

· 综 述 ·

原人参二醇型皂苷水解酶及制备人参皂苷 Compound K 的研究进展

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摘 要: 人参皂苷 Compound K (CK)是一种具有抗癌抗炎等药理活性的化合物。目前在天然人参中暂未鉴定出, 工业上主要通过原人参二醇型皂苷的去糖基化进行制备。相对于传统的物理、化学的去糖基化法, 利用原人参二醇型皂苷水解酶制备 CK 具有特异性强、绿色环保和高效稳定的优点。本文根据水解酶作用的糖基连接碳原子的差异将原人参二醇型皂苷水解酶分成了 3 类, 发现大多数能制备 CK 的水解酶为 III 型原人参二醇型皂苷水解酶。此外, 对水解酶在制备 CK 中的应用进行了总结评估, 旨在为人参皂苷 CK 的大规模制备及其在食品和药品行业中的开发提供参考。

关键词: 原人参二醇型皂苷水解酶; 酶催化; 应用; 制备; 人参皂苷 Compound K

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Protopanaxadiol-type ginsenoside hydrolases and their application in the preparation of ginsenoside Compound K: a review

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Abstract: Ginsenoside Compound K (CK) has anti-cancer and anti-inflammatory pharmacological activities. It has not been isolated from natural ginseng and is mainly prepared by deglycosylation of protopanaxadiol. Compared with the traditional physicochemical preparation methods, the preparation of CK by hydrolysis with protopanaxadiol-type (PPD-type) ginsenoside hydrolases has the advantages of high specificity, environmental-friendliness, high efficiency and high stability. In this review, the PPD-type ginsenoside hydrolases were classified into three categories based on the differences in the glycosyl-linked carbon atoms of the hydrolase action. It was found that most of the hydrolases that could prepare CK were PPD-type ginsenoside hydrolase type III. In addition, the applications of hydrolases in the preparation of CK were summarized and evaluated to facilitate large-scale preparation of CK and its development in the food and pharmaceutical industries.

Keywords: protopanaxadiol-type ginsenoside hydrolases; enzyme catalysis; application; preparation; ginsenoside Compound K

人参是五加科的一种多年生草本植物，长久以来在全球被用于保持身体活力和延年益寿，素有“中药之王”的美称。大量文献表明，人参中的主要活性成分是人参皂苷，其在抗癌、抗炎和抗氧化等方面显示出巨大的应用价值^[1-5]。截至目前，已有 200 多种人参皂苷及其衍生物被报道^[3]。其中，达玛烷型人参皂苷由相似的达玛烷(四环三萜)骨架和糖基部分组成。由于苷元骨架上糖基的类型、附着位置以及数量的差异，达玛烷型人参皂苷又可分为原人参二醇型皂苷(Rb₁、Rb₂、Rb₃、Rc、Rd、F₂、CK 等)和原人参三醇型皂苷(Re、Rf 等)^[6]。此外，根据去糖基化的程度，人参皂苷又可分为主要人参皂苷和稀有人参皂苷。稀有

人参皂苷是通过主要人参皂苷去糖基化制备，在天然人参植物中不存在或者含量非常低^[7]。其中，稀有人参皂苷(例如 CK)因其具有比主要人参皂苷更高的生物利用度备受学者们的关注。

稀有人参皂苷 CK，于 1972 年首次被发现，是原人参二醇型皂苷去糖基化的主要产物^[8]。半个世纪以来，CK 对癌症、肿瘤、炎症、糖尿病等多种疾病的(辅助)治疗能力已在许多研究中得到证实^[9]。CK 具有高度的安全性和多样化的生物学功能，可能是许多疾病的潜在治疗剂。目前，一种含有稀有人参皂苷 CK 的药片作为类风湿关节炎的候选药物，已在我国完成全部的 0 期和 I 期临床试验^[10]。随着 CK 市场

