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磷酸酰胺天然产物氮磷键的生物合成研究进展

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摘要 磷酸酰胺天然产物是指生物体产生的含有氮磷键的小分子化合物. 因其结构和细胞内磷酸化的生物小分子或羧酸小分子相似, 它们竞争生物生命活动某些必需酶的功能, 从而表现出各种各样的生物活性. 目前已知的磷酸酰胺天然产物氮磷键生物合成机制可以分为3类: MccB蛋白催化类、APS腺苷酰基转移酶(adenylyl sulphate: ammonia adenylyltransferase, APSAT)催化类和丙酮酸磷酸二激酶(pyruvate phosphate dikinase, PPDK)同源蛋白催化类. 本文就这3类氮磷键生物合成机制进行综述.

关键词 磷酸酰胺, 氮磷键, 天然产物, 生物合成

磷酸酰胺(phosphoramidate)是指含有氮磷键(N–P)的化合物, 磷酸酰胺天然产物就是指生物体产生的含有氮磷键的小分子化合物. 本文所指的磷酸酰胺天然产物不包括磷酸酰胺化的DNA, RNA和蛋白质等生物大分子. 磷酸酰胺天然产物的结构和细胞内磷酸化的生物小分子, 以及某些羧酸小分子的结构非常相似. 因为结构的相似性, 磷酸酰胺天然产物容易被细胞内某些酶错误地识别、结合, 但是不能被有效地催化. 如前所述, 磷酸酰胺天然产物表现出各种各样的生物活性. 这其中不乏对人类生产和生活非常有用的活性, 如菜豆菌毒素(Phaseolotoxin, 图1)是一种非常有潜力的广谱性生物除草剂[1], 本课题组[2]的研究揭示菜豆菌毒素产生菌*Pseudomonas syringae*对菜豆菌毒素的抗性机制. 其水解后的毒素核心PSOrn(N^{δ}-(N'-sulfodiaminophosphinyl)-*L*-ornithine)是膦基乙酰基鸟氨酸(N^{δ}-(phosphonoacetyl)-*L*-ornithine, PALO)的相似物, 能够特异性地抑制植物细胞鸟氨酸氨甲酰基转移酶(ornithine carbamoyltransferase, OCTase)从而阻断精氨酸的合成. 放线形土壤杆菌*Agrobacterium radiobacter*产生的土壤杆菌素Agrocin 84(图1)是一种广泛使用的生防制剂[3,4], 其水解产生的毒素核心TM84是亮氨酰单磷酸腺苷酸(leucyl-adenosine-5′-monophosphate, Leu-AMP)的类似物, 能够特异性地抑制亮氨酰tRNA合成酶(leucyl-tRNA synthetases, LeuRSs), 从而抑杀作物的冠瘿瘤病原菌根癌农杆菌*Agrobacterium tumefaciens*. 磷酸酰胺天然产物金属蛋白酶抑制剂FMPI[5,6], 小菌素Microcin C7[7], 磷连氮霉素Fosfazinomycin[8], FR-900137[9], 磷酸阿米酮Phosphoramidon[10], 鱼卵毒素Dinogunellins[11], Phosmidosine[12,13], 1100-50[14], *L*-(*N*-phosphonyl) methionine-*S*-sulfoximinyl-*L*-alanyl-*L*-alanine[15], EM 2487[16], *S*ulphostin[17], JU-2[18]也表现出各种各样的生物活性(图1).

目前已知的磷酸酰胺天然产物中氮磷键生物合成机制可以分为3类: (1) 由MccB蛋白催化合成的氮磷键, 代表性化合物有小菌素Microcin C7; (2) APS腺苷酰基转移酶(adenylyl sulphate: ammonia adenylyltransferase, APSAT)催化合成的腺苷酰硫酸

