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·专题论坛·

植物中松脂醇-落叶松脂素还原酶催化特征研究进展

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摘要 松脂醇-落叶松脂素还原酶(PLR)是植物中木脂素生物合成的关键酶, 能够催化松脂醇转化为落叶松脂素, 并进一步催化落叶松脂素生成开环异落叶松脂素, 且存在底物立体选择性, 是一种NADPH依赖型还原酶。PLR的催化产物位于不同类型8-8′木脂素的源头, 其底物选择性直接决定木脂素的骨架类型, 如呋喃、二苄基丁烷、二苄基丁内酯和芳基四氢萘木脂素。因此, PLR的催化特性和表达特征在植物木脂素组成及其生物活性多样性中发挥重要作用。该文综述了PLR在植物木脂素生物合成中的作用、对映异构体选择性及其催化机制, 以期为进一步研究*PLR*基因的生物学功能以及催化机制奠定基础, 并为不同类型木脂素的精确生物合成指明方向。

关键词 松脂醇-落叶松脂素还原酶, 木脂素, 催化特征, 对映体选择性, 催化机制

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木脂素是一类由两分子苯丙素衍生物(即C_6-C_3单体)氧化聚合而成的次生代谢产物, 在非维管植物(Takeda et al., 1990; Cullmann and Becker, 1999; Scher et al., 2003)及维管植物(Wada et al., 1992)中均有分布。按照其结构类型可分为8大类: 芳基萘(AN)、芳基四氢萘(AT)、二苯并环辛二烯(DCO)、二苄基丁烷(DB)、二苄基丁内酯(DBLL)、二苄基丁酸乳酯(DBL)、呋喃(FR)和双并四氢呋喃(FF) (Umezawa, 2003; Teponno et al., 2016)。木脂素在植物中的生物学功能主要有抗真菌(Li et al., 2019)、抗菌(Céspedes et al., 2006; Favela-Hernández et al., 2012)及拒食(Kraus and Spiteller, 1997)等。一些木脂素表现出很强的毒性, 如鬼臼毒素(Bohlin and Rosen, 1996), 推测其主要功能可能与保护植物免受食草动物和病原微生物的侵袭有关。许多木脂素具有多种生物活性, 如抗病毒(Gordaliza et al., 2004; Allen et al., 2007)、抗肿瘤(刘长军和侯嵩生, 1997; 程丽姣等, 2006)、抗氧化(Hano et al., 2017)及抗菌消炎(Kassuya et al., 2005; Zheng et al., 2014), 对人类健康有积极影响。

木脂素类化合物通常含有多个手性中心, 具有旋光性, 其生物活性与化学结构的构型密切相关(Suzuki et al., 2002)。例如, Guo等(2019)通过体外细胞毒性实验, 发现(+)-crataegifin B (IC_{50}=25.47 μmol·L^{-1})对肝癌细胞Hep3B毒性作用明显强于(−)-crataegifin B (IC_{50}=59.37 μmol·L^{-1}); (+)-crataegifin B诱导细胞凋亡的作用也比(−)-crataegifin B更显著; (+)-crataegifin C (IC_{50}=34.29 μmol·L^{-1})对HepG2细胞的毒性作用强于(−)-crataegifin C (IC_{50}>100 μmol·L^{-1})。Zhou等(2018)发现化合物(±)-rasidasin II能减弱H_2O_2诱导的神经毒性, 相比其对映异构体(以下简称对映体)(−)-rasidasin II具有更显著的神经保护作用。松脂醇-落叶松脂素还原酶(pinoresinol-lariciresinol reductases, PLR)是木脂素生物合成中的关键酶, 不仅影响木脂素的结构类型, 而且影响对映体的形成(Umezawa, 2003)。本文对PLR在木脂素生物合成中的作用、催化特征及催化机制进行综述, 以期为进一步研究*PLR*基因的生物学功能以及催化机制奠定基础, 并为通过合成生物学或植物次生代谢工程实现不

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同类型木脂素对映体的精准生物合成提供思路。

1 PLR在木脂素生物合成中的作用

目前, 木脂素上游生物合成途径已比较清晰(图1)。木脂素的合成首先由苯丙烷途径合成木脂素类化合物的前体松柏醇, 随后松柏醇在漆酶(laccase, Lac)和一类具有dirigent结构域的指导蛋白(dirigent protein, DIR)共同作用下发生氧化耦合反应, 形成松脂醇(pinoresinol, PIN)。在耦合过程中同时进行位置选择(region selectivity)和立体选择(stereo selectivity) (陈瑞兵, 2018)。

PLR是在两分子木脂素单体初始二聚后参与木脂素生物合成的第1个酶, 属于NADPH依赖型还原酶, 能够催化松脂醇生成落叶松脂素(lariciresinol, LAR), 再将落叶松脂素转化为开环异落叶松脂素(secoisolariciresinol, SEC) (van Fürden et al.,