需求量不断增大, 如何大批量地生产这种在人参植株中不存在或者含量非常低的代谢物成为国内外学者们的研究方向。

以往的许多研究专注于使用热处理法、酸碱处理法、微生物转化法或酶促转化法将原人参二醇型主要人参皂苷转化为稀有原人参皂苷^[11-13]。其中, 加热或酸碱处理法对于糖苷键的水解是随机的, 导致产物的结构和类型难以预期。而且, 尚无文献报道使用酸水解法制备 CK。这可能是由于位于 C-20 的糖基在物理化学条件下可被非特异性裂解, 而位于 C-3 的糖基则不能^[9]。此外, 物理化学法制备过程中产生的过高的能耗和废弃化合物对环境具有严重的污染性。微生物法为制备 CK 提供了一种环境友好型的方案。但是, 菌株有限的生命力和较长的生长周期增加了工业生产的成本。相比较于这些方法, 具有特异选择性的酶促催化

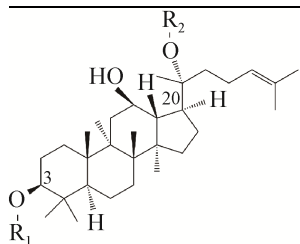
法在绿色、稳定生产 CK 时具有显著优势^[14]。为了满足人参皂苷 CK 不断增加的需求量和工业生产的低成本要求, 对于更高效、更稳定以及底物适用范围更广泛的原人参二醇型皂苷水解酶的挖掘和研究仍然是迫切的。因此, 本文主要就近年来原人参二醇型皂苷水解酶的发展状况及其在制备 CK 中的应用展开详述。

1 原人参二醇型皂苷水解酶

原人参二醇型皂苷水解酶, 是一种可以水解原人参二醇型皂苷的糖苷水解酶。以往的研究表明, 原人参二醇型皂苷中涉及的糖基包含 β -D-吡喃葡萄糖基、 β -D-吡喃木糖基、 α -L-阿拉伯呋喃糖基和 α -L-阿拉伯吡喃糖基, 通过 C-3 和/或 C-20 与原人参二醇型苷元进行连接(化学结构如表 1 所示)^[6]。截至目前, 对原人参二醇型皂苷水解酶分类的研究较少^[15-16]。以往对原人

表 1 原人参二醇型皂苷的化学结构^[6]

Table 1 Chemical structure of PPD-type ginsenosides^[6]

Chemical structure	PPD-type ginsenosides	R ₁	R ₂
	Rb ₁	Glc(2→1)Glc	Glc(6→1)Glc
	Rd	Glc(2→1)Glc	Glc
	F ₂	Glc	Glc
	Rb ₂	Glc(2→1)Glc	Glc(6→1)Arap
	CO	Glc	Glc(6→1)Arap
	CY	H	Glc(6→1)Arap
	Rb ₃	Glc(2→1)Glc	Glc(6→1)Xyl
	CMx ₁	Glc	Glc(6→1)Xyl
	CMx	H	Glc(6→1)Xyl
	Rc	Glc(2→1)Glc	Glc(6→1)Araf
	CMc ₁	Glc	Glc(6→1)Araf
	CMc	H	Glc(6→1)Araf
	Gyp XVII	Glc	Glc(6→1)Glc
	Gyp LXXV	H	Glc(6→1)Glc
	CK	H	Glc
	Rg ₃	Glc(2→1)Glc	H
	Rh ₂	Glc	H
	20 (S)-PPD	H	H

Glc: β -D-glucopyranosyl; Arap: α -L-arabinopyranosyl; Araf: α -L-arabinofuranosyl; Xyl: β -D-xylopyranosyl.

参二醇型皂苷水解酶的分类是依据水解酶作用的糖基与苷元骨架连接还是与糖基连接, 把原人参二醇型皂苷水解酶分为 6-8 种。为了便于理解与归类, 我们根据水解酶作用的糖基连接

的碳原子的不同, 将原人参二醇型皂苷水解酶分为 3 种类型(图 1A)。其中, 仅能水解 C-3 上糖基的水解酶被定义为 I 型原人参二醇型皂苷水解酶。仅能水解 C-20 上糖基的水解酶被定义为

