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·综 述·

蔗糖磷酸化酶及其在糖基化反应中的应用

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摘 要: 糖基化反应能有效改善化合物的水溶性、稳定性、生物利用度等性质，利用糖苷水解酶类和糖基转移酶类对生物活性化合物进行糖基化修饰已成为研究热点。相比于糖基转移酶类，糖苷水解酶类在大规模催化中具有来源丰富、成本低的优势。其中，蔗糖磷酸化酶因其卓越的糖基化活性和广泛的底物特异性，在化工领域受到人们的广泛关注。文中综述了蔗糖磷酸化酶的结构与催化特性，概述了蔗糖磷酸化酶的定向改造，同时系统性地总结了蔗糖磷酸化酶在糖基化反应中的应用及与其他酶的联合应用。并且，基于蔗糖磷酸化酶的研究现状，结合笔者研究团队的多年工作经验，探讨了该课题的未来发展方向。

关键词: 糖基化作用，碳水化合物活性酶，蔗糖磷酸化酶，酶改造，结构修饰

Application of sucrose phosphorylase in glycosylation

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Abstract: Water solubility, stability, and bioavailability, can be substantially improved after glycosylation. Glycosylation of bioactive compounds catalyzed by glycoside hydrolases (GHs) and glycosyltransferases (GTs) has become a research hotspot. Thanks to their rich sources and use of cheap glycosyl donors, GHs are advantageous in terms of scaled catalysis compared to GTs. Among GHs, sucrose phosphorylase has attracted extensive attentions in chemical engineering due to its prominent glycosylation activity as well as its acceptor promiscuity. This paper reviews the structure, catalytic characteristics, and directional redesign of sucrose phosphorylase. Meanwhile, glycosylation of diverse chemicals with sucrose phosphorylase and its coupling applications with other biocatalysts are summarized. Future research directions were also discussed based on the current research progress combined with our working experience.

Keywords: glycosylation, carbohydrate-active enzymes, sucrose phosphorylase, enzymatic redesign, structural modification

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糖基化作用是自然界中最重要的生化反应之一[1]，能赋予天然产物结构多样性，改善化合物水溶性、稳定性、生物利用度等性质，所得产物能广泛应用于食品、医药及个人护理等领域[2-3]。有机小分子化合物的糖基化方法包括化学法和生物酶法。化学法常被用于合成不同的糖苷化合物，其根据供体底物的不同主要分为 4 类：银盐活化的糖基卤化物 (Koenigs-Knorr 糖苷化反应)[4-6]、路易斯酸活化的糖基三氯乙酰亚氨酯[7]、亲电子试剂活化的含硫甙以及 *n*-戊烯基糖苷[8]。化学糖基化反应的区域选择性难以有效预测、控制[9]，反应过程还伴随着许多副产物 (如糖基供体水解副产物) 的生成，影响产率[10]。与化学法相比，生物酶法专一性高，避免了对非作用基团的保护措施，且反应条件温和，是一类高效、绿色的糖基化修饰方法。De Roode 等计算得出酶法糖基化反应产生的废料比化学法低 5 倍,时空产量高 15 倍[11]。生物体有 1%–3%的基因编码与糖基化反应相关的酶[12], 这些酶在碳水化合物活性酶 (Carbohydrate-active enzyme，CAZy) 数据库中[13]，根据序列相似性被分为两大类：糖基转移酶类 (Glycosyltransferases，GTs) 和糖苷水解酶类 (Glycoside hydrolases，GHs)[14]。GTs 具有广泛的底物特异性，参与自然界中大部分的糖基化反应 (图 1A)。GTs 的催化效率很高，但其大规模应用被糖基供体高昂的价格所限制[15]。GHs 包含糖苷酶和转糖苷酶，能够参与糖苷键的水解和合成 (图 1B)。GHs 分布广泛，糖基供体廉价，在生物技术和生物医学中有广泛的应用[16]。

GHs 中存在许多出色的能够以蔗糖 (Sucrose，Suc)为供体底物的葡萄糖基转移酶，如葡聚糖蔗糖酶 (Glucansucrase)、淀粉蔗糖酶 (Amylosucrase，ASase，EC 2.4.1.4) 和蔗糖磷酸化酶 (Sucrose phosphorylase，SPase，EC 2.4.1.7) 等。葡聚糖蔗糖酶是 GH70 家族的一系列能够催化 Suc 生成 α-葡聚糖的生物催化剂。同时，这类酶具有广泛的受体底物特异性，能够实现非碳水化合物的糖基化修饰[17]。但是，葡聚糖蔗糖酶类的一个显著特征是能够转移多个葡萄糖基至一个受体底物中，形成的混合产物不仅会影响糖基化反应的效率，还会增加下游分离难度[18]。淀粉蔗糖酶是葡聚糖蔗糖酶类中的一种，不同于该类酶中的其他酶，ASase 属于 GH13 家族，可以催化以 Suc 为底物的聚合、异构化和水解反应[19]，并且 ASase 具有将 Suc 的葡萄糖基转移至其他糖基受体 (如糖原、淀粉、黄酮类化合物等) 的能力[20-22]。ASase 的糖基化产率往往高于其他酶[23]，但是大多数的 ASase 表现出较差的热稳定性，而工业中碳水化合物的转化反应通常在高温条件下进行以避免微生物污染[24]。同属于 GH13 家族的 SPase 能够可逆地催化 Suc 与无机磷酸盐反应生成 D-果糖 (D-Fru) 和 α-D-葡萄糖-1-磷酸 (α-D-Glc-1-P)[25-26]。SPase 具有广泛的受体底物特异性，能将 Suc 的葡萄糖基转移至多种受体化合物，根据参与反应的类型可将其作用受体分为 3 类 (图 2)：水；无机磷酸；含醇羟基、酚羟基或羧基的糖基受体化合物[27]。SPase 在糖基化应用中具有突出表现，尤其是对非碳水化合物的糖基

