</sup>
C-Mc	Rc	$\beta$ -Glucosidase	<i>Terrabacter ginsenosidimitans</i>	An <sup>[39]</sup>
C-Mc <sub>1</sub>	Rc	$\beta$ -Glucosidase	<i>Cellulosimicrobium cellulans</i>	Yuan <sup>[49]</sup>
C-Mx	Rb3	$\beta$ -Glucosidase	<i>Terrabacter ginsenosidimitans</i>	An <sup>[39]</sup>
Rg2	Re	$\beta$ -Glucosidase	<i>Microbacterium esteraromaticum</i>	Quan <sup>[50]</sup>
Rg2	Re	$\beta$ -Glucosidase	<i>Mucilaginibacter</i>	Cui <sup>[51]</sup>
Rg2	Re	$\beta$ -Glucosidase	<i>Pseudonocardia</i>	Du <sup>[36]</sup>
Rh1	Rg1	$\beta$ -Glucosidase	<i>Microbacterium esteraromaticum</i>	Quan <sup>[50]</sup>
Rh1	Rf	$\beta$ -Glucosidase	<i>Pyrococcus furiosus</i>	Oh <sup>[52]</sup>
Rh1	Rf	$\beta$ -Glucosidase	<i>Aspergillus niger</i>	Ruan <sup>[53]</sup>
Rh1	Rg2	$\alpha$ -L-Rhamnosidase	<i>Absidia</i>	Yu <sup>[54]</sup>
Rh1	R2	$\beta$ -Xylosidase	<i>Thermoanaerobacterium</i>	Shin <sup>[55]</sup>
F1	Rg1	$\beta$ -Glucosidase	<i>Fusarium moniliforme</i>	Kim <sup>[56]</sup>
F1	Rg1	$\beta$ -Glucosidase	<i>Penicillium sclerotiorum</i>	Wei <sup>[57]</sup>
F1	Rg1	$\beta$ -Glucosidase	<i>Sanguibacter keddiei</i>	Kim <sup>[58]</sup>

G17: gypenoside XVII; G75: gypenoside LXXV; C-O: compound O; C-Y: compound Y; C-Mc<sub>1</sub>: compound Mc<sub>1</sub>; C-Mc: compound Mc; C-Mx: compound Mx; C-K: compound K.

生成相应的皂苷 LXXV (G75)、Compound Y (C-Y)、Compound Mx (C-Mx)、Compound Mc (C-Mc)和 C-K。此外,一些糖苷水解酶能够同时水解二醇型人参皂苷中 C-20 和 C-3 位糖基。从 *Arthrobacter chlorophenolicus*<sup>[41]</sup>中克隆得到的重组葡萄糖苷酶能将人参皂苷 Rb1、Rb2 和 Rc 转化成 F2。*Fusobacterium K60*<sup>[42]</sup>、endophytic fungi GE 17-18<sup>[43]</sup>、*Sulfolobus acidocaldarius*<sup>[44]</sup>、*Aspergillus niger*<sup>[45]</sup>和 *Microbacterium esteraromaticum*<sup>[46]</sup>中的糖苷水解酶则能够水解人参皂苷 Rb1 制备 C-K。

三醇型人参皂苷中 C-6 和 C-20 位的糖基也能被糖苷水解酶水解。人参皂苷 Rg2 可通过糖苷酶水解 Re 分子中的 C-20 的葡萄糖获得,利用重组技术从 *Microbacterium esteraromaticum*<sup>[50]</sup>、*Mucilaginibacter*<sup>[51]</sup>和 *Pseudonocardia*<sup>[36]</sup>中克隆得到的重组葡萄糖苷酶不仅可以将人参皂苷 Re 转化成 Rg2,也可以将人参皂苷 Rg1 转化成 Rh1。通过水解人参皂苷 Rf、Rg2 和 R2 的 C-6 位外侧的葡萄糖、鼠李糖和木糖均可以转化制备 Rh1<sup>[52-55]</sup>。与人参皂苷 Rh1 不同,人参皂苷 F1 只有一个葡萄糖连在其苷元的 C-20 位。

