

人工金属酶研究进展*

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摘要 金属酶由金属辅因子和蛋白骨架构成。金属辅因子提供催化活性,是金属酶发挥功能的关键,而蛋白骨架为金属辅因子提供附着位点,同时提供手性环境。天然金属酶种类较多,可催化羟基化、环氧化等反应,其辅因子主要以金属离子或金属配体的形式存在,所含金属元素以 Fe、Cu、Zn 居多,部分天然金属酶也含 Mn 等金属元素。然而,由于天然金属酶难以催化非天然底物,且部分金属酶体外催化效率低、自身稳定性较差,无法得以广泛应用。近年来研究发现,通过对起催化功能的金属辅因子和提供酶促反应微环境的蛋白骨架进行理性设计构建人工金属酶 (artificial metalloenzymes, ArMs),可提高金属酶的催化效率或使其能够催化多种天然和非天然反应。此外,利用纳米技术修饰人工金属酶可以提高金属酶的稳定性和可调控性,为人工金属酶的优化提供了新思路。总结近年来人工金属酶领域取得的成果,着重介绍构建人工金属酶策略方面的研究进展,包括金属辅因子的改造、蛋白骨架的设计、基于纳米技术的修饰等,并展望了设计改造人工金属酶所面临的机遇和挑战,以期为人工金属酶的设计和应用提供参考。

关键词 人工金属酶 辅因子 蛋白骨架 理性设计 纳米技术

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自然界中的酶因高效性和高选择性受到研究人员的关注。其中,金属酶在生物系统中发挥着重要作用,如电子转移、O₂ 的结合传递、催化 C-H 氧化和卤化反应等^[1-3]。金属酶主要由蛋白骨架和金属辅因子组成,通过与底物基团配位发挥催化作用^[4],其中金属辅因子以单独金属离子或金属络合物等形式存在。首个发现的天然金属酶是来自蜡样芽孢杆菌的头孢菌素酶^[5],属于 Zn 依赖型金属酶,该酶的金属活性中心与 β-内酰胺羰基结合产生一个四面体复合物,继而使 β-内酰胺环的 C-N 键发生水解断裂,使 β-内酰胺类抗生素失效^[6]。随后,一些含有 Cu、Fe 等的金属酶相继被发现^[1]。目前已报道的金属酶约占已知天然酶的 1/3^[7],包括氧化还原酶类、水解酶类、异构酶类、合成酶类、裂解酶类和转移酶类。金属酶可催化很多利用

化学方法难以实现的反应,具有反应条件温和、副产物少、对环境危害小等优势。但部分天然金属酶存在着催化效率低、底物特异性高、金属中心在蛋白骨架中的反应性有限等问题,不利于对其深入理解和探索。

近年来,通过对金属酶进行蛋白骨架或金属辅因子的改造、组合、重构等,研究人员开发出许多具有新功能的人工金属酶。20 世纪 70 年代,Yamamura 等^[8]报道了第一个人工金属酶,利用 Cu(II)代替羧肽酶 A 中的 Zn(II),使原本具有肽链外切酶活性的羧肽酶 A 转变为具有氧化酶活性的人工金属酶。但由于当时缺乏对金属酶作用机理的了解以及可用于蛋白质工程的工具有限,人工金属酶研究在较长一段时间内发展缓慢^[9-10]。近年来随着生物技术、化学合成手段的进步以及对蛋白质结构和作用机理更深入的解析,具有新功能的人工金属酶不断被开发。2018 年,Mirts 等^[11]将 [4Fe-4S] 辅因子与细胞色素 c 结合,使其具有亚硫酸盐还原酶的功能,并通过理性调节 [4Fe-4S] 与底物结合位点附近的次级配体之间的相互作用,提高了该酶活性。此外,设计简单稳定的多肽支架是模拟复杂金属酶的一个有力途径,从头设计的