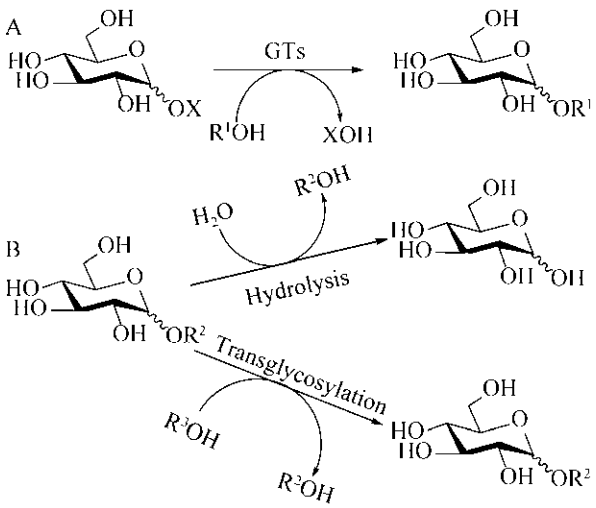


图 1　GTs 和 GHs 催化的生物反应

Fig. 1　Reactions catalyzed by (A) GTs and (B) GHs. X: the activating group of a donor substrate including pyrophosphate, phospholipid and nucleoside diphosphate.

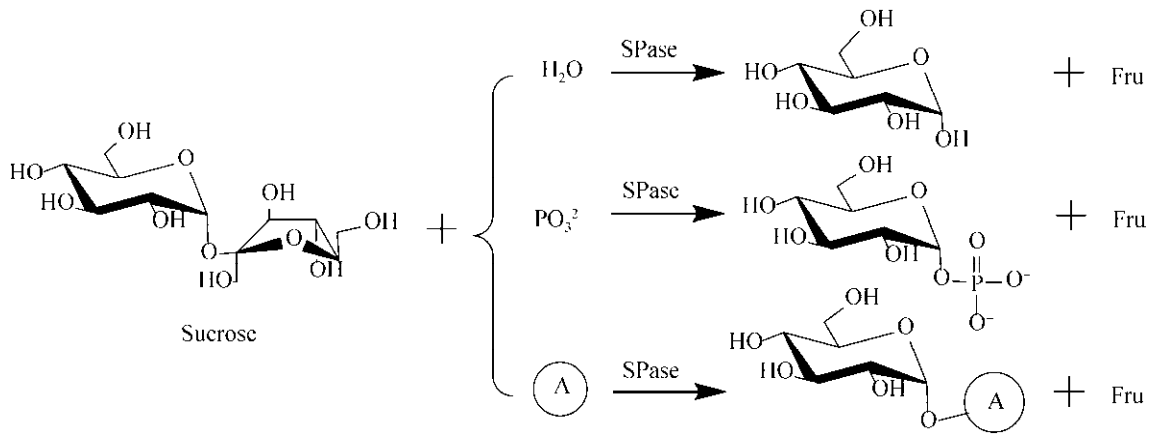


图 2 SPase 催化反应类型

Fig. 2 Three types of reactions catalyzed by sucrose phosphorylases including hydrolysis, phosphorylysis and transglycosylation. A: acceptors.

化活性使其逐渐成为酶工程研究的热点[27]。

以下从 SPase 的结构与催化特性、SPase 的定向改造、SPase 在糖基化反应中的应用 3 个方面系统性地总结了 SPase 的研究进展。最后，在此基础上对未来该课题研究的发展方向进行了展望。

1 SPase 的结构和催化特性

1.1 SPase 的结构

SPase 主要分布于肠膜明串珠菌 *Leuconostoc mesenteroides*[28]、嗜糖假单胞菌 *Pseudomonas saccharophila*[29]、变形链球菌 *Streptococcus mutans*、长双歧杆菌 *Bifidobacterium longum*[30]、青春双歧杆菌 *Bifidobacterium adolescentis* 等微生物中[31-35]，以单体或同型二聚体的形式发挥作用 (表 1)。*B. adolescentis* 来源的 SPase (*Ba*SPase) 在结构上是一个同型二聚体，其中每个单体由 4 个结构域组成：结构域 A (残基 1–85，167–291，356–435) 是由 8 个交替平行的 β-折叠和 α-螺旋组成的 $(\beta/\alpha)_8$-桶结构；结构域 B (残基 86–166) 主要由 2 个反向平行的 β-折叠和 2 个短的 α-螺旋构成；结构域 B' (残基 292–355) 主要是一长一短的 2 个 α-螺旋；结构域 C 含有 5 个反相平行的 β-折叠，拓扑学结构为 1,1,1,1 (图 3A)[36]。催化相关位点定点突变体的动力学研究证实了 SPase 的活性中心是结构域 A 中的催化三联体结构 (Asp^{192}：催化亲核试剂；Asp^{290}：过渡态稳定剂、Glu^{232}：酸/碱催化剂) (图 3B)，其在序列和结构上都具有保守性[37-39]。结构域 B 和 B'是结构元件，与 SPase 的功能密切相关，位于其上的两个回环 (Loop A，^{336}AAASNLDLY^{344} 和 Loop B，^{132}YRPRP^{136}) 接近催化活性中心，在催化过程中参与酶构型的转变[40]。结构域 C 的长回环顶端接近结构域 B'，封

表 1 SPase 的分布与结构[27]

Table 1 Distributions and structures of SPase[27]

Organism	Amino acid	Natural molecular mass (kDa)	Oligomeric state	Domains	3D structure status	Residues involved in catalysis	Reference
B. adolescentis	504	129.0	dimeric	A, B, B′, C	$(\beta/\alpha)_8$-barrel	Asp^{192}, Glu^{232}, Asp^{290}	[32]
L. mesenteroides	492	56.1	monomeric			Asp^{196}, Glu^{237}, Asp^{295}	[33]
P. saccharophila	498	78.0	dimeric			Asp^{200}, Glu^{240}, Asp^{298}	[34]
S. mutans	480	55.7	monomeric			Asp^{193}, Glu^{234}, Asp^{292}	[35]