*Fusarium moniliforme*<sup>[56]</sup>、*Penicillium sclerotiorum*<sup>[57]</sup>和 *Sanguibacter keddiei*<sup>[58]</sup>中的葡萄糖苷酶可以特异性水解人参皂苷 Rg1 的 C-6 位葡萄糖,生成人参皂苷 F1。

本实验室旨在研究长白山特色天然药物的有效成分及其化学性质和生物学活性,近年来,在人参皂苷生物转化研究中取得了一些进展。在真菌研究中,对植物病原真菌转化人参皂苷的效率和转化产物进行了系统分析<sup>[59]</sup>,从 *Penicillium oxalicum*<sup>[47]</sup>和 *Cladosporium fulvum*<sup>[48]</sup>中分离纯化出转化人参皂苷 Rb1 为 Rd 的糖苷水解酶。对于细菌的研究,本实验室从长白山林地的人参种植土壤中筛选出 24 种能够转化人参皂苷的细菌,并对它们的转化能力和转化产物进行了系统分析,发现 *Cellulosimicrobium cellulans* sp. 21 水解能力最强<sup>[60]</sup>。从中克隆获得的重组糖苷水解酶 CcBgl1A 能够特异性水解人参二醇型皂苷 C-3 位外侧连接的葡萄糖,将人参皂苷 Rb1、Rb2、Rc 和 Rd 完全水解为 G17、C-O、C-Mc<sub>1</sub> 和 F2<sup>[49]</sup>。目前,我们通过组合使用多种重组糖苷水解酶实现了百克级

规模制备 Rg3、Rh2、C-K、Rg2 和 F1 等多种稀有人参皂苷，为人参的开发利用和工业生产奠定了基础。

糖苷水解酶不仅应用于转化制备活性稀有人参皂苷，同时也被广泛应用于水解修饰甘草、大豆和薯蓣等皂苷(表 2)。从 *Streptococcus* LJ-22<sup>[61]</sup> 和 *Penicillium purpurogenum* Li-3<sup>[62]</sup> 中分离纯化的葡萄糖醛酸苷酶能够水解甘草皂苷生成单葡萄糖醛酸甘草酸，且没有副产物甘草次酸的生成。Morana 等<sup>[63]</sup> 使用来源于 *Aspergillus niger* 的葡萄糖醛酸苷酶，可将甘草皂苷彻底水解生成甘草次酸。从 *Aspergillus oryzae*<sup>[64]</sup> 中分离纯化的大豆皂苷水解酶能够水解大豆皂苷 I 生成大豆皂醇 B。而 *Neocosmospora vasinfecta*<sup>[65]</sup> 中的一种新大豆皂苷水解酶能将大豆皂苷 I、II、III 都转化成大豆皂醇 B，为制备具有抗氧化和调血脂的大豆皂苷提供了有效的工具。在甾体皂苷中，对薯蓣皂苷糖链水解修饰的研究比较系统。Inoue 等<sup>[66]</sup> 从 *Costus speciosus* 中分离纯化出能够水解原薯蓣皂苷生成薯蓣皂苷的葡萄糖苷酶。Liu 等<sup>[67]</sup> 从 *Aspergillus oryzae* 中分离纯化并克隆得到重组原薯蓣皂苷水

解酶，该酶能水解原薯蓣皂苷中葡萄糖基和  $\alpha$ -1,4 鼠李糖基，生成薯蓣次苷 III。Feng 等<sup>[68]</sup> 从 *Curvularia lunata* 中分离纯化的  $\alpha$ -L-鼠李糖苷酶能够水解薯蓣皂苷中的  $\alpha$ -1,2 鼠李糖基，生成薯蓣次苷 V。Qian 等<sup>[69]</sup> 从新鲜牛肝中分离纯化出一种  $\alpha$ -L-鼠李糖苷酶，该酶能够水解薯蓣皂苷中的  $\alpha$ -1,2 和  $\alpha$ -1,4 两个鼠李糖基，生成葡萄糖基-薯蓣皂苷元。Fu 等<sup>[70]</sup> 则从 *Absidia* 中分离纯化出能将薯蓣皂苷彻底水解为薯蓣皂苷元的薯蓣皂苷水解酶。