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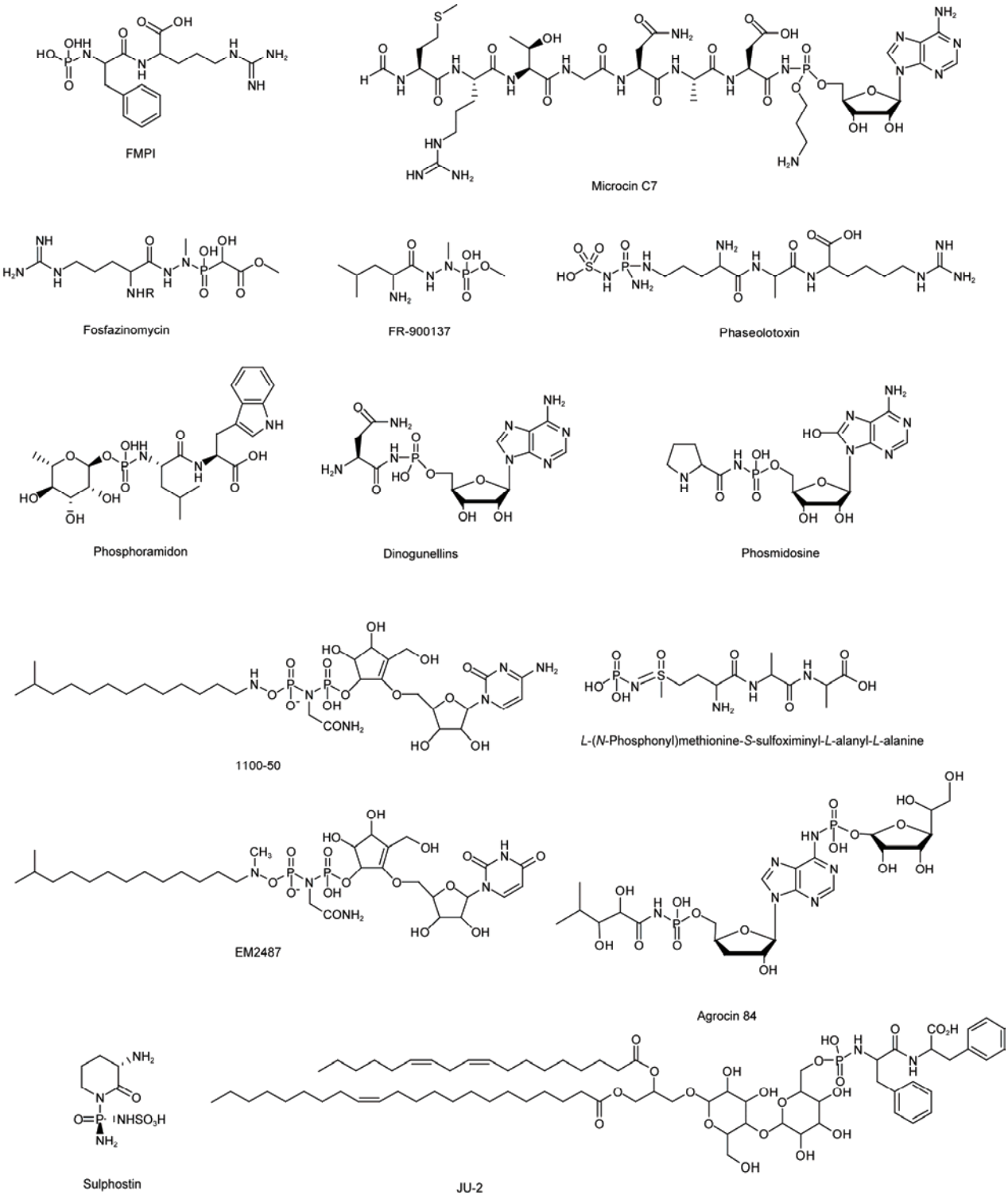

图 1 常见的磷酸酰胺天然产物化学结构

Figure 1 Chemical structures of phosphoramidate natural products

(adenosine-5′-phosphosulfate, APS)氨解反应，代表性化合物有腺苷-5′-磷酸酰胺(AMP-NH_2); (3) 丙酮酸磷酸二激酶(pyruvate phosphate dikinase, PPDK)同源蛋白催化的氮磷键合成，代表性化合物有菜豆菌毒素

Phaseolotoxin. 本文就这3类氮磷键的生物合成机制进行综述.