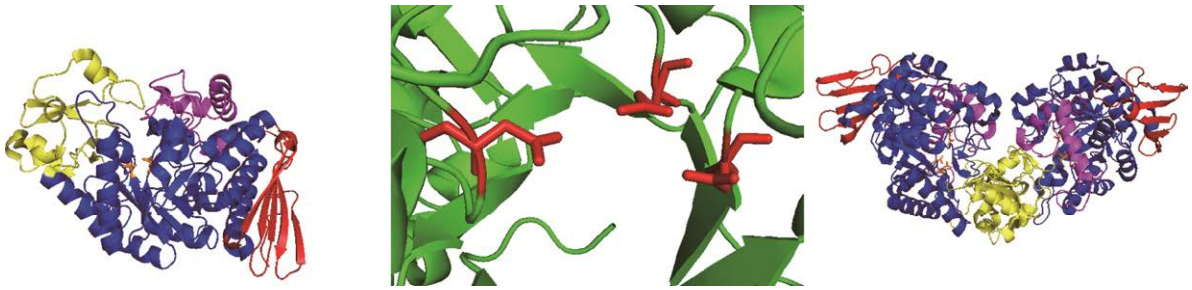


图 3 *Ba*SPase 的三维结构[36]

Fig. 3 The three-dimensional structure of *Ba*SPase[36]. (A) Monomeric structure of *Ba*SPase. (B) Catalytic triad of *Ba*SPase. (C) Structure of *Ba*SPase as a homodimer.

锁了相当于 ASase 的寡糖结合位点的区域，因此导致了两种酶催化特性的差异[41]。单体之间通过结构域 B 相互作用在二聚体内部形成空腔，底物因此能进入活性位点 (图 3C)[36]。

1.2 SPase 的催化特性

SPase 遵循保留型双取代作用机制，催化过程中存在共价连接的 β-葡萄糖基-酶中间体[34,42-43]。如图 4 所示，在催化的起始阶段，催化谷氨酸 Glu^{232} 对 Suc 糖苷键的质子化以及天冬氨酸 Asp^{192} 对葡萄糖基异头碳原子的亲核攻击同时进行，形成 β-葡萄糖基-酶中间体，释放 D-Fru。随后，受体化合物 (如磷酸盐) 与中间体反应，生成 α-D-葡萄糖基化合物。

SPase 的催化亚位点残基在催化过程中也起着至关重要的作用。Wildberger 等研究发现在 *L. mesenteroides* 来源的 SPase (*Lm*SPase) 中底物 Glc-1-P 在活性位点的定位是通过与催化三联体残基及催化亚位点残基 Arg^{137} 形成氢键实现的，并且

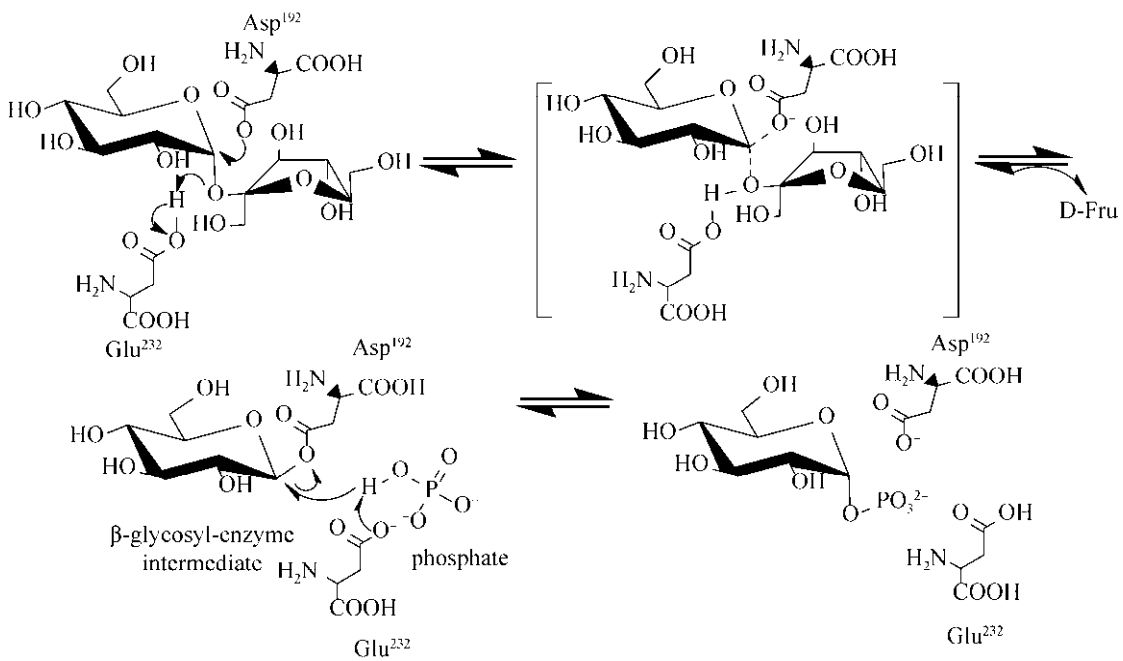


图 4 SPase 的催化机制[34]

Fig. 4 The catalytic scheme of SPase[34].