## 2.2 黄酮苷的生物转化

黄酮类化合物是植物体内广泛分布的多酚类物质，多以糖苷形式存在。研究发现，具有生物活性的黄酮类化合物是食用植物中最重要的有效成分，具有护肝、抗氧化、抗肿瘤、抗病毒等多种药理学活性<sup>[71-72]</sup>，其活性与结构密切相关<sup>[73]</sup>。由于大部分黄酮苷难以通过小肠壁进入到血液中，生物利用度低，因此对天然黄酮化合物进行结构修饰成为目前研究的热点。利用糖苷水解酶水解黄酮苷的糖基成为一种提高黄酮化合物活性的有效途径(表 3)。常见的黄酮苷包括芦丁、橙皮

表 2. 糖苷水解酶转化其他皂苷

Table 2. Biotransformation of other saponins by glycosidase

Product	Substrate	Reaction	Organism	Reference
GAMG	Glycyrrhizin	$\beta$ -Glucuronidase	<i>Streptococcus</i>	Park <sup>[61]</sup>
GAMG	Glycyrrhizin	$\beta$ -Glucuronidase	<i>Penicillium purpurogenum</i>	Zou <sup>[62]</sup>
Glycyrrhetic acid	Glycyrrhizin	$\beta$ -Glucuronidase	<i>Aspergillus niger</i>	Morana <sup>[63]</sup>
Soyasapogenol B	Soyasaponin I	Soybean saponin hydrolase	<i>Aspergillus oryzae</i>	Kudou <sup>[64]</sup>
Soyasapogenol B	Soyasaponin	Soybean saponin hydrolase	<i>Neocosmospora vasinfecta</i>	Watanabe <sup>[65]</sup>
Dioscin	Protodioscin	$\beta$ -Glucosidase	<i>Costus speciosus</i>	Inoue <sup>[66]</sup>
Progenin III	Protodioscin	Protodioscin-glycosidase	<i>Aspergillus oryzae</i>	Liu <sup>[67]</sup>
Progenin V	Dioscin	$\alpha$ -L-Rhamnosidase	<i>Curvularia lunata</i>	Feng <sup>[68]</sup>
Diosgenyl-glucoside	Dioscin	$\alpha$ -L-Rhamnosidase	Bovine liver	Qian <sup>[69]</sup>
Diosgenin	Dioscin	Dioscin-glycosidase	<i>Absidia</i>	Fu <sup>[70]</sup>

GAMG: Glycyrrhetic acid mono-glucuronide.