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α_3 DIV-L21C 骨架具有类似红素氧还蛋白的金属结合位点,进一步引入 Mo 原子增加了三螺旋束肽的热稳定性^[12]。相对于天然金属酶,从头设计的人工金属酶结构相对简单,底物识别范围广,逐渐得到了研究人员的青睐^[13]。

本文总结了人工金属酶设计的常用策略和最新进展,着重介绍了人工金属酶的构建方法,包括辅因子改造、蛋白骨架改造和基于纳米技术的修饰(图1),并对扩展现有构建人工金属酶的方法进行了思考与展望。

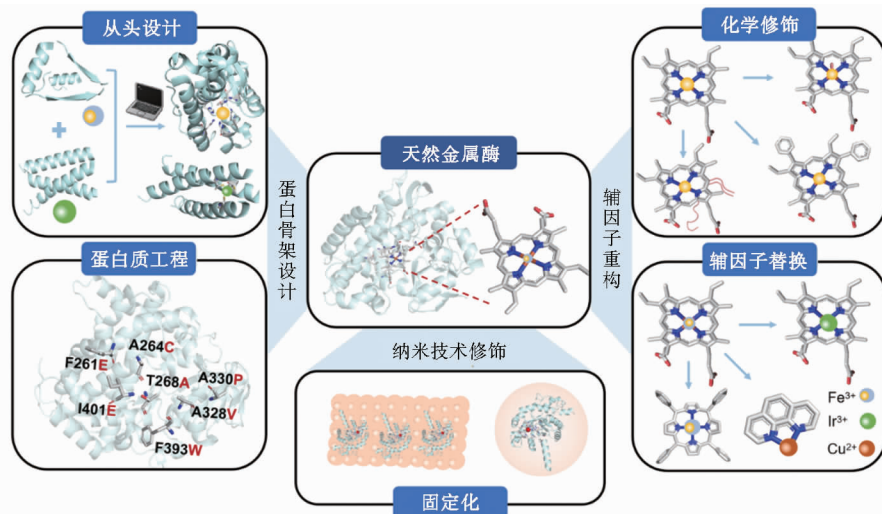


图1 人工金属酶的构建策略

Fig.1 Strategies of artificial metalloenzymes construction

Protein scaffolds design: Using *de novo* design to obtain scaffolds or using protein engineering to improve catalytic efficiency and develop new functions of artificial metalloenzymes. Cofactors reconstitution: Using chemical modification to improve the microenvironment of cofactors; introducing or replacing metal ligands to construct new artificial metalloenzymes. Enzymes modification assisted by nanotechnology: Encapsulating artificial metalloenzymes in nanoparticles or adsorbing on the surface to immobilize enzymes, allowing to increase stability and activity. The protein structure was from the Protein Data Bank (PDB) and edited by PyMOL

1 辅因子改造在人工金属酶构建中的应用

天然金属酶利用辅因子催化反应^[13-14],辅因子是保持酶活性必需的离子或有机分子。基于作用机理,辅因子可分为三类:催化型辅因子、载体型辅因子和底物型辅因子^[15]。大多数金属酶中的辅因子属于催化型辅因子,位于酶的活性中心,是金属酶发挥催化功能的核心。因此,辅因子是人工金属酶设计的关键,其与蛋白骨架的有效结合是构建新功能人工金属酶的重要思路^[13, 16-17]。

1.1 天然辅因子的化学修饰

通过改变辅因子的微环境,调节辅因子的还原势能,可构建高效的电子转移途径^[18]。利用吸电子、供电子或芳香基团等对辅因子进行修饰,可在一定程度上改善金属辅因子的空间和立体电子效应,这是设计人工金属酶的策略之一^[19]。Fruk 等^[20]设计了不同长度、不同碱基组成的 DNA 寡聚物用于修饰血红素辅因子,将其与脱辅基肌红蛋白(myoglobin, Mb)结合,修饰后的 Mb 对 2, 2-联氮-二(3-乙基-苯并噻唑-6-磺酸)二铵