氨基酸残基 Phe^{53} 与过渡态的稳定作用密切相关[44]。催化亚位点 Asp^{49}、Arg^{395} 能与被转移的葡萄糖基形成带电荷的氢键网络，从而有利于催化反应的进行[45]。Verhaeghe 等[46]通过丙氨酸扫描技术绘制了 *Ba*SPase 的受体位点图谱，发现与 SPase 磷酸盐底物特异性相关的位点是 Pro^{134}、Arg^{135}、Tyr^{196}、His^{234}、Leu^{343}、Tyr^{344}、Gln^{345}，与 Fru 底物特异性相关的位点是 Tyr^{132}、Pro^{134}、Tyr^{196}、His^{234}、Asp^{342}、Gln^{345}。

SPase 的糖基供体底物特异性较专一，只有 Suc、Glc-1-P 及 α-D-葡萄糖-1-氟化物能够作为其糖基供体底物[47]。但其能够作用的糖基受体范围较广，尤其是 *Lm*SPase 和 *Ba*SPase[28-29,48-51]。SPase 的最小作用受体底物是乙二醇，其参与糖基化反应的区域及立体选择性取决于受体底物的结构[52]，如 SPase 作用于 1,2-丙二醇类受体化合物时，能够催化 2 位羟基的区域选择性糖基化反应[53]。Wildberger 等证实了 SPase 参与的甘油酰胺的糖基化反应存在完全的立体选择性和非对映体选择性，因此能够实现外消旋甘油酰胺的手性拆分[54]。

Silverstein 等研究了 *P. saccharophila* 来源的 SPase (*Ps*SPase) 的催化反应动力学，发现在不同受体浓度下，供体底物浓度倒数 (1/[S]) 与初始反应速率倒数 (1/[*v*]) 组成的线形图谱是相互平行的，该现象与双取代作用机制一致[34]。双取代作用机制存在着一个显著的缺点，即反应体系中的水会与葡萄糖基-酶共价中间体反应造成糖基供体底物不可逆的水解。因此，SPase 的催化过程存在糖基化反应与水解反应的竞争关系。糖基供体底物的水解产物葡萄糖会抑制 SPase 的催化活性[34,55]，影响最终产率。虽然底物的转化水平受到热力学限制，最终的糖基化产率也受到糖基化/水解反应选择性的影响，但是对反应过程进行动力学控制 (如提高受体底物的浓度等) 能够推动反应向理想方向进行[52,56-57]。

2 SPase 的定向改造

虽然 SPase 的糖基受体底物特异性广泛，具有较好的工业应用前景，但其对一些受体底物(如芳香类化合物)的亲和力不佳，导致产率低[58]，且 SPase 的有机溶剂与热稳定性也有待提高。因此，需要借助酶工程手段 (如酶的固定化技术) 改变 SPase 的作用形式，或通过理性突变、定向进化等策略进行天然 SPase 的基因修饰，实现其稳定性和催化特性的定向改造[59-62]。

2.1 SPase 的热稳定性改造

SPase 的热稳定性可以通过酶固定化技术、定点突变和定向进化技术进行改善。SPase 与酶载体 Sapebeads EC-HFA 的共价连接使其在 60 ℃条件下的半衰期超过 10 h[63]。并且，其在 60 ℃的填充床反应器中进行 Glc-1-P 的生物合成时，2 周时间内未见酶活损失[64]。将 SPase 固定化为交联酶聚集体形式能使其最适温度提高 17 ℃，且其在 60 ℃下孵育 10 d 活性不丧失[65]。*Lm*SPase 与硅结合模块 Z_{basic2} 的融合表达能够实现前者以固定化酶形式参与微生物反应器中的连续流式生物催化反应，从而提高酶活性利用率[66]。Fujii 等通过向 *S. mutans* 来源的 SPase (*Sm*SPase) 中引入随机突变的策略构建突变体文库，经高温筛选得到热稳定性改善的突变体[67]。Cerdobbel 等将 *Ba*SPase 结构中最易弯曲的氨基酸残基替换为相应序列位点最常见的氨基酸，同时结合定点突变技术促进蛋白质晶体结构中的静电相互作用，最终获得 60 ℃条件下半衰期延长 1 倍以上的突变体，值得一提的是，该突变体的有机溶剂稳定性也有所改善[68]。

2.2 SPase 的催化性质改造

SPase 的转糖苷活性、底物特异性、糖基化反应的区域及立体选择性等性质可以通过定点突变和定向进化技术得以改造。*Lm*SPase 经定点突变获得的突变体 E237Q 在酚类化合物的糖基化反应中无 Suc 水解活性，转化率因此提高了约 7 倍[39]。

何贺贺等通过向 *Lm*SPase 的活性中心近距离位点引入突变，得到了转糖苷活性增强的突变体[69]。Dirks-Hofmeister 等将热解糖热厌氧杆菌 *Thermoanaerobacterium thermosaccharolyticum* 来源的 SPase (*Tt*SPase) 活性回环中位阻效应较大的 Arg^{134} 突变为 Ala，获得的突变体 *Tt*SPase (R134A) 能实现白藜芦醇饱和水溶液中受体化合物的完全转化[70]。Kraus 等将 *Ba*SPase 的 Gln^{345} 突变为 Phe，结构域继而发生可逆性的移动，使其底物特异性发生改变[71-72]。该突变体能够高效催化一系列芳香族化合物的糖基化反应，尤其是以白藜芦醇作为受体底物时糖基化反应产率可达到 97%[73]。并且，*Ba*SPase (Q345F) 参与糖基化反应的区域选择性也发生了转变，从而实现稀有二糖黑曲霉糖 (3-*O*-a-D-吡喃葡萄糖基-D-葡萄糖，3-*O*-a-D-glucopyranosyl-D-glucose) 的高效合成[74]。Verhaeghe 等通过半理性突变和低通量筛选的方法获得了糖基化区域选择性改变的双突变体 *Ba*SPase (L341I_Q345S)，实现了益生元曲二糖 (2-*O*-α-D-吡喃葡萄糖基-D-葡萄糖，2-*O*-α-D-glucopyranosyl-D-glucose) 的选择性合成[75]。并且利用该突变体，高效、规模化的曲二糖生产流程得以建立[76]。