1 MccB蛋白催化的氮磷键合成

迄今为止, 唯一一个被阐释清楚的氮磷键生物合成途径是Microcin C7的第7个氨基酸残基天冬酰胺的*N*-磷酸化后修饰(图2(a))[19]. 该氮磷键的合成受MccB蛋白催化, MccB与E1泛素连接酶(E1 ubiquitin ligase)同源, 催化底物的腺苷酰化反应. 首先, Microcin C7的七肽前体物的第7个氨基酸残基天冬酰胺在MccB的催化下, 消耗一分子ATP, 环化形成琥珀酰亚胺. 然后, 亚氨基对另一分子的ATP的α磷酸基团进行亲核进攻形成氮磷键, 从而将琥珀酰亚胺和单磷酸腺苷两个结构单元连接起来, 形成"单磷酸腺苷-*N*-琥珀酰亚胺"(AMP-*N*-succinimide)(图2(a)). 为了简化表述, 本文中将此类氮磷键称为AMP-NHR类氮磷键(R表示氨基酸或内酰胺).

分析图1所列举的14种磷酸酰胺天然产物的结构可以发现, 土壤杆菌素Agrocin 84[20]、鱼卵毒素Dinogunellin[21]和Phosmidosine[22]与Microcin C7相似, 含有AMP-NHR结构单元. 因为Dinogunellin和Phosmidosine的生物合成基因簇尚未被克隆, 我们只能推测其氮磷键合成机制可能和Microcin C7的相似. Agrocin 84的化学结构中含有2根氮磷键, 一个是连接2,3-二羟基-4-甲基-戊酰胺的氨基与3′-脱氧一磷酸腺苷酸(3′dAMP)的磷酸基团的氮磷键(为表述方便称为第1根氮磷键), 另一个是连接3′dAMP嘌呤环上的氨基与磷酸化的羟基呋喃六糖(*D*-glucofuranosyloxy-phosphoryl)上的磷酸基团的氮磷键(为表述方便称为第2根氮磷键). 对Agrocin 84的生物合成基因簇进行分析, 可以找到MccB蛋白编码基因*mccB*的同源基因*agnA*. 基于相似的结构单元、高度同源的基因, 有文献报道认为Agrocin 84中第1根氮磷键与Microcin C7的氮磷键合成机制相同[23](图2(b)).