A PPD-type ginsenoside hydrolases type I

Hydrolyses of C-3 sugars in PPD-type ginsenosides

PPD-type ginsenoside hydrolases type II

Hydrolyses of C-20 sugars in PPD-type ginsenosides

PPD-type ginsenoside hydrolases type III

Hydrolyses of C-3 and C-20 sugars in PPD-type ginsenosides

B

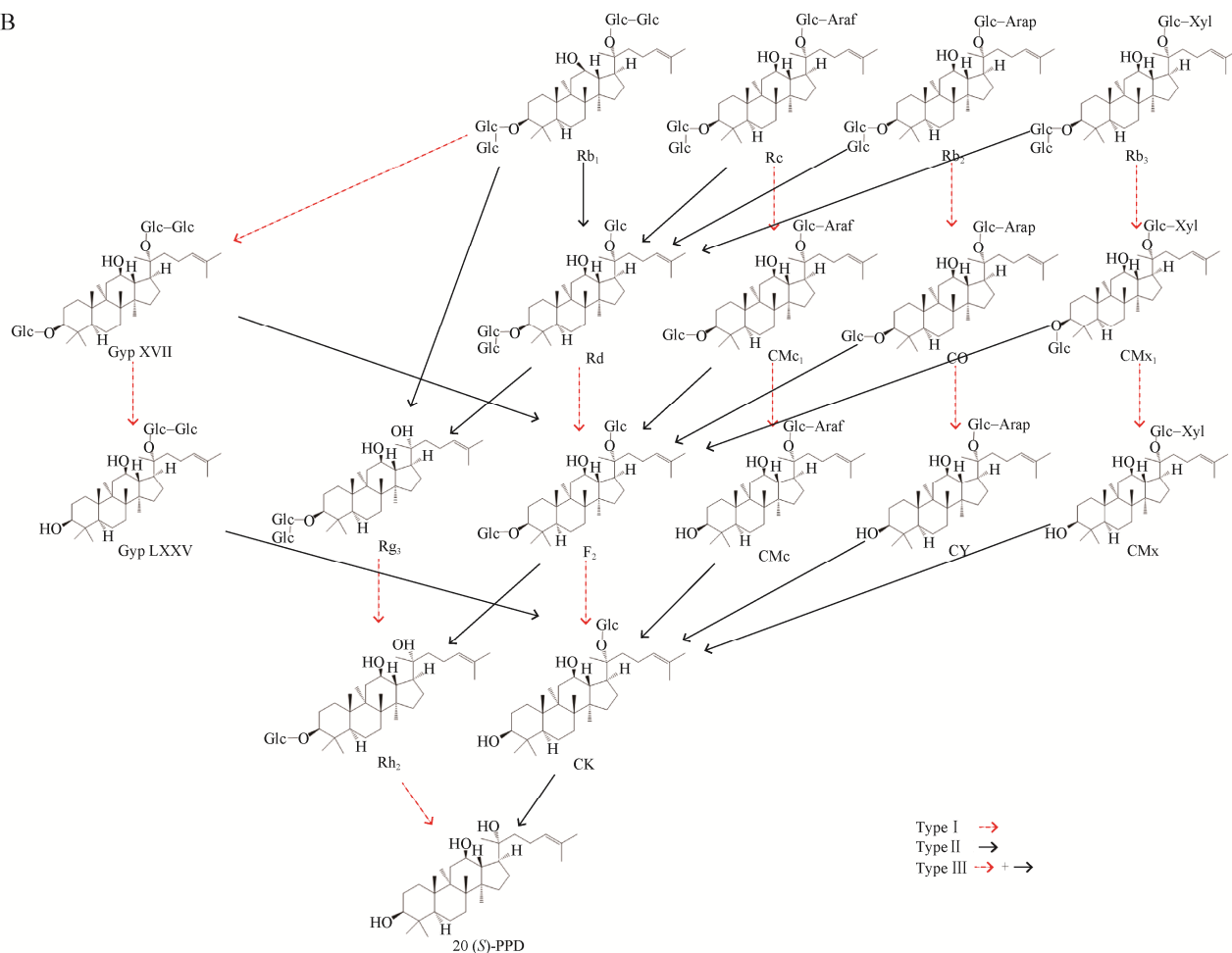


图 1 原人参二醇型皂苷水解酶的分类(A)及原人参二醇型皂苷的转化途径(B)

Figure 1 Classification of PPD-type ginsenoside hydrolases (A) and transformation pathway of PPD-type ginsenosides (B).