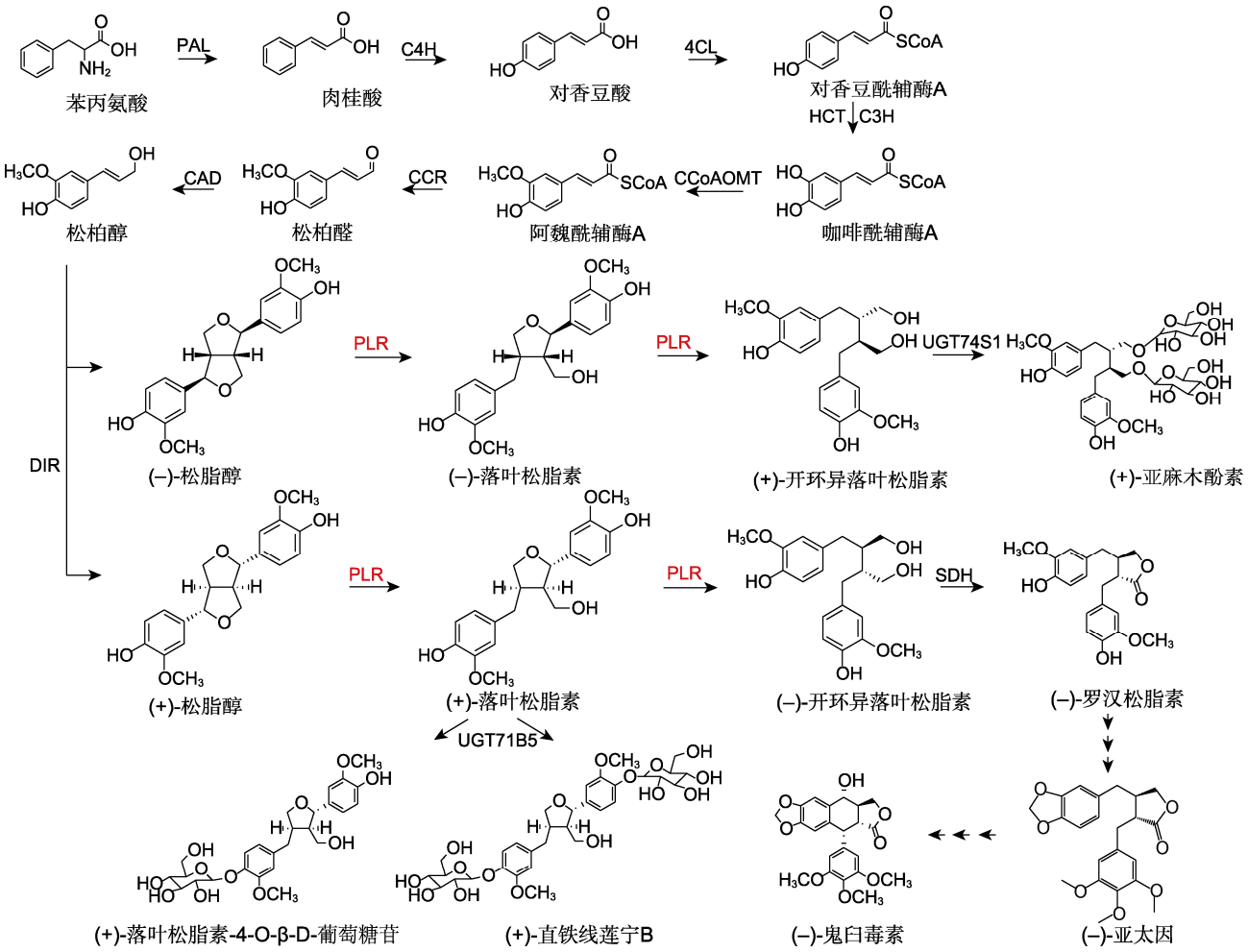

图1 木脂素生物合成途径

PAL: 苯丙氨酸解氨酶; C4H: 肉桂酸-4-羟化酶; 4CL: 4-香豆酸:辅酶A连接酶; HCT: 羟基肉桂酰转移酶; C3H: 香豆酸-3-羟化酶; CCoAOMT: 咖啡酰辅酶A-O-甲基转移酶; CCR: 肉桂酰辅酶A还原酶; CAD: 肉桂醇脱氢酶; DIR: 聚合蛋白酶; PLR: 松脂醇-落叶松脂素还原酶; SDH: 开环异落叶松脂醇脱氢酶; UGT: 依赖尿苷二磷酸的糖基转移酶

Figure 1 Lignan biosynthetic pathways

PAL: Phenylalanine ammonia-lyase; C4H: Cinnamate-4-hydroxylase; 4CL: 4-coumarate:coenzyme A ligase; HCT: Hydroxycinnamoyltransferase; C3H: Coumarate-3-hydroxylase; CCoAOMT: Caffeoyl coenzyme A-O-methyltransferase; CCR: Cinnamoyl-CoA reductase; CAD: Cinnamyl alcohol dehydrogenase; DIR: Dirigent protein; PLR: Pinoresinol-lariciresinol reductase; SDH: Secoisolariciresinol dehydrogenase; UGT: Uridine diphosphatedependent glycosyltransferase