2 APSAT催化的氮磷键合成

1981年, Fankhauser等人[24]首次从真核生物小球藻细胞中, 通过不同浓度的$(NH_4)_2SO_4$沉淀、葡聚糖

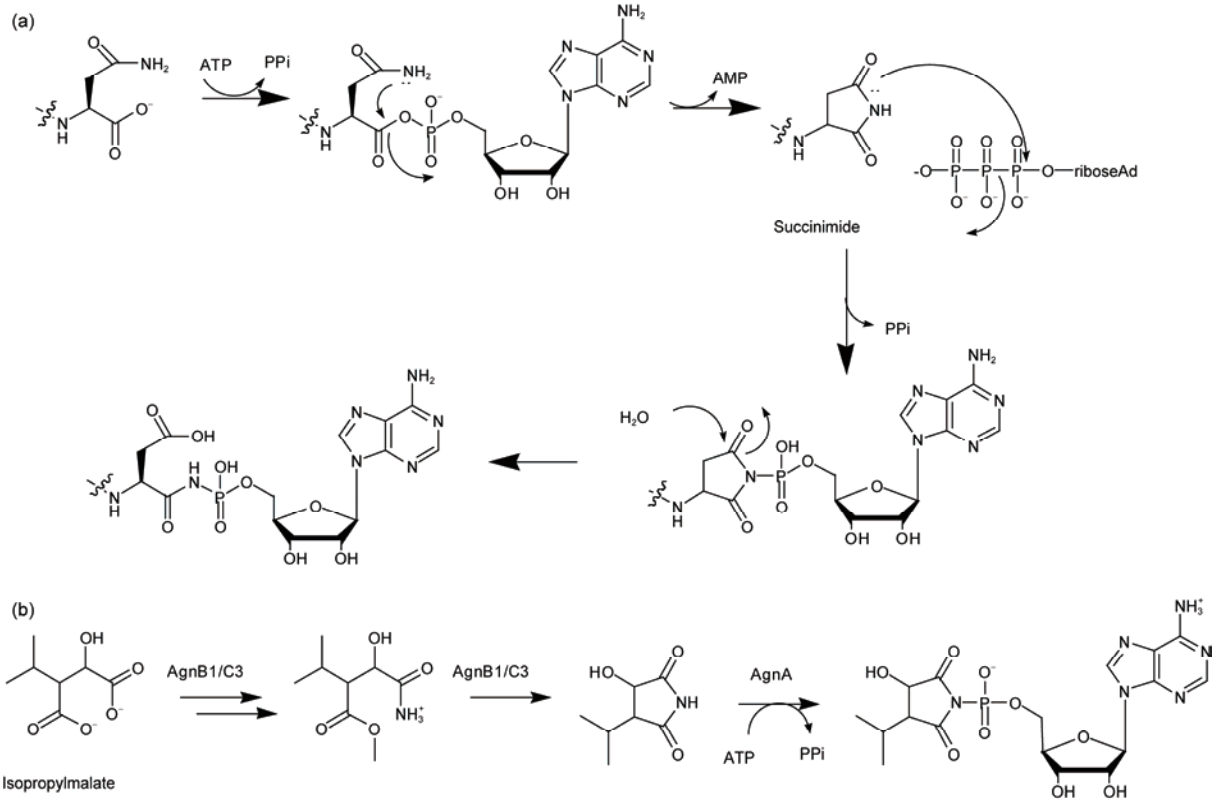

图 2 AMP-NHR类磷酸酰胺天然产物氮磷键生物合成示意图. (a) Microcin C7, (b) Agrocin 84

Figure 2 Biosynthetic pathway of AMP-NHR phosphoramidate N-P bond in Microcin C7 (a) and Agrocin 84 (b)

凝胶层析分离纯化得到了APS腺苷酰基转移酶APSAT(EC 2.7.7.51), 并针对其生化特性及产生的化合物结构进行了研究, 证实了APS和氨在APSAT作用下发生酶促反应, 可以生成含有氮磷键的AMP-NH_2(图3), 这也是首次分离得到AMP-NH_2. 在小球藻培养液中喂养[^{14}C]APS, 可分离检测到[^{14}C]AMP-NH_2, 也揭示了AMP-NH_2是由APSAT催化APS和氨产生[25]. 将原核生物大肠杆菌、真核生物小眼虫、盘基网柄菌及大麦和菠菜叶片的细胞抽提物, 利用$(NH_4)_2SO_4$梯度沉淀纯化蛋白, 各沉淀组分进行APSAT酶活反应, 结果表明上述所有细胞抽提物中, 均有$(NH_4)_2SO_4$沉淀组分可以表达APSAT活性, 与APS和氨作用生成AMP-NH_2. AMP-NH_2不仅在真核生物和原核生物中广泛存在, 在自然条件下, APS和氨在煮沸的情况下也可以产生AMP-NH_2[24]. APS中的磺酸基团作为一个离去基团, 在NH_3的亲核进攻下, 很容易离去从而产生氮磷键[30].

鉴于AMP-NH_2的广泛存在, APSAT催化的酶学反应也应该具有一定的普遍性. 但迄今为止, APSAT蛋白合成基因在原核生物中尚未被克隆, 在真核生物中, 脆性组氨酸三联体*Fhit*(*Fragile Histidine Triad*)作为一种新的抑癌基因[26], 其所编码的蛋白Fhit不仅具有催化二腺苷三磷酸(ApppA)水解生成AMP和ADP的活性[27], 还具有腺苷酰基转移酶的活性. Fhit可以催化APS和腺苷5′-氟代磷酸(F-pA)或二腺苷三磷酸(ApppA)发生氨解反应, 产生AMP-NH_2, 并依据已经揭示的Fhit作用于磷手性底物的特性, 提出假设, Fhit催化氨解反应是由NH_3进攻Fhit-核苷酸共价结合体[28], 但APS氨解的酶学机制还需经严格的实验数据验证.

3 PPDK同源蛋白催化的氮磷键合成

Phaseolotoxin和Agrocin 84化学结构和基因簇信息都表明, Phaseolotoxin的3根氮磷键和Agrocin 84第2根氮磷键的合成机制并不属于上文列举的两类机制. 分析Phaseolotoxin和Agrocin 84的生物合成基因簇, 可以发现Phaseolotoxin的基因簇中含有磷酸烯醇式丙酮酸合酶(phosphoenolpyruvate synthase, PS)基因的同源物*phtE*和*ppdk*基因的同源物*phtL*; Agrocin 84的生物合成基因簇中含有*PS*基因的同源物*agnD1*, *ppdk*基因的同源物*agnD2*和丙酮酸激酶(pyruvate kinase, PK)基因的同源物*agnE2*. PS, PPDK和PK都是激酶, 催化烯醇式丙酮酸的磷酸化经过中间状态: 磷酸(或者焦磷酸)基团以氮磷键共价结合的形式挂载在对应的催化酶(PS, PPDK或PK)的保守的组氨酸残基上(图4(a))[29].