表 3. 糖苷水解酶转化黄酮苷

Table 3. Biotransformation of flavonoid glycosides by glycosidase

Product	Substrate	Reaction	Organism	Reference
Isoquercitrin, Prunin, Hesperetin glucoside	Rutin, Naringin, Hesperidin	$\alpha$ -Rhamnosidase	<i>Aspergillus niger</i>	Manzanares <sup>[74]</sup>
		$\alpha$ -Rhamnosidase	<i>Aspergillus nidulans</i>	Manzanares <sup>[75]</sup>
	$\alpha$ -Rhamnosidase	<i>Aspergillus aculeatus</i>	Manzanares <sup>[76]</sup>	
	$\alpha$ -Rhamnosidase	<i>Clostridium stercorarium</i>	Kaur <sup>[77]</sup>	
Quercetin	Rutin	$\beta$ -Rutinosidase	<i>Penicillium rugulosum</i>	Narikawa <sup>[78]</sup>
Quercetin	Rutin	$\beta$ -Glycosidase	<i>Penicillium decumbens</i>	Mamma <sup>[79]</sup>
Quercetin	Rutin	$\beta$ -Heterodisaccharidase	<i>Fagopyri herba</i>	Baumgertel <sup>[80]</sup>
Quercetin	Rutin	$\beta$ -Rutinosidase	<i>Aspergillus niger</i>	Šimčíková <sup>[81]</sup>
Naringenin	Naringin	Naringinase	<i>Aspergillus niger</i>	Thammawat <sup>[82]</sup>
Naringenin, Quercetin	Naringin, Rutin	Naringinase	<i>Aspergillus niger</i>	Puri <sup>[83]</sup>
Hesperetin	Hesperidin	Diglycosidase	<i>Acremonium</i>	Piñuel <sup>[84]</sup>
Hesperetin	Hesperidin	Diglycosidase	<i>Actinoplanes missouriensis</i>	Neher <sup>[85]</sup>
Daidzein	Daidzin	$\beta$ -Glucosidase	Unculturable microbes	Li <sup>[86]</sup>
Daidzein	Daidzin	$\beta$ -Glucosidase	<i>Sulfolobus solfataricus</i>	Kim <sup>[87]</sup>
Daidzein	Daidzin	$\beta$ -Glucosidase	<i>Aspergillus oryzae</i>	Horri <sup>[88]</sup>
Daidzein	Daidzin	$\beta$ -Glucosidase	<i>Pyrococcus furiosus</i>	Yeom <sup>[89]</sup>
Daidzein, Genistein	Daidzin, Genistin	$\beta$ -Glucosidase	<i>Bacillus subtilis</i>	Xue <sup>[90]</sup>
		$\beta$ -Glucosidase	<i>Thermotoga maritima</i>	Kuo <sup>[91]</sup>
Daidzein, Genistein, Glycitein	Daidzin, Genistin, Glycitin	$\beta$ -Glucosidase	<i>Dalbergia</i>	Chuankhayan <sup>[92]</sup>
		$\beta$ -Glucosidase	<i>Bacteroides thetaiotaomicron</i>	Byun <sup>[93]</sup>
Baicalein	Baicalin	$\beta$ -Glucuronidase	<i>Scutellaria viscidula</i>	Zhang <sup>[94]</sup>
Tilianin	Linarin	Naringinase	<i>Penicillium decumbens</i>	Cui <sup>[95]</sup>
Butin	Butrin	$\beta$ -Glucosidase	Almond	Jassbi <sup>[96]</sup>
Phloretin	Phlorizin	$\beta$ -Glycosidase	Sheep small intestine	Day <sup>[97]</sup>

苷和柚皮苷, 它们的糖基部分通常为芸香糖( $\alpha$ -1,6 连接的鼠李糖和葡萄糖)和新橙皮糖( $\alpha$ -1,2 连接的鼠李糖和葡萄糖), 因此糖苷水解酶对其进行水解修饰的方式主要包括外切和内切两种。从 *Aspergillus niger*<sup>[74]</sup>和 *Aspergillus nidulans*<sup>[75]</sup>中分离纯化出能水解  $\alpha$ -1,2 和  $\alpha$ -1,6 鼠李糖苷键的  $\alpha$ -鼠李糖苷酶, 该酶能水解芦丁、柚皮苷和橙皮苷, 分别生成异栎素、洋李苷和橙皮素葡萄糖苷。从 *Aspergillus aculeatus*<sup>[76]</sup>和 *Clostridium stercorarium*<sup>[77]</sup>中克隆得到的重组  $\alpha$ -鼠李糖苷酶也具有水解黄酮苷中鼠李糖的活性。对于水解上述三种黄酮苷,