II型原人参二醇型皂苷水解酶。能同时水解C-3和C-20上糖基的水解酶被定义为III型原人参二醇型皂苷水解酶。图1B显示了原人参二醇型皂苷之间的转化关系。表2列出了近几年挖掘的原人参二醇型皂苷水解酶,这些水解酶来源于不同的菌株,最适催化条件也有较大差别。

1.1 I型原人参二醇型皂苷水解酶

如图1B所示,用虚线箭头指示的转化途径需要用I型原人参二醇型皂苷水解酶进行催化。

Zhang等报道了一种来自短双歧杆菌(*Bifidobacterium breve*) ATCC 15700的GH3家族的 β -葡萄糖苷酶,该I型水解酶可在12h内将5 mmol/L Rd全部转化为CK,能够逐步水解连在Rd的C-3上的内外两个 β -D-吡喃葡萄糖基^[17]。Shin等发现一种来自阿拉斯加鞘氨醇盒菌(*Sphingopyxis alaskensis*)的GH3家族的 β -葡萄糖苷酶,该I型水解酶可在1h内将8 mg/mL Rb₁转化为6.8 mg/mL Gypenoside XVII (Gyp XVII),可逐步水解连在Rb₁的C-3上的内外两个 β -D-吡喃葡萄糖基^[18]。Yuan等发现一种来自纤维纤维微菌(*Cellulosimicrobium cellulans*) 21的GH1家族的 β -葡萄糖苷酶,该I型水解酶可在2h内将1g原人参二醇型皂苷转化为292 mg Gyp XVII、134 mg CO、184 mg Cmc₁和62 mg F₂,可水解连在C-3上的内外两个 β -D-吡喃葡萄糖基^[19]。

综上所述,I型原人参二醇型皂苷水解酶在酶量和时间充足的情况下,可将C-3上连接的内外 β -D-吡喃葡萄糖基逐步水解。

1.2 II型原人参二醇型皂苷水解酶

如图1B所示,用实线箭头指示的转化途

径需要用II型原人参二醇型皂苷水解酶进行催化。

Quan等报道一种来自酯香微杆菌(*Microbacterium esteraromaticum*)的GH3家族的 β -葡萄糖苷酶,该II型水解酶在1h内能将10 mg/mL Rb₁转化为6.9 mg/mL Rg₃,可逐步水解C-20上的内外两个 β -D-吡喃葡萄糖基^[20]。Quan等发现一种来自*M. esteraromaticum* GS514的GH2家族的 β -糖苷酶,该II型水解酶可在2h内将1 mg/mL Rb₂转化为0.47 mg/mL Rg₃,水解连在C-20上的 α -L-阿拉伯呋喃糖基和 β -D-吡喃葡萄糖基^[21]。Li等发现一种来自约氏黄杆菌(*Flavobacterium johnsoniae*)的 β -葡萄糖苷酶,该II型水解酶在24h内能将Rb₁转化为Rg₃(摩尔转化率为61%),可逐步水解C-20上的内外两个 β -D-吡喃葡萄糖基^[22]。Zhang等报道一种来自嗜嗜热菌(*Thermotoga petrophilia*)的GH1家族的 β -糖苷酶,该II型水解酶可在3h内将10 mg/mL人参提取物转化为3.93 mg/mL Rg₃,可逐步水解C-20上的内外两个 β -D-吡喃葡萄糖基^[23]。Xie等发现一种来自嗜嗜热菌(*Thermotoga thermarum*) DSM5069的GH51家族的 α -L-阿拉伯呋喃糖苷水解酶,该II型水解酶可在1h将25 mg/mL Rc转化为21.8 mg/mL Rd,水解C-20上的 α -L-阿拉伯呋喃糖基^[24]。

综上所述,II型原人参二醇型皂苷水解酶可作用于C-20上连接的 β -D-吡喃葡萄糖基、 α -L-阿拉伯呋喃糖基、 α -L-阿拉伯吡喃糖基和 β -D-吡喃木糖基。此外,从类似*M. esteraromaticum*等具有转化效力的微生物中挖掘出多种II型原人参二醇型皂苷水解酶。