基于生物信息学分析结果, 推测Phaseolotoxin氮磷键的合成与Agrocin 84的第2根氮磷键的合成, 具有一定的相似性. 哈佛大学Roush[30]提出假设认为, 在Phaseolotoxin合成时, 鸟氨酸的游离氨基对PhtL组氨酸残基上的磷酸基团进行亲核进攻, 形成氮磷键(图4(b)). 同理可以假设在Agrocin 84合成时, 3′dAMP嘌呤环上的氨基对AgnD1(或者AgnD2)组氨酸残基上的磷酸基团进行亲核进攻, 形成Agrocin 84的第2根氮磷键(图4(c)). 这是磷酸酰胺天然产物氮磷键生物合成的一种全新假设, 从磷酸酰胺天然产物的化学结构和生物合成基因簇推理发现该机制具有一定的普遍性, 但是具体的酶学机制尚待深入的实验研究.

4 结论

天然产物是人类预防和治疗疾病的重要的药物源泉, 对一千多个临床使用的小分子药物的统计结果表明, 59.3%的抗感染药物(细菌)和53.4%的抗癌药物来源于天然产物或其衍生物[31]. 传统的活性指导的天然产物药物筛选, 因为资金和劳动成本耗费大, 复筛率高, 面临着严重的挑战. 焦磷酸法高通量测序