盐的氧化相对活性是天然 Mb 活性的 4 倍以上,证实了对辅因子进行化学修饰的可行性。2019 年, Hayashi 等^[21]通过将含苯环的小分子化合物引入血红素-丙酮酯侧链的末端,在血红素入口处构建疏水口袋,使得 Mb 对小分子底物(儿茶酚、对苯二酚和创愈木酚)的氧化相对活性提高到天然 Mb 活性的 14 ~ 32 倍。

综上,对辅因子进行化学修饰可使金属酶的结构、电子特性、催化活性等在一定程度上发生改变,进而提高其活性。未来,通过在辅因子周围引入一些吸电子(斥电子)基团或者亲水(疏水)基团来改变金属酶的催化活性是构建人工金属酶的重要方向。

1.2 非天然辅因子的掺入

为开发金属酶新的催化活性,研究人员试图将非天然辅因子构建到蛋白骨架中。在保留蛋白骨架结构基本不变的同时,使用非天然辅因子代替天然辅因子或许能更好地调节金属酶的催化性能^[22]。2003 年, Ohashi 等^[23]将 Cr(III)席夫碱配合物引入脱辅基 Mb 突变体中制备人工金属酶,实现了对苯甲硫醚的选择性氧化。虽然该人工金属酶的反应活性和对映选择性还

有待提升,但展示了非天然辅因子在人工金属酶设计中的潜力。非天然辅因子的掺入主要通过更换金属离子和更换金属配体这两种方式实现。

金属离子是金属酶发挥催化功能的核心,将其更换为其他金属有望实现新的催化功能^[2]。Morra 等^[24]利用含氮供体 Rh(III) 络合物代替布氏嗜热厌氧菌乙醇脱氢酶(TbADH)中的 Zn(II),人工金属酶的稳定性得到了提高。Sreenilayam 等^[25]利用 Mn 和 Co 分别取代血红素中的 Fe,使得 Mb 突变体可以催化二氢异苯并呋喃和重氮乙酸乙酯分子间的卡宾插入反应,这是野生型 Mb 无法实现的。2023 年,研究人员发现通过改变反应 pH 与酶和光敏剂的浓度,可实现 Co-PPIX 取代的肌红蛋白(CoMb)酶活性和产物选择性的提高,将其用于光驱动水相中 CO₂ 的还原,获得的转化数(turnover number, TON)高达 2 000^[26]。Dydio 等^[27]报道了利用 Ir 卟啉[Ir(Me)-PIX]代替 CYP119 中的 Fe 卟啉(Fe-PIX),得到的人工金属酶(Ir-CYP119)活性与天然酶相似,对 Ir-CYP119 进行定点突变,所得 Ir(Me)-CYP119-Max 及其突变体能够以高达 90% 以上的对映体过量百分数(enantiomeric excess, ee)催化一系列重氮酯类底物的分子内卡宾插入反应(图 2)。后续通过改造大肠杆菌 Nissle 1917(EcN)构建了 Ir-CYP119 体内组装的

有效平台。在该系统中,Ir(Me)-MPIX 由 EcN 的外膜受体转运至细胞内,随后体内组装的 Ir-CYP119 催化卡宾插入反应,这种全细胞筛选方法加速了人工酶 Ir-CYP119 的定向进化效率^[28]。此外,利用微生物从头合成非天然辅因子使得人工金属酶的构建更加简单便捷。Perkins 等^[29]利用大肠杆菌 BL21 在 Fe 缺陷、富 Co 的生长条件下合成 Co 卟啉(Co-PPIX),在添加 CoCl₂ 的基本培养基中,代谢产生的 Co-PPIX 被直接结合到多个蛋白中,得到了五种 Co 取代蛋白:Mb 突变体(Mb-H64V/V68A)、染料脱色过氧化物酶、醛脱氢酶、CYP119 和过氧化氢酶。一些天然金属酶中含有两个或以上的金属离子^[30],对金属中心分别研究有助于加深对其催化机理的理解。为了探究非血红素金属离子对血红素铜氧化酶催化的作用效果,Bhagi-Damodaran 等^[31]以 Mb 为研究模型,在保留血红素催化中心的同时,在另一位置引入其他金属离子,构建了含不同金属离子的双金属蛋白。其中,Fe(II)-Fe_BMb 和 Cu(II)-Fe_BMb 的氧化还原能力分别比野生型 Mb 提高了约 11 倍和 30 倍。Hosseinzadeh 等^[32]在天青蛋白的 Cu(II)催化中心引入额外的 Ni(II),得到一种含双金属的人工金属酶,其覆盖的氧化还原电势范围由 +100 mV ~ +800 mV 扩大至 -954 mV ~ +970 mV,该蛋白被广泛应用于生物化学的研究中。