1.3 III型原人参二醇型皂苷水解酶

如图 1B 所示, 包含两种类型箭头的转化途径需要用III型原人参二醇型皂苷水解酶进行催化。

Quan 等报道了一种来自 *M. esteraromaticum* 的 GH3 家族的 β -葡萄糖苷酶, 该III型水解酶在 1 h 内能将 1 mg/mL Rb₁ 转化为 0.46 mg/mL CK, 可水解 Rb₁ 的 C-3 和 C-20 上的糖基生成 CK^[25]。Noh 等发现一种来自硫磺矿硫化叶菌 (*Sulfolobus solfataricus*) 的 β -糖苷酶, 该III型水解酶在 12 h 内将 1.9 mg/mL Rb₁、0.52 mg/mL Rb₂、0.92 mg/mL Rc 和 0.23 mg/mL Rd 转化为 1.63 mg/mL CK, 可同时水解 Rb₁、Rb₂、Rc 和 Rd 的 C-3 和 C-20 上的糖基生成 CK^[26]。Shin 等报道了一种来自 *S. solfataricus* 的 β -糖苷酶, 该III型水解酶在 1.5 h 内能将 2 mg/mL Rb₁ 完全转化为 1.12 mg/mL CK, 可水解 Rb₁ 的 C-3 和 C-20 上的糖基生成 CK^[27]。Cui 等发现一种来自奇迹束丝放线菌 (*Actinosynnema mirum*) KACC 20028T 的 β -葡萄糖苷酶, 该III型水解酶可将天然皂苷 Rb₁、Rb₂、Rc 和 Rd 转化为稀有人参皂苷 Rg₃、CK 和 20 (S)-PPD, 水解多种主要人参皂苷的 C-3 和 C-20 上的糖基生成稀有人参皂苷^[28]。Ko 等发现一种来自米曲霉 (*Aspergillus oryzae*) 的乳糖酶, 该III型水解酶可将天然皂苷 Rb₁、Rb₂、Rc、Rb₃ 和 Rd 转化为稀有人参皂苷 Rg₃、CK 和 20 (S)-PPD, 水解多种皂苷的 C-3 和 C-20 上的糖基生成稀有人参皂苷^[29]。

综上所述, III型原人参二醇型皂苷水解酶可作用于 C-3 和 C-20 上连接的 β -D-吡喃葡萄糖基、 α -L-阿拉伯呋喃糖基、 α -L-阿拉伯吡喃糖基和 β -D-吡喃木糖基。此外, 从类似 *M. esteraromaticum* 和 *S. solfataricus* 等具有转化效力的微生物中挖掘出多种III型原人参二醇型皂

苷水解酶。

2 原人参二醇型皂苷水解酶在 CK 生产上的应用

一个多世纪以来, 酶在工业中的使用引起了人们的关注, 工业酶的全球市场不断增长, 预计在 2022 年可达到 85 亿美元的市场^[16]。这种绿色、高效且特异性强的生物催化剂为生产增值化合物提供了一个综合性的强大方法库。基于对原人参二醇型皂苷水解酶的分类, 近几年水解酶在 CK 生产上的案例也被总结归纳。

2.1 天然酶水解制备 CK

有氧条件下的微生物转化为人参皂苷的生产提供了一种高效且廉价的工艺。究其根本, 具有人参皂苷转化效力的微生物具有特异性的酶催化剂, 可以水解原人参二醇型皂苷生产 CK。

拟青霉 (*Paecilomyces bainier*) 229 的胞外酶经分离纯化得到 7 种组分, 每种组分都具有水解人参皂苷的能力, 其中一种被定义为 β -葡萄糖苷酶的组分可特异性地将 Rb₁ 水解为 CK^[30]。Chang 等报道了一株黑曲霉 (*Aspergillus niger*) 的胞外酶可将 Rb₁ 水解为 Rg₃ 和 CK^[31]。来源于蜜环菌 (*Armillaria mellea*) 菌丝体的胞内酶与 1 mg/mL 的 Rb₁ 和 Rb₂ 反应 96 h, 皂苷被全部水解为 CK^[32-33]。Kim 等从发酵大豆砖中分离出一种可生产 CK 的塔宾曲霉 (*Aspergillus tubingensis*), 其经分离纯化的胞外 β -葡萄糖苷酶与原人参二醇型皂苷 (包含 Rb₁、Rb₂、Rb₃ 和 Rc) 反应 20 h 后, 可将 13.4 mmol/L 原人参二醇型皂苷完全转化为 CK (图 2A), 这是第一个将所有原人参二醇型皂苷完全转化为 CK 的食品安全 (generally recognized as safe, GRAS) 酶^[34]。除此之外, 多项研究报道了微生物天然