2.3 SPase 的高通量筛选与生产

不论是在自然界优质酶的筛选过程中，还是在工业酶的定向进化过程中，高通量筛选策略常常被用于高活性目标的高效筛选。Choi 等发现 SPase 作用于 Glc-1-P 释放的无机磷酸根离子能与钼酸铵络合成蓝色复合物，通过比色法能够实现 SPase 高活性菌株的快速筛选[77-78]。Vilozny 等利用选择性碳水化合物传感系统检测 SPase 的作用产物 Fru，实现了 SPase 的高通量筛选[79]。此外，Aerts 等开发了基于组成型启动子的高通量筛选系统，有效减少了筛选过程中的移液步骤[80]。

在宿主细胞中克隆不可培养生物体来源的 SPase 基因以实现 SPase 在工程菌株中的异源表达能够提高 SPase 在生物催化领域的应用潜力[81]。不同生物体的 SPase 的基因可通过基因工程技术获得，包括聚合酶链式反应[82]以及利用密码子优化技术实现目的基因的合成[83]等。工业上 SPase 的生产主要通过工程菌的发酵来实现，结合发酵条件 (如初始细胞密度、诱导剂浓度等) 优化，能够实现 SPase 在宿主细胞中的过量表达[84]。Su 等通过 SPase 与磷脂酶 C 的共表达，实现了胞质蛋白在胞外的高效表达，从而简化 SPase 生产中的蛋白提纯步骤[85]。

3 SPase 的应用

3.1 SPase 在糖基化反应中的应用

SPase 的糖基化受体根据涉及的反应基团的不同可分为 3 类 (表 2)：多羟基化合物、含酚基化合物、含羧基化合物。

3.1.1 SPase 参与多羟基化合物的糖基化反应

SPase 能够作用于一系列单糖、糖醇类受体，如：D-Fru[34]、D-Glc[86]、L-山梨糖[29]、L-阿拉伯糖[32]、木糖醇[49]、L-阿拉伯醇[87]，生成的产物能够作为食品、化妆品添加剂[49]。SPase 还能催化二糖、寡糖的糖基化反应，如乳糖、纤维二糖、麦芽三糖、异麦芽三糖[88]，糖基化寡糖能抑制淀粉酶和糖苷酶的活性，因此具有潜在的血糖调节功能[89]。

SPase 还能作用于非碳水化合物的醇羟基。*Lm*SPase 能催化 Suc 和甘油，实现 2-*O*-α-D-吡喃葡萄糖基-*sn*-甘油 (2-*O*-a-D-glucopyranosyl-*sn*-glycerol，2-αGG) 的高效合成[56]。2-αGG 具有强效保湿、抗衰老、舒缓修复、激活细胞等生理活性，被广泛应用为化妆品添加剂[90]。并且其甜度是 Suc 的 0.55 倍，可被应用为无致龋性的甜味剂[91]。

B. longum 来源的 SPase (*Bl*SPase) 能以 L-抗坏血酸作为糖基受体合成 2-*O*-α-D-吡喃葡糖基-L-抗坏血酸 (2-*O*-α-D-glucopyranosyl-L-ascorbic acid，AA-2G)[92]。AA-2G 有更好的体外稳定性，

在体内 AA-2G 能被 α-糖苷酶水解为 L-抗坏血酸，从而发挥其抗氧化活性[93]。利用 SPase 生物合成 AA-2G 的过程具有特殊的 pH 依赖性：pH 为 7.0–7.5 时，主要发生 Suc 的水解，副产物 AA-6G 积累；当 pH 低于 6.0 时，*Bl*SPase 高效催化 AA-2G 的合成，不产生 AA-6G，而且随着 pH 的降低，催化活性逐渐增强，在 pH 为 5.2 时，AA-2G 的合成活性达到最大[94]。

*Lm*SPase 能催化 Suc 与曲酸反应生成 5-*O*-α-D-吡喃葡萄糖基-曲酸 (Kojic acid 5-*O*-α-D-glucopyranosyl-kojic acid，KA-5G) 和 7-*O*-α-D-吡喃葡萄糖基-曲酸 (Kojic acid 7-*O*-α-D-glucopyranosyl-kojic acid，KA-7G)。其中，KA-7G 不仅保留了酪氨酸酶的抑制活性，并且其口味、水溶性、稳定性均优于曲酸，能应用于化妆品、食品等行业[51,95]。

5-乙基-4-羟基-2-甲基-3(*2H*)-呋喃酮(5-ethyl-4-hydroxy-2-methyl-3(*2H*)-furanone，EHM F)与 4-羟基-2,5-二甲基-3(*2H*)-呋喃酮(4-hydroxy-2,5-dimethyl-3(*2H*)-furanone，HDMF) 不仅能被应用为食品加工行业的芳香物质，还能抑制致癌物诱发的小鼠前胃癌的发生[96-97]。但是 EHMF 和 HDMF 极易被氧化，且易挥发[98]。*Lm*SPase 能够分别催化 EHMF 和 HDMF 与 Suc 反应，生成稳定性显著改善的 2-乙基-5-甲基-3(*2H*)-呋喃酮-4-*O*-α-D-葡萄糖苷 (2-ethyl-5-methyl-3(*2H*)-furanone-4-*O*-α-D-glucopyranoside，EMG-G) 和 2,5-二甲基-3(*2H*)-呋喃酮-4-*O*-α-D-葡萄糖苷 (2,5-dimethyl-3(*2H*)-furanone-4-*O*-α-D-glucopyranoside，DMF-G)，其在生物体内经 α-糖苷酶水解后发挥抗氧化活性[99]。

*Tt*SPase 经酶改造后获得的突变体 R134A 能够催化 3-羟基-β-内酰胺类化合物的糖基化反应[100]。该催化反应具有高度的对映体选择性，为其他手性化合物的合成奠定了基础。