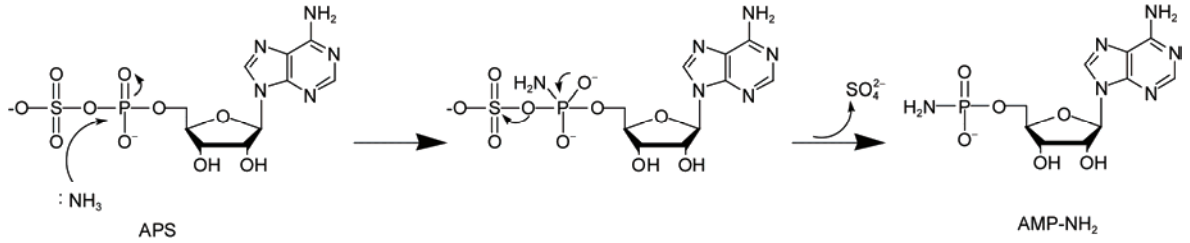

图 3 腺苷酰基转移酶催化AMP-NH_2的氮磷键生物合成示意图

Figure 3 Biosynthetic pathway of AMP-NH_2 N-P bond catalyzed by APSAT

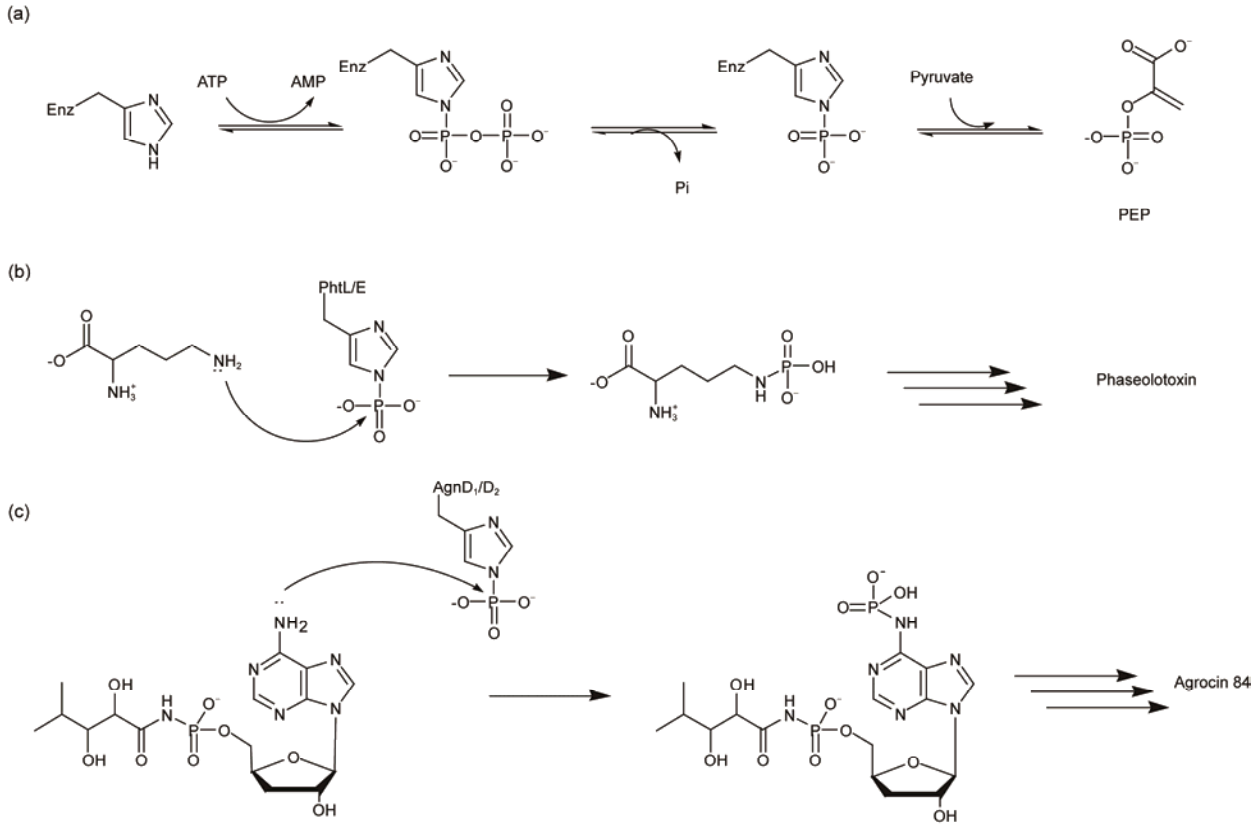

图 4 Phaseolotoxin和Agrocin 84中氮磷键生物合成机制的新假设. (a) PPDK催化的反应机制; (b) Phaseolotoxin中N–P键可能的合成机制; (c) Agrocin 84中N–P键可能的合成机制

Figure 4 Proposed biosynthetic pathway of Phaseolotoxin and Agrocin 84 N–P bonds. (a) The reaction diagram catalyzed by PPDK; (b) proposed biosynthetic pathway of Phaseolotoxin N–P bond; (c) proposed biosynthetic pathway of Agrocin 84 N–P bond

技术的兴起, 掀起了通过基因组挖掘(genome mining)寻找新的药物先导化合物的浪潮. 膦酸(phosphonate, 指含有碳磷键的化合物)天然产物的挖掘就是一个很好的例子. 以酸烯醇式丙酮酸变位酶基因(*pepM*)为探针, 并结合^{31}P NMR和MS等检测方法, Evans等人[32]发现了新的膦酸天然产物Phosacetamycin; Ju等人[33]发现了Argolaphos, Valinophos和Phosphonocystoximates等新的膦酸天然产物; Gao等人[34]发现了新的磷连氮霉素Fosfazinomycin产生菌株. Fosfazinomycin是目前发现的唯一一个含有C-P-N结构单元的天然产物, 但是因为其产量低、产生菌株难以获得等原因, 对它的生物合成研究一直无法展开. 发现新的Fosfazinomycin产生菌株以后, Huang等人[35,36]对Fosfazinomycin的生物合成途径进行了研究, 这为揭示C-P-N结构单元的生物合成机制奠定了基础. 通过基因组挖掘找到新的天然产物或者已知天然产物的新的产生菌株, 可以促进天然产物的生物合成研究; 而生物合成研究揭示的生物合成机制又可以进一步地指导基因组挖掘.

磷酸酰胺天然产物氮磷键生物合成规律的揭示将为基因组挖掘提供基因水平的切入口, 为发现数量更多、活性更好的磷酸酰胺天然产物打下理论基础, 也为通过代谢工程和组合生物合成创造活性更好的非天然磷酸酰胺天然产物提供酶学工具. 在核酸药物的开发和基因治疗的过程中, 我们经常面临核酸分子(DNA或RNA)进入细胞后被降解(磷酸二酯键被水解)的困难. 通过化学合成用氮磷键替代磷脂键($P(O)(OH)(NH_2)_2$单元替代$OP(OH)_3$单元), 制造核糖磷酸酰胺骨架, 不失为应对降解问题的有效策略[37,38], 揭示磷酸酰胺天然产物氮磷键的生物合成规律, 可能会为通过生物的手段制造核糖磷酸酰胺骨架提供有益的启示.