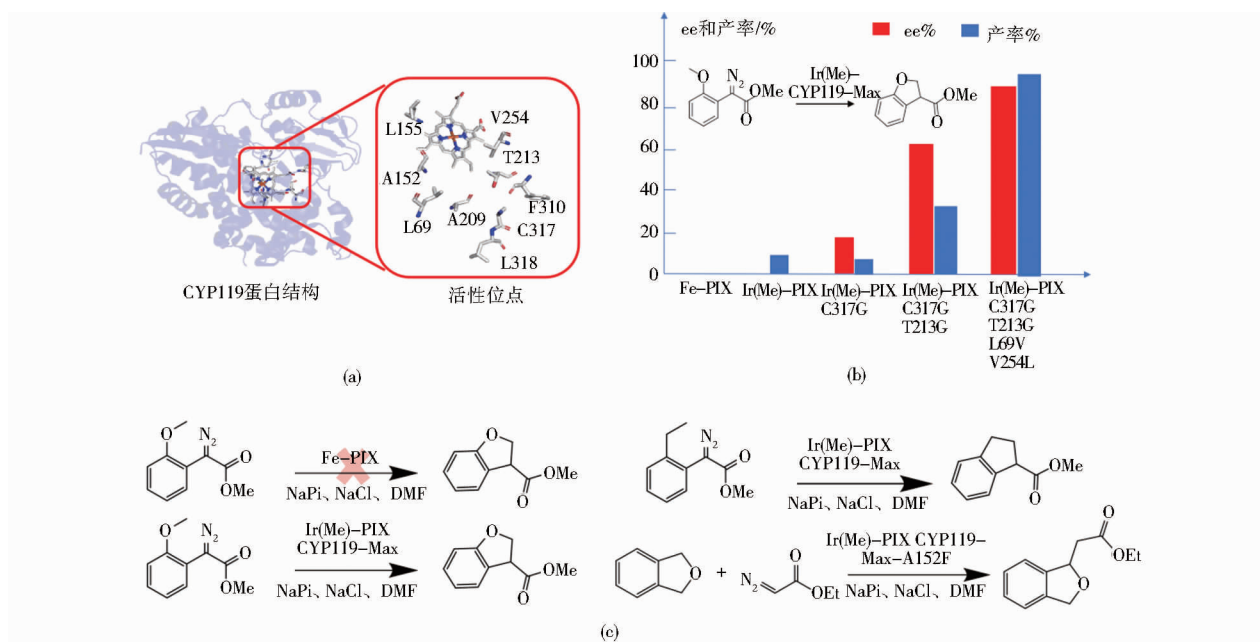


图 2 利用卟啉替换构建人工金属酶^[27]

Fig. 2 Artificial metalloenzymes construction via porphyrin substitution^[27]

(a) The structure and active sites of CYP119 (PDB ID: 11O7) (b) Enantioselectivity and yield of the conversion of diazoesters to dihydrobenzofuran catalyzed by evolved CYP119 variants (c) Extended reaction type catalyzed by CYP119 mutants, Ir(Me)-PIX CYP119-Max; L69V, T213G, C317G, V254L. The protein structure is from PDB and edited with PyMOL.