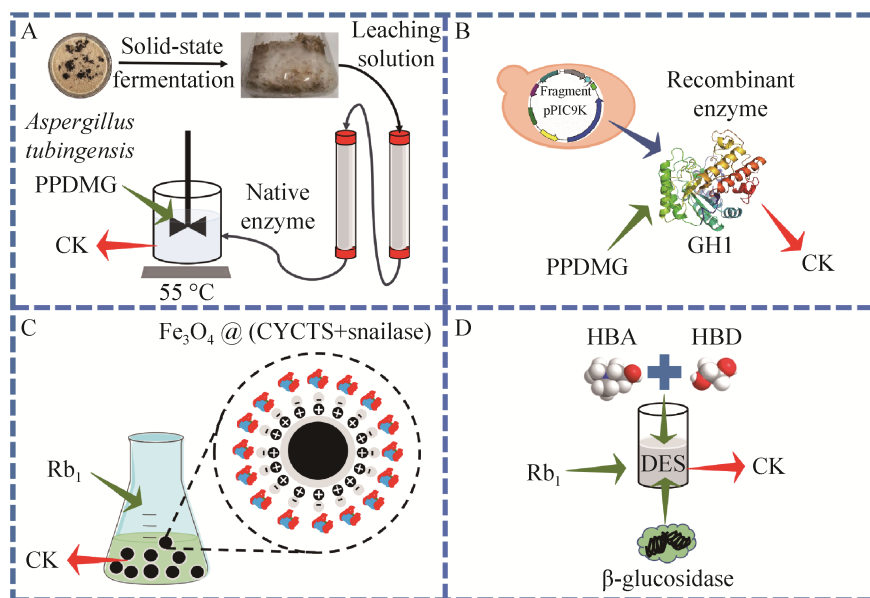


图 2 天然酶(A)、重组酶(B)、固定化酶(C)、溶剂工程在 CK 生产中的应用(D)

Figure 2 Application of native enzymes (A), recombinant enzymes (B), immobilized enzymes (C), and solvent engineering (D) in the production of CK.

酶对人参皂苷的水解能力^[35-39], 微生物资源丰富, 是尚待深入挖掘新酶的宝库。

所筛选的微生物通过发酵的方式被大量制备, 这些菌体被处理后得到大量的天然生物催化剂。生物催化剂通过纯化的方式, 得到纯度较高的酶制剂。但是, 天然酶制剂的纯化制备过程复杂, 需要耗费大量的人力物力。随着基因工程技术的不断发展, 可通过克隆表达的方式大量制备具有水解特异型的酶制剂, 且表达产物具有纯度高、活性强的优点。

2.2 重组酶水解制备 CK

研究表明^[40-45], 大多数能制备 CK 的水解酶为 III 型原人参二醇型皂苷水解酶, 而且来自 GH1 和 GH3 家族的 β -葡萄糖苷酶对原人参二醇型皂苷的水解作出了巨大贡献(表 1)。

来自黄杆菌(*Flavobacterium chilense*)的 GH3 家族的 β -葡萄糖苷酶在大肠杆菌(*Escherichia coli*) BL21 中成功表达^[46], 该酶可用于制备 CK, 且最适温度为 37 °C、最适 pH 7.0。但是, 该酶

在温度超过 45 °C 时, 酶活性仅能维持甚至低于初始酶活性的 20%。较高的酶促反应温度会促进水溶性较差底物(例如, 大多数人参皂苷)在反应液中的溶解度, 这种正向的促进作用会加快底物与酶制剂之间的传质速率从而降低生产的时间成本。相似地, 在 *E. coli* BL21 中成功表达的 β -葡萄糖苷酶可耐受高达 40 g/L 的人参皂苷 Rb^[17]。这种高浓度的底物耐受性有利于在工业生产中降低酶制剂的使用成本。此外, 在谷氨酸棒状杆菌(*Corynebacterium glutamicum*) ATCC 13032 中表达的重组 C3a-MT619, 仅具有在 *E. coli* BL21 中异源表达的 MT619 的 61.9% 的酶活性^[7]。因此, 不同的表达系统对于同种酶的表达效果并不相同。来源于尖海龙共附生真菌(*Talaromyces amestolkiae*)的 β -葡萄糖苷酶在毕赤酵母(*Pichia pastoris*)中被高效表达, 75 U/mL 的生产水平是文献中报道的重组 β -葡萄糖苷酶的较高水平^[47]。由此得出, 在筛选最优表达系统时, 表达系统的