2005)。大多数PLR催化这2个连续的还原步骤(von Heimendahl et al., 2005)。而拟南芥(*Arabidopsis thaliana*)中的PLR只能催化松脂醇生成落叶松脂素，因此被称为松脂醇还原酶(pinoresinol reductases, PrR) (Nakatsubo et al., 2008)。PLR的催化产物落叶松脂素和开环异落叶松脂素是合成不同类型8-8′木脂素的起点，包括呋喃、二苄基丁烷、二苄基丁内酯和芳基四氢萘木脂素等(Dinkova-Kostova et al., 1996)。作为光学活性木脂素生物合成的关键节点，PLR不仅影响木脂素的结构类型，而且影响木脂素对映体的形成(Umezawa, 2003)。例如，抗肿瘤药物依托泊苷(etoposide)的前体化合物(−)-鬼臼毒素((−)-podophyllotoxin) (Stadler and Bach, 2008)；用于治疗雌激素依赖性疾病的亚麻木酚素((+)-secoisolariciresinol diglucoside, (+)-SDG) (Calado et al., 2018)；具有抗增殖活性的亚太因((−)-yatein) (Kuo et al., 2006)；具有抗病毒抗炎功效的直铁线莲宁B (clemastanin B, (+)-LDG)和(+)-落叶松脂素-4-O-β-D-葡萄糖苷((+)-lariciresinol-4-O-β-D-glucopyranoside, (+)-L4G) (Chen et al., 2021) (图1)。调控*PLR*基因的表达对木脂素生物合成有重要影响。Ayella等(2007)在小麦(*Triticum aestivum*)中异源表达金钟连翘(*Forsythia intermedia*)中的*FiPLR*基因，使SDG的积累显著增加。Xiao等(2015)在菘蓝(*Isatis indigotica*)毛状根中过表达*IiPLR1*基因，使落叶松脂素产量达野生型的6.3倍。SDG是亚麻(*Linum usitatissimum*)种皮中的主要木脂素，Renouard等(2014)利用RNAi技术下调亚麻种子*LuPLR1*基因的表达，使SDG的积累急剧减少。Hemmati等(2007)采用RNAi技术下调宿根亚麻(*Linum perenne*)毛状根中*LpPLR1*基因的表达，导致爵床脂素B (justicidin B)的积累减少近75%。综上，PLR在木脂素生物合成中具有重要作用。

2 植物*PLR*基因的克隆及序列分析

植物*PLR*基因最早在金钟连翘中被发现。近年来，在拟南芥、菘蓝以及亚麻属(*Linum*)植物等中均有报道。对植物中的*PLR*基因进行汇总(表1)，显示每种植物

表1 植物中的*PLR*基因

Table 1 *PLR* genes from different plant species

植物名称	基因名称	GenBank登录号	cDNA长度(bp)	开放阅读框长度(bp)	参考文献
金钟连翘(*Forsythia intermedia*)	*FiPLR1*	U81158	1047	939	Dinkova-Kostova et al., 1996
拟南芥(*Arabidopsis thaliana*)	*AtPrR1*	NM102944	1129	954	Nakatsubo et al., 2008
	AtPrR2	NM117440	1206	954	
菘蓝(*Isatis indigotica*)	*IiPLR1*	JF264893	1062	954	Xiao et al., 2015
亚麻(*Linum usitatissimum*)	*LuPLR1*	AJ849359	–	939	von Heimendahl et al., 2005
	LuPLR2	EU029951	1203	993	Hemmati et al., 2010
白亚麻(*L. album*)	*LaPLR1*	AJ849358	1482	981	von Heimendahl et al., 2005
黄亚麻(*L. flavum*)	*LfPLR*	MK599138	–	975	Akira et al., 2016
长萼亚麻(*L. corymbulosum*)	*LcPLR1*	EU107358	1244	948	Bayindir et al., 2010
宿根亚麻(*L. perenne*)	*LpPLR1*	EF050530	1145	945	Hemmati et al., 2007
桃儿七(*Sinopodophyllum hexandrum*)	*ShPLR*	EU855792	983	936	Wankhede et al., 2013
六角莲(*Dysosma pleiantha*)	*DpPLR*	KJ000045	–	933	Kuo et al., 2014
北美乔柏(*Thuja plicata*)	*TpPLR1*	AF242503	1190	942	Fujita et al., 1999
	TpPLR2	AF242504	1151	939	
	TpPLR3	AF242505	1308	945	
	TpPLR4	AF242506	1287	939	
台湾杉(*Taiwania cryptomerioides*)	*TcPLR1*	MG264424	–	975	Chiang et al., 2018
	TcPLR2.2	MG264425	1138	942	
	TcPLR3	MG264426	1102	939	
山茶(*Camellia sinensis*)	*CsPLR1*	MH037247	1325	939	Wu et al., 2019
	CsPLR2	MH037248	1126	939	