除了外切糖苷酶外, 内切糖苷酶也有大量研究报道。从 *Penicillium rugulosum*<sup>[78]</sup>、*Penicillium decumbens*<sup>[79]</sup>和 *Fagopyri herba*<sup>[80]</sup>中分离纯化出的二糖苷酶, 以及从 *Aspergillus niger*<sup>[81]</sup>中克隆获得的重组芦丁酶, 均可水解芦丁生成抗氧化活性更好的槲皮素。从 *Aspergillus niger* BCC 25166<sup>[82]</sup>中可分离纯化出能水解柚皮苷生成柚皮苷元的柚皮苷酶, *Aspergillus niger* 1344<sup>[83]</sup>中的柚皮苷酶可同时水解柚皮苷和芦丁, 分别生成柚皮苷元和槲皮素, 但不能水解橙皮苷。而 *Acremonium* sp. DSM24697<sup>[84]</sup>和 *Actinoplanes missouriensis*<sup>[85]</sup>中的二糖苷酶能

水解橙皮苷中的新橙皮糖，生成高活性的橙皮素产物。

异黄酮是黄酮类化合物的一种，主要存在于豆科植物中，有助于疾病的预防 and 人类健康。大豆异黄酮的主要成分为大豆苷、大豆苷元、染料木苷、染料木素、黄豆黄素和黄豆黄素苷元，其中去糖基化的苷元具有更好的生物活性。从红树林土壤的基因文库<sup>[86]</sup>、*Sulfolobus solfataricus*<sup>[87]</sup>、*Aspergillus oryzae*<sup>[88]</sup>和 *Pyrococcus furiosus*<sup>[89]</sup>中克隆获得能水解大豆苷生成大豆苷元的重组  $\beta$ -葡萄糖苷酶；从 *Thermotoga maritima*<sup>[90]</sup>和 *Bacillus subtilis*<sup>[91]</sup>中克隆得到的重组  $\beta$ -葡萄糖苷酶能水解大豆苷和染料木苷生成大豆苷元和染料木素；从 *Dalbergia*<sup>[92]</sup>中分离纯化和从 *Bacteroides thetaiotaomicron*<sup>[93]</sup>中克隆重组的糖苷酶可以水解大豆苷、染料木苷和黄豆黄素生成大豆苷元、染料木素和黄豆黄素苷元。

糖苷水解酶在其他黄酮苷水解中也有报道和应用(表 3)。研究表明，黄芩苷具有抗肿瘤、抗感染等功效。从 *Scutellaria viscidula* Bge<sup>[94]</sup>中分离纯化的  $\beta$ -葡萄糖苷酶能够水解黄芩苷生成黄芩素，

去糖基化的产物黄芩素具有更好的药理学活性。田蓟苷也是一种稀有黄酮苷，具有抗高血压和镇静等活性，但难以通过直接提取和化学合成的方式获得。Cui 等<sup>[95]</sup>使用柚皮苷酶可将蒙花苷中的鼠李糖水解，生成田蓟苷。此外，Jassbi 等<sup>[96]</sup>使用  $\beta$ -葡萄糖苷酶将紫柳苷水解生成紫柳亭，抗氧化实验结果表明去糖基化的紫柳亭比紫柳苷具有更好的活性。Day 等<sup>[97]</sup>从羊小肠中分离纯化的糖苷酶则可以水解根皮苷生成根皮素。

### 2.3 其他氧糖苷的生物转化

除了皂苷和黄酮苷外，糖苷水解酶也被应用于水解修饰其他氧糖苷(表 4)。梔子果实是一种传统的中药，用于治疗心脑血管、肝胆等疾病。梔子果实中存在大量京尼平苷，但有效成分是京尼平苷的去糖基化产物京尼平，含量不足 0.01%。从 *Penicillium nigricans*<sup>[98]</sup>和 *Aspergillus niger*<sup>[99]</sup>中分离纯化出的  $\beta$ -葡萄糖苷酶能转化京尼平苷制备京尼平，以满足大量获取京尼平的需求。牛蒡子具有预防或治疗慢性肾功能衰竭等功效，其有效成分为牛蒡子苷和牛蒡子苷元。*Grifola frondosa*<sup>[100]</sup>和 *Rhizoctonia solani*<sup>[101]</sup>中的  $\beta$ -葡萄糖苷酶能够转化