此外,替换卟啉骨架也是一种提高人工金属酶催化特性的有效策略。Sreenilayam 等^[33]利用 Fe-二氢卟吩 e6 复合物[Fe(Ce6)]替代 Mb 突变体(Mb-H64V/V68A)中的天然 Fe-PPIX 辅因子以促进乙烯基芳烃环丙烷化反应,催化效率达到了 6 970 TON,该反应在有氧条件下具有更高的选择性(ee 达到 99%)。该研究团队将非天然氨基酸作为近端配体引入 Mb,可结合非天然 Fe-PPIX 类辅因子[Fe(DADP)Cl 和 Fe(Ce6)Cl 等],得到了一系列人工卡宾转移酶。该策略能够有效

改善基于 Mb 的环丙烷化生物催化剂的反应活性^[34],并有望被广泛用于人工酶改造。

金属辅因子的改造在开发人工金属酶中至关重要^[35-37],近年来通过金属辅因子改造策略构建的人工金属酶如表 1 所示。在选择金属辅因子时还需要考虑是否与蛋白骨架相匹配、是否占据了底物通道等一系列问题,因此非天然金属辅因子的替换仍然是人工金属酶的研究热点和难点。

表 1 含金属辅因子的人工金属酶

Table 1 Summary of artificial metalloenzymes containing metal cofactors

蛋白骨架	原辅因子	新辅因子	功能	参考文献
Apo-SiRCcP. 1	—	[4Fe-4S]	催化亚硫酸盐的还原反应	[11]
HSA	—	Au	催化氢胺化反应	[38]
Homo-oligomeric protein	—	Cu(bpy)	催化多质子/电子介导的氧化还原反应	[39]
MC6 * a	—	Fe、Mn	过加氧酶活性	[40]
αRepA3	—	Cu(II)	催化 Diels-Alder 反应	[41]
αRep	—	Co(III)-porphyrin complex	光诱导制氢和二氧化碳还原反应	[42]
MDRs	—	Cu(II)、BpyA-Cu(II)	催化 Friedel-Crafts 烷基化	[43]
mAbs	—	BIQ-Cu、BIQ-PdCl ₂ 、BIQ-Pd(OAc) ₂ 、BIQ-PtCl ₂	催化 Friedel-Crafts 烷基化反应	[44]
LmrR	—	Fe(III)-CPPIX	催化环丙烷化反应	[45]
LmrR	—	Cu(II)-phen complex	催化 Friedel-Crafts 烷基化反应	[46]
LmrR	—	Cu(II)-phenanthroline complex	催化 Friedel-Crafts 烷基化和 Diels-Alder 反应	[47]
LmrR	—	Cu(II) complexes	催化迈克尔加成反应	[48]
Sav-SOD	—	Cp * Ir(biot-p-L)Cl	催化不对称氢转移反应	[49]
Nitrobindin (NB)	—	Cp * Rh(III) complexes	催化环加成反应	[50]
Nitrobindin (NB)	—	Cp * Rh(III)-dithiophosphate complex	催化环加成反应	[51]
POP	—	Dirhodium complexes	催化环丙烷化反应	[52]
POP	—	Ru(II) polypyridyl complexes	催化环加成反应	[53]
POP	—	Dirhodium complexes	催化重氮化偶合反应	[54]
(A3A3') Y26C	—	Mn(III)-tetraphenylporphyrin	过氧化物酶和单加氧酶的活性	[55]
Four-helix bundle	—	Zn-PPIX	过氧化物酶活性	[56]
Four-helix bundle	—	Ru(II)(η ⁶ -arene)(bipyridine) complexes	催化氢转移反应	[57]
Van and DADA complexes	—	[IrCp * (m-I)Cl]Cl	催化环亚胺的不对称加氢反应	[58]
Heptaepetidic	—	Methyl salicylate Pd complexes	催化去炔丙基化和 Suzuki-Miyaura 交叉偶联反应	[59]
TbADH	Zn(II)	Cp * Rh(III) complexes	催化还原反应	[24]
Azurin	Cu	Ni	催化碳碳耦合及硫酯合成反应	[60]
P450-BM3	Fe-PPIX	Ir(Me)-deuteroporphyrin IX	催化烯烃环丙烷化反应	[61]
CYP119	Fe-PPIX	Ir(Me)-PIX、Ir(Me)-MPIX	催化环丙烷化反应	[28, 37, 62]
Mb	Fe-PPIX	Fe-2, 4-diacetyl deuteroporphyrin IX	催化烯烃的不对称环丙烷化	[63]
Mb	Fe-PPIX	CuCP	DNA 切割活性	[64]