– 未知 – Unknown

中*PLR*的数量为1–4个, 开放阅读框(open reading frames, ORFs)长度为933–993 bp。用ESPript 3.0软件(Robert and Gouet, 2014)对已有功能验证的PLR蛋白进行氨基酸序列比对, 并对已获得蛋白晶体结构的PLR二级结构域进行比对(图2), 表明PLR为NADPH依赖型还原酶, 拥有完全保守的NADPH结合基序GXXGXXG。

3 PLR的底物和对映体的选择性

大多数PLR能有效地将松脂醇和落叶松脂素分别还原为落叶松脂素和开环异落叶松脂素。然而, PLR对松脂醇和落叶松脂素的亲和力也不尽相同, 表现出底物选择性。此外, 植物中的PLR多数表现出底物手性选择性。PLR作为还原酶催化2个连续的还原反应, 同时考虑到松脂醇和落叶松脂素各自的对映体作为底物, 反应共涉及4个底物。因此要想深入研究PLR及其所参与的还原反应, 必须同时了解PLR对松脂醇和落叶松脂素的底物选择性和对映体选择性。

Nakatsubo等(2008)从拟南芥中鉴定并注释了2个*PLR*基因——*AtPrR1*和*AtPrR2*, 并用大肠杆菌(*Escherichia coli*)制备的重组酶进行体外酶活检测, 发现AtPrR1对松脂醇的催化活性是落叶松脂素的35倍, 而AtPrR2在孵育1小时后仍未能将落叶松脂素还原成开环异落叶松脂素, 表明AtPLR1/2对松脂醇表现出严格的底物选择性。而其它植物的PLR能有效地将松脂醇和落叶松脂素分别还原为落叶松脂素和开环异落叶松脂素。因此, 拟南芥的2个PLR被命名为松脂醇还原酶(PrR) (Umezawa, 2003)。此外, AtPrR1可将(+)-松脂醇和(−)-松脂醇分别还原为(+)-落叶松脂素和(−)-落叶松脂素, 且具有相似的动力学参数; 而AtPrR2只能将(−)-松脂醇还原为(−)-落叶松脂素, 表现出严格的对映体选择性(Nakatsubo et al., 2008)。

研究发现, 植物中的PLR可以选择性催化(+)-松脂醇和(+)-落叶松脂素分别生成(+)-落叶松脂素和(−)-开环异落叶松脂素, 或催化(−)-松脂醇和(−)-落叶松脂素分别生成(−)-落叶松脂素和(+)-开环异落叶松脂素的单一或连续还原反应。我们对植物PLR的对映体选择性进行了汇总(图3)。偏好催化(+)-松脂醇→(+)-落叶松脂素→(−)-开环异落叶松脂素反应的PLR包括FiPLR1 (Dinkova-Kostova et al., 1996)、LaPLR1 (von Heimendahl et al., 2005)、LcPLR1 (Bayindir et al., 2008)、LuPLR2 (Hemmati et al., 2010)、PhPLR (Lau and Sattely, 2015)、PpPLR (Kuo et al., 2014)、TcPLR1、TcPLR2.2、TcPLR3 (Chiang et al., 2018)及CsPLR2 (Wu et al., 2019)。其中, FiPLR1、LaPLR1 (von Heimendahl et al., 2005)和LcPLR1 (Bayindir et al., 2010)具有相似的行为, 均优先选择(+)-松脂醇作为底物, 当(+)-松脂醇全部转化为(+)-落叶松脂素且有剩余活性PLR时才会生成(−)-开环异落叶松脂素; LuPLR2将(+)-松脂醇全部转化为(−)-开环异落叶松脂素, 没有中间体(+)-落叶松脂素的积累, 表明其对(+)-落叶松脂素的催化效率高于(+)-松脂醇(Hemmati et al., 2010)。而偏好催化(−)-松脂醇→(−)-落叶松脂素→(+)-开环异落叶松脂素的主要有AtPrR2 (Nakatsubo et al., 2008)和LuPLR1 (von Heimendahl et al., 2005)。其中, AtPrR2只能催化(−)-松脂醇生成(−)-落叶松脂素(Nakatsubo et al., 2008); LuPLR1对(−)-松脂醇的催化效率明显高于(−)-落叶松脂素(von Heimendahl et al., 2005)。2个方向均能催化的主要有AtPrR1 (Nakatsubo et al., 2008)、TpPLR1 (Fujita et al., 1999)、TpPLR2 (Fujita et al., 1999)、CsPLR1 (Wu et al., 2019)和LpPLR1。其中, TpPLR1将(−)-松脂醇全部转化为(+)-开环异落叶松脂素, 没有中间体(−)-落叶松脂素的积累, 将(+)-松脂醇还原为(+)-落叶松脂素, 但不能继续将(+)-落叶松脂素还原为(−)-开环异落叶松脂素, 且对(−)-落叶松脂素的催化效率高于(±)-松脂醇; TpPLR2对(+)-松脂醇的催化效率约为(−)-松脂醇的7倍(Fujita et al., 1999), 但不能将(−)-落叶松脂素还原为(+)-开环异落叶松脂素; LpPLR1可以还原松脂醇和落叶松脂素对映体, 特别是LpPLR1对松脂醇和落叶松脂素表现出完全相反的对映专一性, 即优先选择(+)-松脂醇和(−)-落叶松脂素作为底物(Hemmati et al., 2007)。