表 4. 糖苷水解酶转化其他氧糖苷

Table 4. Biotransformation of other O-glycosides by glycosidase

Product	Substrate	Reaction	Organism	Reference
Genipin	Geniposide	$\beta$ -Glucosidase	<i>Penicillium nigricans</i>	Xu <sup>[98]</sup>
Genipin	Geniposide	$\beta$ -Glucosidase	<i>Aspergillus niger</i>	Gong <sup>[99]</sup>
Arctigenin	Arctiin	$\beta$ -Glucosidase	<i>Grifola frondosa</i>	Kim <sup>[100]</sup>
Arctigenin	Arctiin	$\beta$ -Glucosidase	<i>Rhizoctonia solani</i>	Kuo <sup>[101]</sup>
Arctigenin	Arctiin	$\beta$ -Glucosidase	Commercial	Liu <sup>[102]</sup>
Resveratrol	Polydatin	$\beta$ -Glucosidase	<i>Aspergillus oryzae</i>	Chen <sup>[103]</sup>
Resveratrol	Polydatin	$\beta$ -Glucosidase	<i>Lactobacillus kimchi</i>	Ko <sup>[104]</sup>
Resveratrol	Polydatin	$\beta$ -Glucosidase	Unculturable microbes	Mai <sup>[105]</sup>
10-Deacetylpaclitaxel	7-Xylosyl-10-deacetylpaclitaxel	$\beta$ -Xylosidase	<i>Cellulosimicrobium cellulans</i>	Dou <sup>[106]</sup>



牛蒡子生成牛蒡子苷元。Liu 等<sup>[102]</sup>使用商品化  $\beta$ -葡萄糖苷酶也可以将牛蒡子完全水解获得牛蒡子苷元产物。牛蒡子苷转化为牛蒡子苷元后能有效提高生物利用度。白藜芦醇具有预防肿瘤和动脉粥样硬化等功能, 从 *Aspergillus oryzae* sp. 100<sup>[103]</sup> 和 *Lactobacillus kimchi*<sup>[104]</sup> 中分离纯化的  $\beta$ -葡萄糖苷酶, 以及 Mai 等<sup>[105]</sup> 从红树林土壤的宏基因组中克隆获得的重组  $\beta$ -葡萄糖苷酶能够转化虎杖苷生成白藜芦醇。紫杉醇为红豆杉植物的次生代谢产物, 对卵巢癌和乳腺癌等具有良好的治疗效果。豆杉中紫杉醇的干重仅为 0.02%, 而作为废物丢弃的 7-木糖-10-去乙酰紫杉醇的含量则是紫杉醇的 10 倍以上。Dou 等<sup>[106]</sup> 利用 *Cellulosimicrobium cellulans* strain F16 分泌的胞外木糖苷酶将 7-木糖-10-去乙酰紫杉醇转化成 10-去乙酰紫杉醇, 再通过一步酰化反应即可生成紫杉醇。

### 3 碳糖苷及硫糖苷的生物转化

除了氧糖苷外, 糖苷水解酶同样被应用于碳糖苷和硫糖苷的水解修饰研究中(表 5)。碳糖苷是由苷元酚羟基所活化的邻位或对位的氢与糖基脱水缩合而形成。碳苷黄酮具有抗炎抑菌、抗肿瘤、降血糖和增强免疫等多种活性<sup>[107]</sup>。与氧苷黄酮相比, 碳苷黄酮具有更高的稳定性, 可能被完整吸收, 成为潜在的药物分子<sup>[108]</sup>。由于碳糖苷键难以