生物安全水平、表达量以及对基因编码的偏好性等都需要评估的条件。此外,挖掘报道的水解酶多用于水解单一底物,对于酶的底物适用范围应该被扩大研究。新的生物催化剂在绿色化学和可持续化学工程中占有重要地位,有望克服现有生物催化剂的不足^[48]。那些被报道的具有转化人参皂苷效果的微生物,是发现新型生物催化剂的巨大宝库(图 2B)。未来,学者们对于新型生物催化剂的持续开发,是推动稀有人参皂苷产业不断前进的基础且关键的一步。

此外,人参皂苷结构多样,不同类型的人参皂苷水解酶可特异性水解人参皂苷中的不同的糖基部分^[16]。不同类型的人参皂苷水解酶组合使用策略为实现 CK 的高效生产提供了参考^[15]。基于 BglSk 与 BglPm 的组合酶催化策略可制得(277.61±4.27) mg 的 CK,高于单一酶促反应的产量^[49]。还有研究表明,可以通过随机突变或者理性设计的方式改变蛋白质的氨基酸序列,从而改善蛋白的理化能力和催化性能^[50]。通过对来源于 *S. solfataricus* 的 β -糖苷酶进行理性设计,CK 的产率由 306 mg/(L·h) 提升至 843 mg/(L·h)^[27]。Shin 等对源于番茄溃疡病菌(*Clavibacter michiganensis*)的 β -葡萄糖苷酶 512 位氨基酸残基进行了靶向诱变,拓宽了突变体的区域选择性,使变体酶具有了将 Rb₁ 转化为 CK 的能力(以前不能)^[51]。这种基于酶的定向进化的大量变体库生成后,可筛选和选择表现出所需特征的最佳变体^[52]。未来,可以通过蛋白质工程优化相关蛋白的催化性能和理化功能,为 CK 的制备降低酶制剂成本。

2.3 固定化酶水解制备 CK

异源表达的方式使短期内大量制备纯度高、特异性强的酶制剂成为可能。但是,在实

际的工业生产中,如何稳定发挥酶的催化性能以及重复使用酶制剂仍是有待解决的问题^[53]。因此,易于回收且能维持酶稳定性和活性的固定化酶受到研究者的关注。这也为重复、高效使用酶制剂催化生产 CK 提供了参考。

使用 GRAS 菌株 *C. glutamicum* ATCC 13032 表达了一种来源于 *M. testaceum* 且融合纤维素结合模块的新型 β -葡萄糖苷酶^[7]。融合蛋白以高密度(最大 984 mg/g 纤维素)有效地固定在无定形纤维素上,使酶浓度提高了 286 倍。高浓度的固定化酶可在 24 h 内制备 7.59 g/L CK。Shi 等将 GH3 家族的 β -葡萄糖苷酶(Tpebg13)负载在 Fe₃O₄-SiO₂-NH₂-Cellu-ZIF₈ 上,这种金属有机骨架磁性颗粒赋予了 Tpebg13 更优异的高温稳定性和耐酸性^[54],而且在 10 个重复循环结束时,固定化酶还保留了 74% 的初始活性。Aslihan 等研究制备了一种基于藻酸盐的固定化 β -葡萄糖苷酶的酶微反应器,该系统在连续流动条件下运行,可持续将 Rb₁ 水解为 CK^[55]。Li 等合成了 50 nm 的球形 Fe₃O₄@CYCTS 纳米粒子^[56]。如图 2C 所示,这种用羧化壳聚糖包封磁铁矿制备的纳米颗粒为蜗牛酶的固定提供了大的比表面积,固定化的蜗牛酶可用于连续生产 CK,在 9 个连续循环后固定化酶仍保留了约 56% 的初始催化活性。Wu 等将来自 *A. niger* 的 β -葡萄糖苷酶通过化学亲和的方法固定在氨基二氧化硅中,固定化酶拥有比游离酶更宽的 pH 范围和更高的热稳定性,并且在重复使用制备 CK 的 45 个循环后仍可保持 97% 的初始活性^[57]。