利用MEGA 7.0软件(Sudhir et al., 2016)对不同来源的PLR构建系统发育树(图3)。系统发育分析表明, PLR的底物或对映体选择性与其进化距离不直接相关。因此, 要进一步了解其催化特征的内在机制需要从解析PLR的蛋白空间结构入手。植物中PLR的催化特性导致不同植物中各种类型木脂素的积累有差异(Umezawa et al., 1991; von Heimendahl et al., 2005); 同时这些PLR在不同植物器官中的表达水平

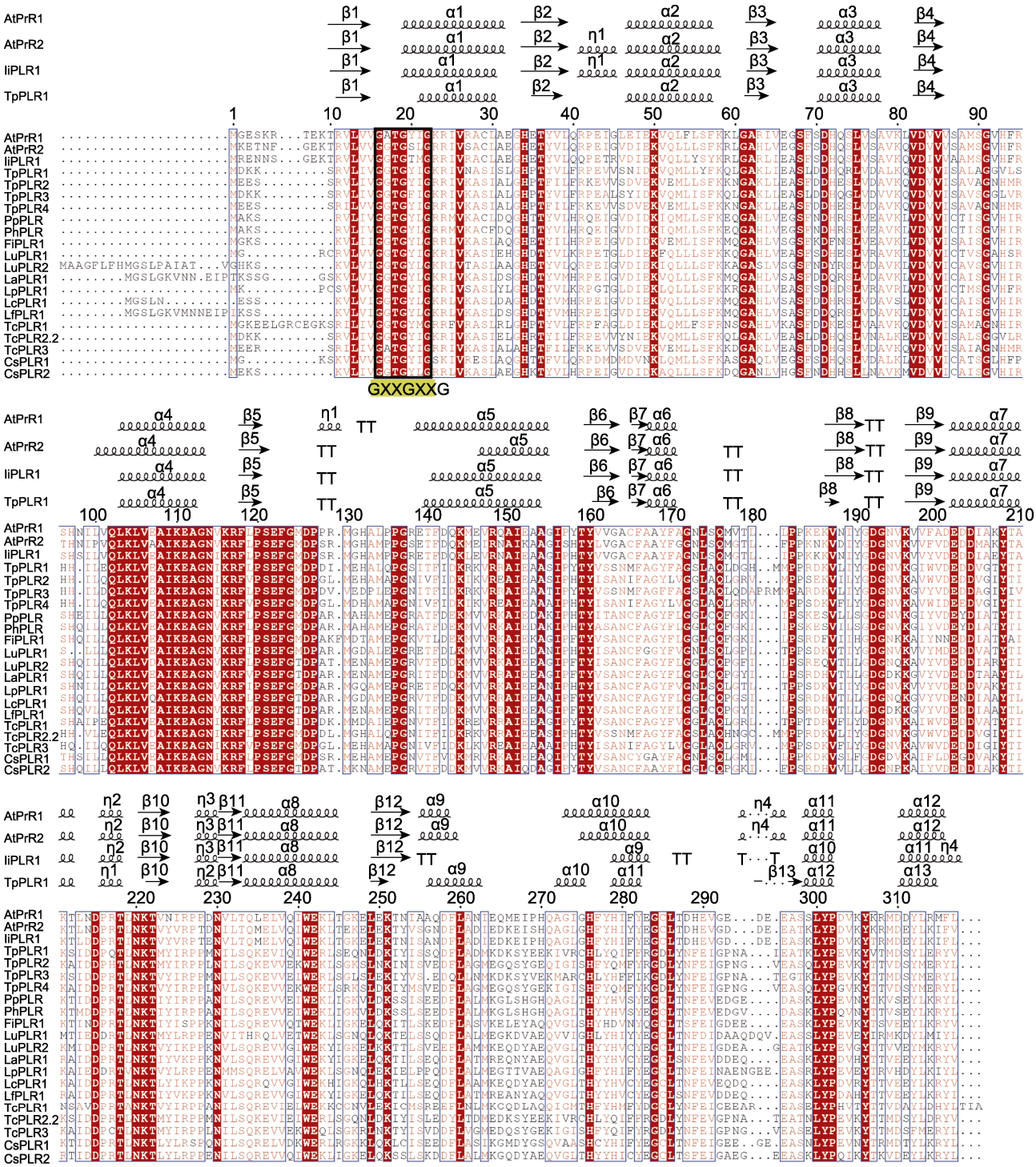

图2 PLRs蛋白氨基酸序列及二级结构域比对(GXXGXXG: NADPH结合基序)