被水解, 因此碳苷黄酮的水解研究报道较少。Sanugul 等<sup>[109]</sup> 从人粪便细菌混合物中分离得到一株细菌, 该菌在芒果苷诱导下分泌一种糖苷酶, 能水解芒果苷中碳糖苷键, 生成活性更好的芒果苷元。Nakamura 等<sup>[110]</sup> 从人肠道细菌中分离得到 strain PUE, 该菌种能分离纯化出一种水解葛根素生成苷元的碳糖苷酶。对于碳糖苷酶编码基因的研究中, Braune 等<sup>[111]</sup> 发现 *Eubacterium cellulosolvens* 中蛋白编码基因 *dfgA*、*dfgB*、*dfgC*、*dfgD* 和 *dfgE* 共同表达出能够水解异荛草素生成相应苷元的碳糖苷酶。

硫代葡萄糖苷是一类重要的硫糖苷化合物, 在十字花科植物中广泛存在, 如芥菜、青花菜、大蒜等<sup>[112]</sup>。研究表明, 食用十字花科植物可有效预防乳腺癌、肺癌以及结肠癌等多种癌症, 其主要活性成分为硫代葡萄糖苷降解后产生的异硫氰酸酯<sup>[113]</sup>。硫代葡萄糖苷酶又称黑芥子酶, 主要存在于十字花科植物中, 但与硫代葡萄糖苷分布在不同位置。只有当细胞破碎时, 它们才会混合发生反应, 由于内源黑芥子酶含量较少, 难以有效水解硫代葡萄糖苷生成活性产物。萝卜硫素是一种具有药理活性的异硫氰酸酯, Shen 等<sup>[114]</sup> 用外源黑芥子酶将萝卜硫苷成功转化为萝卜硫素。目前, 对碳糖苷和硫糖苷进行水解修饰的研究较少, 未来更多的碳糖苷和硫糖苷的开发及结构修饰将为药物开发提供更多的候选分子。

表 5. 酶法转化碳糖苷和硫糖苷

Table 5. Biotransformation of C-glycosides and S-glycosides by glycosidase

Product	Substrate	Reaction	Organism	Reference
Norathyriol	Mangiferin	C-glucosyl-cleaving enzyme	<i>Bacteroides</i>	Sanugul <sup>[109]</sup>
Daidzein	Puerarin	C-glucosyl-cleaving enzyme	Human intestinal bacterium	Nakamura <sup>[110]</sup>
Luteolin	Homoorientin	C-glucosyl-cleaving enzyme	<i>Eubacterium cellulosolvens</i>	Braune <sup>[111]</sup>
Sulforaphane	Glucoraphanin	Myrosinase	Broccoli seeds	Shen <sup>[114]</sup>

## 4 展望

糖苷具有抗炎症、抗氧化和抗肿瘤等多种生物活性, 拥有开发药品、保健品和化妆品的美好前景。随着现代生物技术的进步, 提取分离及分析测试方法不断改善。酶学的发展开拓了生物转化技术的广泛用途, 利用酶转化糖苷越来越受到重视。通过糖苷水解酶制备新的糖苷与苷元, 将改变糖苷的生物活性, 为药物和保健食品提供丰富的资源。目前对酶催化机制的研究相对薄弱, 尤其从分子水平解析酶的立体结构、探究酶的结构与其选择性的关系研究较少, 还不够深入。分子生物学与结构生物学等现代生物技术将逐步解决这些科学问题, 生物转化制备新的糖苷类化合物将越来越广泛地应用在工农业生产中。

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## Glycosidase: An effective tool for the preparation of active glycosides and aglycone

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**Abstract:** Glycosides widely distributed in nature have many pharmacological activities. A lot of drugs belong to glycoside and the sugar chains are closely related to its pharmacological activities. The modification of sugar chains can change the pharmacological activities of glycosides, which provides tremendous glycoside resource for drug development. Because of high efficiency and low pollution, biotransformation by glycosidase is widely used in the preparation of active glycosides. This review summarized recent advances in the preparation of active glycosides by glycosidase transformation, and provided useful references for preparing active glycosides and related research work.

**Keywords:** glycosides, glycosidase, biotransformation

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