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Summary for "磷酸酰胺天然产物氮磷键的生物合成研究进展"

Progress of nitrogen-phosphorus bond biosynthesis study of naturally occurring phosphoramidates

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Naturally occurring phosphoramidates, which contain nitrogen-phosphorus (N−P) bonds, show high structure similarity with cytoplasmic carboxylates and phosphates. Phosphoramidates exhibit wide bioactivity spectrum attributed to the role as inhibitor of certain necessary enzymes in cell cycle. Three N−P bond biosynthetic pathways, which are respectively catalyzed by MccB, adenylyl sulphate: ammonia adenylyltransferase (APSAT) and Pyruvate phosphate dikinase (PPDK) homologues, are reviewed here. (1) MccB, a homologue of E1 ubiquitin ligase, catalyzed the phosphorylation of the asparagine residue of Microcin C7 precursor peptide. It consumed one molecule of ATP for the cyclization of the asparagine residue to give a succinimide. Then, the succinimidyl nitrogen atom attacked the Pα of a second ATP molecule to create an adenosine monophosphate-*N*-succinimide moiety. Agrocin 84, dinogunellin and phosmidosine shared identical AMP-NHR structural moiety with Microcin C7. Unfortunately, the biosynthetic gene clusters of dinogunellin and phosmidosine have not been identified yet. Agrocin 84 contains two N-P bonds, the first one linked the amino group of 2,3-dihydroxy-4-methyl-pentanamide with the phosphate group of 3′dAMP, and the second one bridged the amino group of the 3′dAMP purine ring with the phosphate group on the phosphorylated D-glucofuranosyloxy-phosphoryl. Analysis of Agrocin 84 biosynthetic gene cluster showed that *agnA* is homologous to *mccB* gene. Based on structural similarity and gene homology, it was proposed that the first N−P bond biosynthetic pathway of agrocin 84 is similar to that of Microcin C7. (2) APSAT catalyzed ammonolysis of adenosine-5′-phosphosulfate to give adenosine-5′-phosphoramidate (AMP-NH_2) and sulfate. AMP-NH_2 was discovered from the extracts of *Chlorella pyrenoidosa*, *Dictyostelium discoideum*, *Euglena gracilis* var. *bacillaris*, spinach, barley and *Escherichia coli*. This APSAT protein was isolated from *Chlorella pyrenoidosa* and the biochemical characters were investigated. To eukaryotes, human Fhit (Fragile Histidine Triad) showed adenylyltransferase activity as well. It not only catalyzed the hydrolysis of diadenosine triphosphate (ApppA) to form AMP and ADP, but also catalyzed the ammonolysis of ApppA to form AMP-NH_2 and ADP. In the hydrolysis of ApppA to AMP and ADP catalyzed by Fhit, first, ApppA and Mg^{2+} covalently bound to the 96th histidine residue of Fhit to form Fhit-AMP covalent intermediate and released ADP; then Fhit-AMP was further hydrolyzed to form AMP and Fhit. The Fhit-AMP could also be aminolyzed to give AMP-NH_2. A possible mechanism for Fhit-catalysed ammonolysis involving nucleophilic attacks of NH_3 to the covalent histidine-bound enzyme-nucleosidyl intermediate. (3) PPDK catalyzed the phosphorylation of pyruvate with an intermediate in which a phosphate group was loaded on the conserved histidine residue of PPDK. PPDK homologues PhtL and AgnD were proposed to catalyze the N-P bonds formation of Phaseolotoxin and Agrocin 84, respectively. The Walsh group of Harvard University have hypothesized that the Phaseolotoxin N-P bond was formed by nucleophilic attack of ornithine free amino to the phosphate group attached to the histidine residue of PhtL. Similarly, it can be assumed that the amino group of 3′dAMP purine ring attacking the phosphate group of AgnD1 (or AgnD2) histidine residue, makes the second N-P bond of Agrocin 84. This is a novel hypothesis of the biosynthesis of the N-P bonds in naturally occurring phosphoramidates, while the detailed enzymatic reactions remain to be studied further.

phosphoramidates, nitrogen-phosphorus bond, natural product, biosynthesis

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