3.1.2 SPase 参与含酚基化合物的糖基化反应

许多酚类化合物都具有如抗肿瘤、抗氧化、抗菌、抗炎症等生物活性，甚至能预防和治疗心脑血管疾病[101]。但酚类化合物普遍存在水溶性差、稳定性不佳、毒性高等缺点，糖基化作用能够有效改善这些缺陷[102-103]。SPase 参与的酚类化合物的糖基化反应总结如下。

对苯二酚能抑制生物体内酪氨酸酶活性从而抑制黑色素的合成，因此具有较强的美白作用[104]。但是对苯二酚对人体有很大毒副作用，且易被氧化成醌。*Lm*SPase 能催化 Suc 和对苯二酚专一性合成 α-熊果苷 (α-arbutin)，该产物对酪氨酸酶的抑制能力是 β-arbutin 的 10 倍以上且对人体无毒[50,105]。

*Ba*SPase 能催化 Suc 和邻苯三酚合成稳定性改善的邻苯三酚-2-*O*-α-D-葡萄糖苷 (Pyrogallol-2-*O*-α-D-glucopyranoside)[106]。并且，De Winter 等还设计了缓冲液/乙酸乙酯 (5/3) 的双相体系作为上述反应的溶剂系统，使受体底物的转化率提高约 7 倍[107]。

白藜芦醇是一种植物抗毒素，能够抵抗多种疾病，包括Ⅱ型糖尿病、肥胖、动脉粥样硬化、阿兹海默症等[108-109]。研究发现白藜芦醇的糖基化修饰能够改善其水溶性、稳定性、生物利用度，有利于该化合物的临床应用[110]。酶改造突变体 *Tt*SPase (R134A) 能够高效作用于白藜芦醇生成水溶性、稳定性均得到改善的糖基化产物[70]。

儿茶素类化合物具有抗炎、抗菌、抗病毒及抗氧化等特性[111-112]。其中，含量最丰富的表没食子儿茶素没食子酸酯 (Epigallocatechin gallate，EGCG) 抗氧化性最显著[113]。但 EGCG 在水中易降解，*Lm*SPase 能以 EGCG 为受体生成稳定性改善的 4′-*O*-吡喃葡萄糖基-表没食子儿茶素没食子酸酯 (4′-*O*-D-glucopyranosyl-epigallocatechin gallate，EGCG-G) 和 4′,4″-*O*-二吡喃葡萄糖基-表没食子儿茶素没食子酸酯 (4′,4″-*O*-D-diglucopyranosyl-epigallocatechin gallate，EGCG-2G)[48]。此外，*Lm*SPase 还能催化 Suc 和儿茶素合成儿茶素-3′-*O*-D-葡萄糖苷 (Catechin-3′-*O*-D-glucopyranoside，C-G)[114]。在

保留其抗氧化能力的同时，C-G 的抗褐变能力、水溶性都显著优于儿茶素[106]。

3.1.3 SPase 参与含羧基化合物的糖基化反应

许多结构上含有羧基的化合物能被运用于食品、化妆品行业，如乙酸、苯甲酸、丁酸等[115-116]，但当中许多化合物具有较强的气味和酸度，或是较差的水溶性。Sugimoto 等利用 *Sm*SPase 催化 Suc 和苯甲酸合成 1-*O*-苯甲酰-α-D-吡喃葡萄糖苷 (1-*O*-benzoyl-α-D-glucopyranoside，BA-G)，并且 *Sm*SPase 对乙酸、甲酸、丙酸、马来酸、丙二酸、富马酸、乳酸、苹果酸、阿魏酸都有糖基化活性[117]。*Sm*SPase 作用于乙酸生成的 1-*O*-乙酰-α-D-吡喃葡萄糖 (1-*O*-acetyl-α-D-glucopyranose) 的酸味阈值是乙酸的 100 倍，在口味上有较好的改善[118]。

咖啡酸具有强大的抗氧化活性，能够作为食品及化妆品添加剂[119]。咖啡酸的应用受到其水溶性、稳定性的限制。*Bl*SPase 能催化 Suc 和咖啡酸合成咖啡酸的单糖苷和二糖苷[120]。

3.2 SPase 与其他酶的联合应用

SPase 还能通过与其他酶的联合应用发挥其生物活性，形成的酶耦合系统主要分为以下 3 类：(1) SPase 能够催化 Suc 与无机磷酸生成 Glc-1-P，为其他酶提供底物，实现高附加值产物的合成(图 5)。如 *Lm*SPase 催化合成 Glc-1-P 的过程联用木糖异构酶 (Xylose isomerase, EC 5.3.1.5)、纤维二糖磷酸化酶 (Cellobiose phosphorylase，EC 2.4.1.20) 能实现食品原料纤维二糖的高产量合成[121-122]。上述催化过程联合纤维糊精磷酸化酶 (Cellodextrin phosphorylase, EC 2.4.1.49) 能高效合成可溶性短链麦芽糊精[123]。葡聚糖磷酸化酶 (Glucan phosphorylase，EC 2.4.1.1) 能作用于 *Sm*SPase 的产物 Glc-1-P 高效合成直链淀粉[124-125]。SPase 产生的 Glc-1-P 与麦芽四糖共同作为葡聚糖磷酸化酶的底物，构成了直链糊精的低成本合成途径[126]。SPase、木糖异构酶、昆布二糖磷酸化酶 (Laminaribiose phosphorylase，EC 2.4.1.31) 的联合应用实现了昆布二糖的生物合成[127]。功能糖 β-1,3-葡寡糖的大规模制备是通过 SPase 和昆布二糖磷酸化酶的联合应用实现的[128]。在 D-葡萄糖基-D-阿洛酮糖苷的合成过程中，SPase、D-塔格糖-3-差向异构酶 (D-Tagatose-3- epimerase) 的共同作用能减少投入成本[129]。Nishimoto 等设计了 SPase 在内的耦合酶催化体系，合成了乳糖-*N*-二糖Ⅰ (Lacto-*N*- biose Ⅰ，LNB Ⅰ)[130]、半乳糖-*N*-二糖 (Galacto-*N*-biose，GNB)[131]、半乳糖基-β1→4-L-鼠李糖 (Galatosyl-β1→4-L- rhamnose，GalRha)[132]。由 SPase、葡萄糖磷酸变位酶 (Phosphoglucomutase, EC 5.4.2.2)、肌醇-1-磷酸合酶 (Inositol 1-phosphate synthase，EC 5.5.1.4)、肌醇单磷酸化酶 (Inositol monophosphatase，EC 3.1.3.25) 组成的多酶体系能以热循环级联催化的方式高效合成肌醇[133]。