2 蛋白骨架在人工金属酶构建中的应用

蛋白骨架为金属辅因子提供发挥作用的环境,同时辅因子周围的氨基酸残基通过氢键、疏水等相互作用控制反应的立体选择性。因此,蛋白骨架的改造在人工金属酶的构建中具有重要作用。蛋白骨架的选择需要考虑蛋白质的稳定性、与金属中心的匹配性和二级结构的明确性等^[65]。此外,将非天然氨基酸引入蛋白骨架中对人工金属酶领域的发展也具有重要意义^[66]。

2.1 天然蛋白骨架的选择及改造

常用的天然蛋白骨架有链霉亲和素、脯氨酰内肽酶、脂肪酶、牛血清白蛋白、溶菌酶、多药耐药性调节剂等^[65],具有可与金属结合的稳定结构。其中,链霉亲和素(streptavidin, Sav)的单体是一种通用的蛋白骨架。Mann等^[67]利用生物素-链霉亲和素技术开发了一个较稳定的人工Cu蛋白,Sav为Cu-OOH周围提供了一个含氢键网络的局部环境,可用于稳定水溶液和晶体中的双氧复合物。

天然酶大多在较为温和的生理条件下发挥作用,热稳定性差,限制了其应用范围。选择更稳定的蛋白

骨架构建人工金属酶为实现更多的非天然酶促反应提供了良好基础。Filice等^[68]利用来自嗜热地芽孢杆菌的脂肪酶作为支架结合大量Cu(II)来构建人工金属酶,该酶在Diels-Alder环加成反应和级联反应中显示出优异的催化性能,其底物转化率可达99%。除脂肪酶外,来自棒状杆菌的青色荧光蛋白mTFP1与Cu(II)结合也可得到具有较高稳定性的人工金属酶,能够催化Diels-Alder和Friedel-Crafts烷基化反应^[69]。多药耐药性调节剂(LmrR)蛋白骨架也是一种常用且稳定的蛋白骨架,被广泛应用于人工金属酶的构建。Chordia等^[47]将Cu(phen)(NO₃)₂配合物与LmrR进行组合得到一种新的人工金属酶,其催化Friedel-Crafts烷基化反应的对映选择性可达92%。Roelfes等^[70]将LmrR和Cu(II)-非咯啉配合物进行组装,通过额外引入结合金属配体的非天然氨基酸,可实现吲哚衍生物与 α 、 β -不饱和2-酰基咪唑类化合物的Friedel-Crafts烷基化反应,产物立体选择性为52%~80%。研究人员进一步将该方法应用于蛋白QacR来构建人工金属酶QacR_Y123BpyA,得到了对映选择性高达94%的Friedel-Crafts烷基化产物(图3)。