为了扩大酶制剂的工业化应用范围,越来越多的研究专注于探索新的固定基质和策略^[58-62]。但是,常见的固定化策略多专注于单一蛋白的固定。未来,应该考虑多种蛋白的组合固定策略。

2.4 溶剂工程用于水解制备 CK

在生物催化的过程中,固定化酶可以起到改善酶性质的作用。但是,对于大多数合成反应来说,疏水性底物(例如,大多数人参皂苷)在反应溶剂中的溶解度也是影响反应进程的一大问题^[63]。有机溶剂在一定程度上可以促进疏水性底物的溶解性,但是与水溶液相比,大多数酶制剂在有机溶剂中的催化效率较低。随着溶剂工程的不断发展,低共熔溶剂(deep eutectic solvent, DES)因其低毒性、生物降解性、可回收性、易燃性和价格低廉逐渐引起学者们的关注^[64]。已有研究表明,低共熔溶剂可用于疏水性化合物的浸提以及生物催化反应^[65-69]。

相对于需要大量的有机溶剂、较长的提取时间和较高能量的传统提取方法,Jeong 等研究了一种包含甘油、L-脯氨酸和蔗糖的 DES,可以从人参提取物中低毒、绿色提取大量人参皂苷^[70]。Li 等开发了一种基于氯化胆碱、1,4-丁二醇和无机盐溶液(K_2HPO_4)的水相两相系统,可以将中药注射剂(康爱注射液)中超过 90%的人参皂苷(包括人参皂苷 Rg_1 、 Re 、 Rf 、 Rg_2 、 Rb_1 、 Rc 、 Rb_2 和 Rd)进行提取回收^[71]。Han 等建立的基于 31.9% (质量分数) DES 和 24.5% (质量分数) K_2HPO_4 的水相两相体系,可从顶部和底部相中回收 75.79% 的催化产物 CK 和 61.14% 的 β -葡萄糖苷酶,被回收的 β -葡萄糖苷酶可再次循环用于 CK 的制备^[72]。这是首次将低共熔溶剂用于生物催化生产 CK 的研究。如图 2D 所示, Ma 等开发了一种基于氯化胆碱和乙二醇的 DES,在 30% (体积分数) DES 存在的条件下, β -葡萄糖苷酶的半衰期增加了 96%, F_2 的溶解度增加了 120%, CK 的产率增加了 54%, 在 60 °C、pH 4.5 的条件下, 8 mmol/L Rb_1 可转化为 CK, 转化率达到 80.6%^[73]。

DES 通过季铵盐或金属盐与氢键供体的络合获得^[74], 已有多项研究证明其在生物催化领域的优势^[75-79], 为高效生产 CK 提供了可行的参考。此外,在实际的应用中,多组分络合形成的 DES 的生物毒性也应该被重视^[80]。

3 总结与展望

微生物来源的原人参二醇型皂苷水解酶,具有来源广泛、高催化效率和性质多样等特点,越来越多的原人参二醇型皂苷水解酶被挖掘并被用于生产稀有人参皂苷。相对于使用传统的物理化学手段制备 CK,酶催化法不但生产工艺绿色环保,而且产量稳定高效。但是,原人参二醇型皂苷水解酶要应用于低成本、高产值的 CK 工业化生产时,还需要在以下 4 个方面不断改进。(1) 继续从自然界筛选具有耐高温、耐高浓度底物、底物适用范围更广泛的原人参二醇型皂苷水解酶。(2) 开发适用蛋白表达范围更广泛的表达系统,达到蛋白的最大表达产量,一定程度减少酶促转化制备 CK 的酶制剂成本。(3) 协同使用几种酶制剂或者制备方法,开发更优的制备策略,已达到 CK 产量的更大值。(4) 通过理性设计或代谢调控提高蛋白的表达量和活性,甚至赋予相关蛋白更优良的催化性能。

此外,随着生物技术的不断革新,可以利用合成生物学的手段将廉价的碳源(例如葡萄糖或甘油)在细胞体内加工为稀有人参皂苷^[81-82]。而且, Wang 等构建的工程酵母菌株在补料分批发酵中产生的 CK 滴度可达到 5.74 g/L,这与 5 年生人参根的总皂苷含量(约 3%)相当^[83]。相较于传统的制备方法,这种更廉价、省力的生产方式是植物药用代物工业生产中的福音。未来,对于新兴技术的探索也在继续。

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