(2) SPase 分解 Suc 为反应提供能量。Myung 等设计了 *Lm*SPase 在内的包含 15 种生物催化剂的耦合酶系统，*Lm*SPase 作用于 Suc 为氢气生物合成过程中的水分解提供了能量[134]。

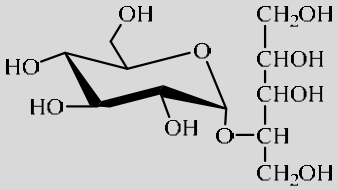
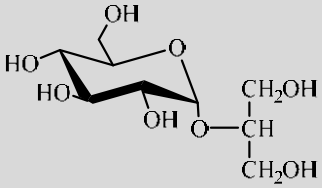
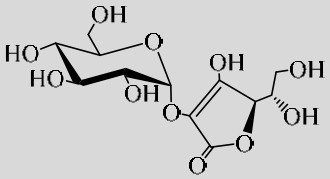
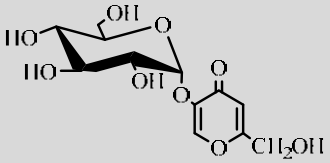
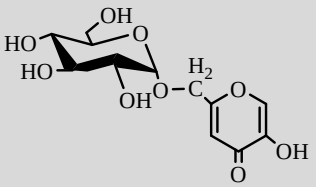
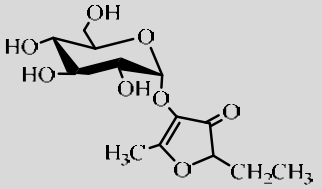
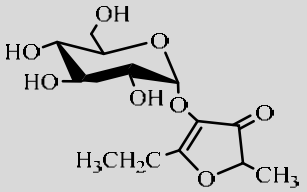
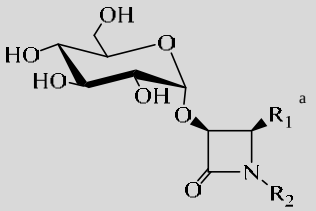
(3) 多酶联用能特异性移除 SPase 的非理想产物，驱动反应向底物消耗的方向进行，提高产率。在纤维二糖的合成过程中，葡萄糖异构酶能够将 SPase 的产物 D-Fru 转化为纤维二糖磷酸化酶的底物 Glc，产量因此显著提高[121]。在氢气的合成过程中，磷酸盐葡萄糖激酶能够去除 SPase 的产物 Glc，提高反应的总体经济[134]。

4 结论与展望

综上所述，SPase 是可作用于廉价供体的葡萄糖基转移酶，具有广泛的糖基受体底物特异性，在食品、化妆品、制药工业中具有广泛的应用。基于国内外科研工作的研究进展，SPase 课题的深入开展可从以下 4 个方面出发：(1) 筛选 SPase 的野生型高活性菌株，或利用基因工程、发酵工程、

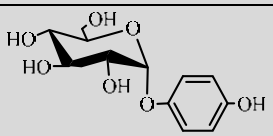
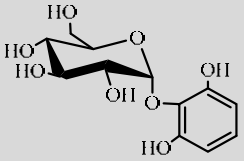
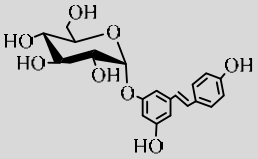
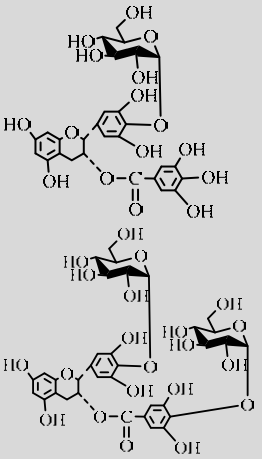
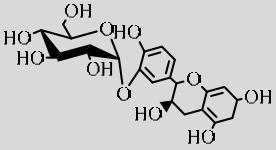
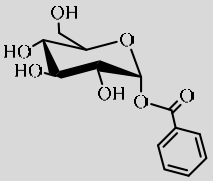
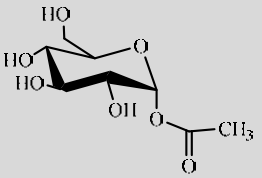
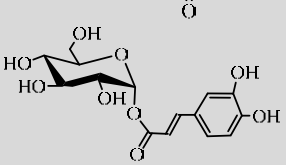
表 2 SPase 在糖基化反应中的应用