Figure 2 Comparison of amino acid sequences and secondary structure domain of PLRs (GXXGXXG: NADPH-binding motif)

对木脂素的空间积累也产生影响。例如, *LuPLR1*在亚麻的根和种子中表达(Renouard et al., 2014), 因此在种皮主要积累(+)-SDG; 而*LuPLR2*在叶和茎中表达(Hemmati et al., 2010), 在叶中主要积累(−)-yatein (Corbin et al., 2018)。研究PLR的对映体选择性为全面了解木脂素类化合物在植物体内的时空分布

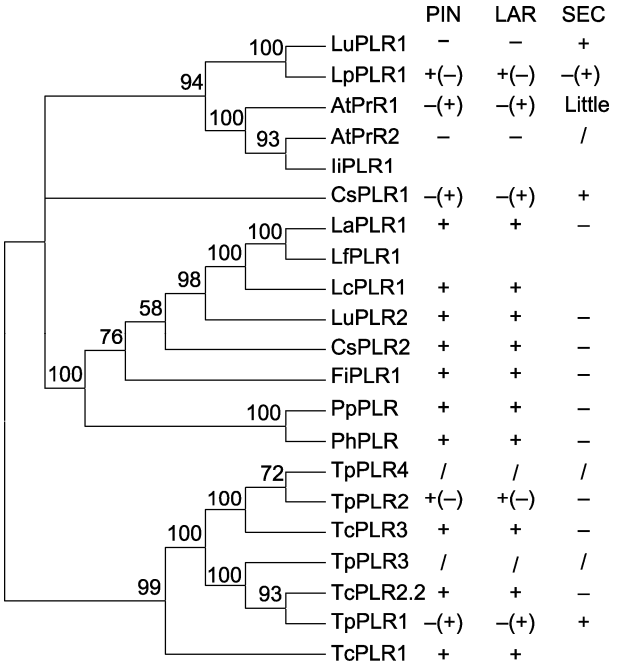

图3 植物中的PLRs系统发育树及其对映体选择性

PIN: 松脂醇; LAR: 落叶松脂素; SEC: 开环异落叶松脂素。右侧为植物中的PLR对松脂醇和落叶松脂素对映体选择性的具体表现形式(/表示无催化活性)

Figure 3 Phylogenetic tree and enantioselectivity of PLRs in plants

PIN: Pinoresinol; LAR: Lariciresinol; SEC: Secoisolariciresinol. On the right side, specific forms of enantioselectivity of PLRs in plants to pinoresinol and lariciresinol (/ indicate no catalytic activity)

及代谢流特征提供了重要依据。

4 基于蛋白晶体结构解析PLR催化机制

PLR底物选择性和对映体选择性的分子机制一直是生物化学领域的研究热点。近年来, 结构生物学的发展极大地推动了基于蛋白晶体结构解析PLR底物选择性的分子机制研究。

Min等(2003)获得了TpPLR1的蛋白晶体结构(PDB ID为1QYD; 分辨率为2.5Å)。TpPLR1为二聚体, 表观分子质量为69.9±0.4; 整个结构由2个结构域组成, 分别是与辅助因子$NADP^+$结合的N-末端结构域和与底物结合的C-末端结构域。较大的N-末端结构域有7个平行的β-折叠, 它们被6个α-螺旋包围, 除第7个β-折叠外, 其余全部由N-末端的氨基酸组成。较小的C-末端结构域包含2个独立的β-折叠和4个较小的α-螺旋, 推测与底物的结合有关。TpPLR1在N端第1个β-α-β单元中含有完全保守的NADPH结合基序^{11}GXXGXXG^{19}。底物结合口袋周围大部分为疏水性氨基酸, 由2个β-链和2个α-螺旋组成。底物结合部位与辅助因子相邻, 底物分子朝向NADPH的烟酰胺环。参与$NADP^+$结合的氨基酸残基有Tyr15、Ile16、Ser41和Arg142, Lys45和Phe160的侧链可以稳定NADPH的烟酰胺环和2'-磷酸基团。在推测的活性部位存在保守的Lys138, 将其突变为Ala使TpPLR1丧失催化松脂醇转化为落叶松脂素的能力, 说明Lys138是TpPLR1的关键活性位点氨基酸。然而, 由于缺乏PLR与底物分子的共晶结构, PLR底物选择性分子机制研究受到极大限制。