Table 2 Applications of SPase in glycosylation

Enzyme	UniProt number	Donor	Acceptor	Conditions (pH, T)	Product	Yield (%)	Reference
*Lm*SP	Q54495	Glc-1-P (20%)	Xylitol (20%)	6.9 37 °C		5.1	[49]
*Lm*SP	Q03Z66	Suc (0.8 mol/L)	Glycerol (2.0 mol/L)	7.0 30 °C		90	[56]
*Bl*SP	V6XTV6	Suc (0.8 mol/L)	L-ascorbic acid (1.2 mol/L)	5.2 40 °C		~50	[94]
*Lm*SP	Q54495	Suc (40%)	Kojic acid (2%)	7.5 42 °C	 	7.5/12.2	[51]
*Lm*SP	Q54495	Suc (50 mg)	EHMF/HDMF (5.0 mg)	7.2 32 °C	 	48/45	[99]
*Tt*SP(R134A)	D9TT09	Suc (34%)	3-hydroxy-β-lactams (15 mmol/L)	55 °C		3–17	[100]

待续

续表 2

Enzyme	UniProt number	Donor	Acceptor	Conditions (pH, T)	Product	Yield (%)	Reference
*Lm*SP	Q54495	Suc (50%)	Hydroquinone (1%)	6.5 37–42 °C		60	[50]
*Ba*SP	Q84HQ2	Suc (68%)	Pyrogallol (10%)	7.5 50 °C		72.3	[106]
*Tt*SP(R134A)	D9TT09	Suc (34%)	Resveratrol (1%)	6.5 55 °C		100	[70]
*Lm*SP	Q54495	Suc (30%)	EGCG (1%)	6.0 42 °C		30.4/39.8	[48]
*Lm*SP	Q54495	Suc (30%)	Catechin (1%)	7.5 42 °C		81	[114]
*Sm*SP	P10249	Suc (30 %)	Benzoic acid (0.8%)	3.9 40 °C		55	[117]
*Sm*SP	P10249	Suc (40%)	Acetic acid (1%)	4.2 37 °C		>90	[118]
*Bl*SP	V6XTV6	Suc (30%)	Caffeic acid (1%)	6.8 42 °C		-[b]	[120]

a: R_1=*i*Pr, Pr, Pr; R_2=Bn, *i*Pr. b: –: Not reported.

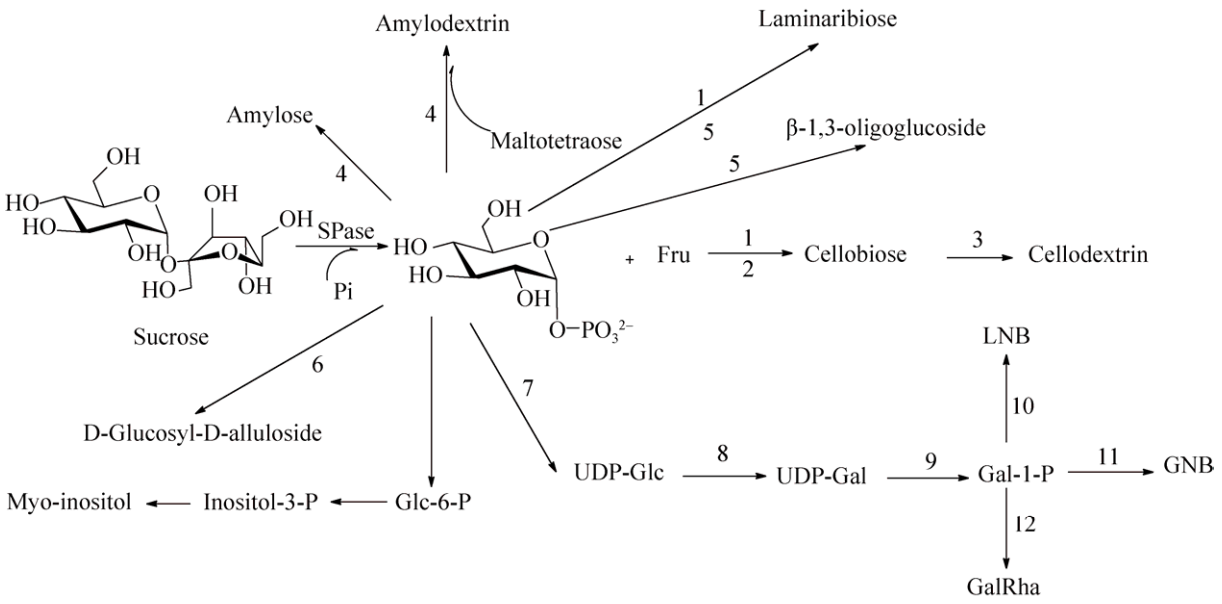


图 5 SPase 与一系列酶偶联催化合成多种化合物

Fig. 5 Applications of SPase combined with other biocatalysts by supplying Glc-1-P. 1: xylose isomerase; 2: cellobiose phosphorylase; 3: cellodextrin phosphorylase; 4: glucan phosphorylase; 5: laminaribiose phosphorylase; 6: D-tagatose-3-epimerase; 7: UDP-glucose-1-phosphate uridylyltransferase; 8: UDP-glucose-4-epimerase; 9: UDP-glucose-1-phosphate uridylyltransferase; 10: lacto-*N*-biose phosphorylase; 11: galacto-*N*-biose phosphorylase; 12: D-galactosyl-β1→4-L-rhamnose phosphorylase; 13: phosphoglucomutase; 14: inositol-1-phosphate synthase; 15: inositol monophosphatase.

代谢工程等手段提高 SPase 产率和活力[81],丰富其来源。(2) 许多蛋白质与机理研究强调了酶活性回环、底物进入通道对催化性质的重要性[135-136]。基于该理念以及改造目的灵活制定半理性、理性突变策略，结合高通量筛选方法实现 SPase 的定向进化，提高其应用潜力。(3) 向催化体系中添加助溶剂能够成功改善疏水性糖基受体在催化体系中溶解度低的问题[137]，需要综合考虑 SPase 的稳定性以及疏水性受体化合物的溶解度问题，设计理想的反应介质以拓展 SPase 的应用范围。(4) 借助酶工程手段 (酶固定化技术) 实现 SPase 的重复使用，降低其在工业应用中的成本。

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