Wu等(2019)从山茶(*Camellia sinensis*)中获得2个CsPLR (CsPLR1和CsPLR2), CsPLR1可将(−)-松脂醇和(−)-落叶松脂素分别转化为(−)-落叶松脂素和(+)-开环异落叶松脂素, 也可将(+)-松脂醇转化为(+)-落叶松脂素; CsPLR2选择性地催化(+)-松脂醇和(+)-落叶松脂素分别生成(+)-落叶松脂素和(−)-开环异落叶松脂素。以TpPLR1的晶体结构为模板构建CsPLR1的同源模型, 通过对CsPLR模型进行分析, 发现底物结合口袋入口处的柔性环(CsPLR 167–178位氨基酸)上的一些氨基酸是CsPLR的关键活性位点, 并可能在一定程度上影响底物选择性。例如, CsPLR1的A165L、N167G和L171P突变体几乎失去酶活性, L174I突变体失去还原(±)-松脂醇的能力, 但保留了还原(−)-落叶松脂素的能力。CsPLR2的G167N突变体对(+)-落叶松脂素的催化活性显著降低, C169S突变体对(+)-松脂醇和(+)-落叶松脂素的催化活性急剧下降。尽管PLR底物选择性的结构基础研究取得了一些进展, 但结构模拟并不能完全展示真实的结构信息, 因此仍缺乏理论依据。

Xiao等(2021)获得了IiPLR1 (图4A)、AtPrR1和AtPrR2蛋白晶体结构(PDB ID分别为7CS2、7CS9和7CSG; 分辨率分别为2.69 Å、2.80 Å和2.00 Å)及它们分别与辅助因子、底物和产物结合的16个晶体结构。IiPLR1和AtPrR1/2的结构与先前报道的TpPLR1同为2个头尾相接的二聚体构象, 每个单体均包含N-末端的$NADP^+$结合域(NBD)和C-末端的底物结合域(SBD) (图4A), 包含NADPH特异性结合基序GXXG-

XXG, 2个结构域中间有个大凹槽。在IiPLR1蛋白结构中，α10-螺旋与相邻单体中的β2-环共同参与底物结合，Val46、Ser98、Met125、Phe166、Tyr169、Phe170、His276和Phe277是IiPLR1的关键活性位点氨基酸(图4B)。IiPLR1的V46A突变体催化松脂醇生成落叶松脂素的效率有所提高，而将落叶松脂素转化为开环异落叶松脂素的效率显著降低。对比IiPLR1_NAP_+PIN (PDB ID为7CS4; 分辨率为2.31 Å)和AtPrR1/2_NAP_+PIN (PDB ID为7CSB和7CSH; 分辨率为2.00 Å和1.59 Å)的晶体结构，发现β4-环在IiPLR1中是柔性的，而在AtPrR1/2中是刚性的。AtPrR1/2的β4-环扭曲，呈8字状，并覆盖NADP$^+$和底物结合的凹槽，环上的His93和His97分别与α5-螺旋和α10-螺旋结合，Val92和Phe94与(+)-Pin相互作用，Arg95与NADP$^+$以及相邻单体上的GXXGXXG基序和α2-螺旋具有强烈的相互作用，β4-环可能与PrR的底物选择性相关。氨基酸序列比对发现，这3个PLR蛋白的β4-环所对应的氨基酸残基十分相似，只有IiPLR1的β4-环的Ser98在AtPrR1/2对应位置被替换为Asn98，空间位阻的增加使AtPrR1/2中β4-环的摆动受到限制，从而影响底物进入与产物释放。AtPrR1的N98S突变体催化落叶松脂素转化为开环异落叶松脂素的效率提高；AtPrR2的N98S突变体获得了将落叶松脂素还原为开环异落叶松脂素的能力。

通过对PLR蛋白晶体结构的解析，Xiao等(2021)精确描绘出PLR的催化反应过程。首先，PLR二聚体的单体通过灵活的β4-环结合游离的NADPH。然后，松脂醇与其中一个单体的底物结合口袋结合，另一个单体用于稳定底物；松脂醇接受从NADPH释放的H还原为落叶松脂素，并被释放。随后，游离的落叶松脂素被另一个PLR单体结合并被相邻单体固定，还原为开环异落叶松脂素并被释放。与PLR相比，PrRs对落叶松脂素的结合要求更加严格，因此难以高效地催化落叶松脂素。

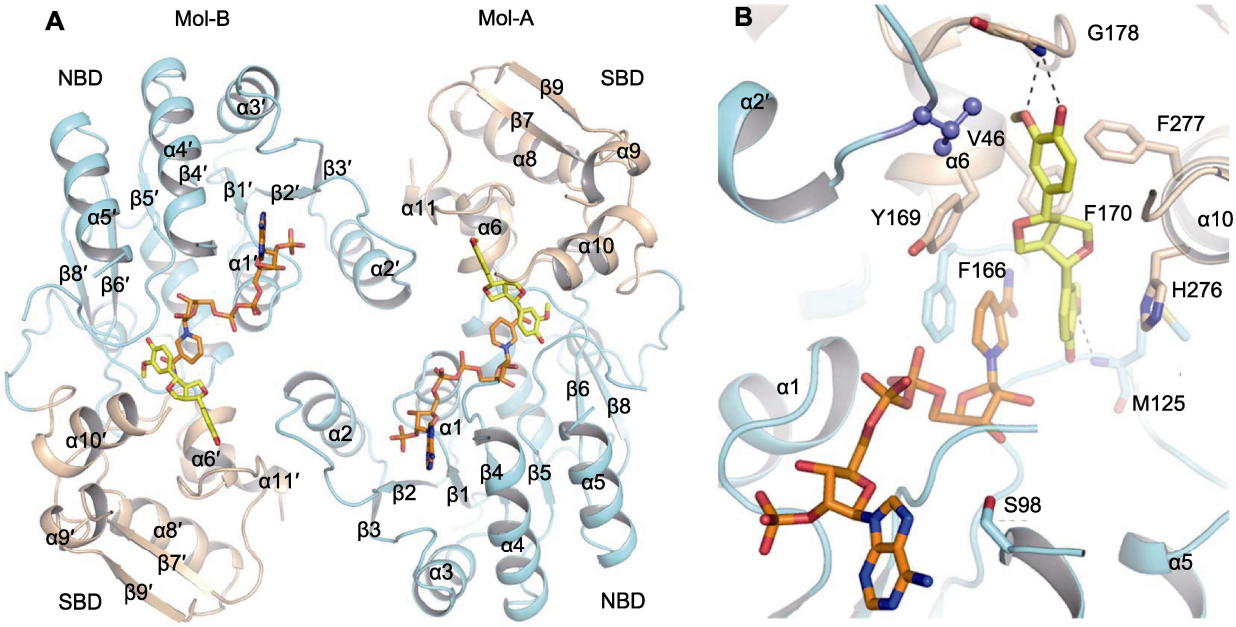

图4 IiPLR1_NAP_+PIN蛋白晶体结构**(A)**和底物结合凹槽细节**(B)**

α: α-螺旋; β: β-折叠。NADPH和+PIN的棒状结构分别为橙色和黄色; N-端的NADP$^+$结合域(NBD)和C-端的底物结合域(SBD)分别为淡绿色和姜黄色; 虚线为+PIN与其周围活性氨基酸可能存在的氢键作用; Mol-A和Mol-B为同源二聚体的2个单体。

Figure 4 Protein crystal structure of IiPLR1_NAP_+PIN **(A)** and detail of substrate bonding groove **(B)**

α: α-helix; β: β-sheet. NADPH and +PIN were highlighted in orange and yellow, respectively; the N-terminal NADP$^+$ binding domain (NBD) and the C-terminal substrate binding domain (SBD) were colored in light green and ginger, respectively; dotted lines denote possible hydrogen bonds between +PIN and active residues around substrate; Mol-A and Mol-B are two monomer of homodimer.

5 总结与展望

木脂素是一类苯丙烷二聚体, 具有独特的立体化学性质, 且与其生物活性密切相关(Rahman, 1990; Davin, 1992)。PLR是木脂素生物合成中的关键酶, 不仅影响木脂素的结构类型, 而且直接影响对映体的形成(Umezawa, 2003)。研究表明, 植物中的PLR包含多个家族成员, 表现出严格的底物选择性和对映体选择性, 其在不同植物或不同器官中的表达差异直接影响不同类型木脂素对映体的积累和分布(Hano et al., 2006; Corbin et al., 2017)。PLR蛋白晶体结构的全面解析极大地加深了人们对PLR催化机制及底物选择结构基础的认识(Xiao et al., 2021)。然而, 植物中PLR对映体选择性的分子机制尚不明确, 而相关研究将对通过合成生物学或植物次生代谢工程实现不同类型木脂素对映体的精准生物合成及可持续生产具有重要的指导意义和应用价值。

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Research Progress on Catalytic Characteristics of Pinoresinol-lariciresinol Reductase in Plants

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Abstract Pinoresinol-lariciresinol reductases (PLRs) are key enzymes involved in the lignan biosynthesis in plants, which convert pinoresinol to lariciresinol and then to secoisolariciresinol. PLRs are NADPH-dependent reductases with substrate stereoselectivity. The catalytic products of PLR are the sources of different types of 8-8′ lignans, and the substrate selectivity directly determines the skeleton types of lignans, such as furano, dibenzylbutane, dibenzylbutyrolactone and aryltetrahydronaphthalene lignans. Therefore, the catalytic and expression characteristics of PLRs play an important role in the composition and biodiversity of lignans in plants. This paper reviewed the research progress on the important role of PLRs in lignans biosynthesis, as well as its enantioselectivity and catalytic mechanism, thus to lay the foundation for further study on the biological function and catalytic mechanism of *PLR* genes, and point out the direction for the precise biosynthesis of different types of lignans enantiomers through synthetic biology.

Key words pinoresinol-lariciresinol reductases (PLRs), lignans, catalytic characteristics, enantioselectivity, catalytic mechanism

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