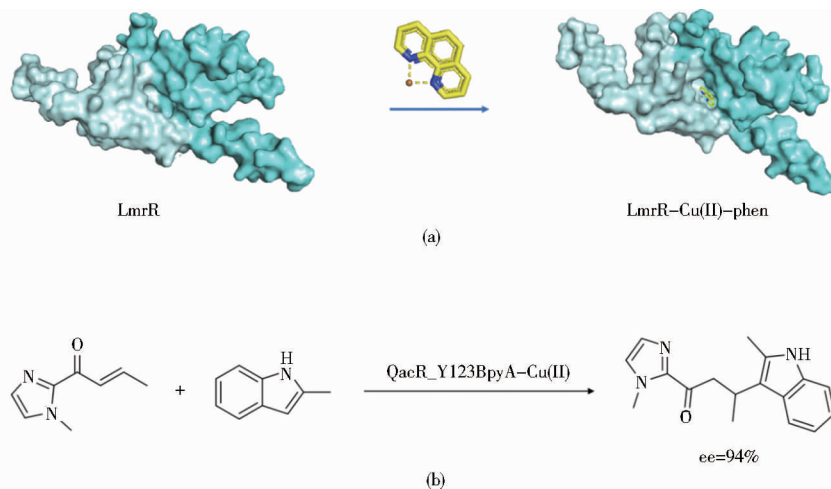


图3 利用蛋白支架构建人工金属酶^[70]

Fig. 3 Artificial metalloenzymes construction employing protein scaffold^[70]

(a) Schematic diagram of ArM assembled by LmrR protein scaffold and copper cofactor (b) Friedel-Crafts alkylation of indoles catalyzed by ArM

对天然蛋白骨架进行工程化改造可以提高金属酶的催化性能。野生型细胞色素P450单加氧酶CYP102A1(P450-BM3)可氧化硅烷生成硅烷醇,但是具有杂泛性。Bähr等^[71]采用定向进化的策略,得到了在温和条件下高效催化硅烷氧化的BM3突变体,成功

提高了硅烷醇的产量。Ensari等^[72]对P450-BM3进行了工程化改造,得到了突变体YE-M1-2(P450-BM3-R47S/Y51W/T235S/N239R/I401M),其对己酸甲酯的初始氧化速率比野生型BM3高23倍,产物3-羟基己酸甲酯的收率增加了1.5倍。一些天然酶中不包含金属

辅因子,研究人员通过改造蛋白基序可以创造金属离子结合位点。Beaumont 等^[73]将咪唑甘油磷酸合成酶(tHisF)的 Glu/His/His 基序进行改造,得到的突变体 tHis^{FEHH}可以在两个不同的位点配位金属离子。进一步构建的人工金属酶 Ni(II): tHis^{FEHH}可以催化槲皮素和杨梅素的 O₂ 依赖性脱羧反应。此外,重组天然蛋白结构域也是构建人工金属酶的有效方法。Kato 等^[74]将脂肪酸结合蛋白(fatty acid binding protein, FABP)的 α -螺旋帽结构域嵌入到 NB 蛋白的 β -桶状结构中作为蛋白骨架,将其与金属辅因子 Cp * Rh(III) 复合物结合来构建人工金属酶。经过多轮定向进化,所得人工金属酶突变体 NBHLH1(Y119A/G149P)的催化性能和稳定性增强,其催化炔与炔烃环加成反应的效率提高了 35 倍以上。

目前许多蛋白的结构还未被表征,借助计算机模拟有助于研究人员进一步了解蛋白质与底物的作用过程,对人工酶的设计具有重要的指导意义^[75]。Martins

等^[76]开发了一个分子对接工作流程,将其应用于设计和优化对 TbADH 辅因子结合位点具有高亲和力的 Ir 催化剂,为设计高效的人工金属酶提供了指导。此外,底物扩散探索模拟软件的开发从模拟底物扩散的角度为设计金属酶的长程突变提供了指导^[77]。随着被表征的蛋白越来越多,可用天然蛋白骨架的数量也将逐渐增加,为进一步构建人工金属酶提供了基础。

2.2 蛋白骨架的从头设计

天然蛋白结构由相同的结构基序按不同的方式组合而成,这使得从头设计人工金属酶成为可能。从头设计金属蛋白结合了蛋白设计和生物无机化学的相关知识,主要思路是将 α 螺旋和 β 折叠等蛋白二级结构进行组合,设计出结构明确的可以插入金属辅因子的蛋白骨架(图 4)。从头设计的蛋白相较于天然蛋白结构更加简单,功能更加明确,有助于进一步探索尚未表征的天然蛋白结构和作用机制^[78]。

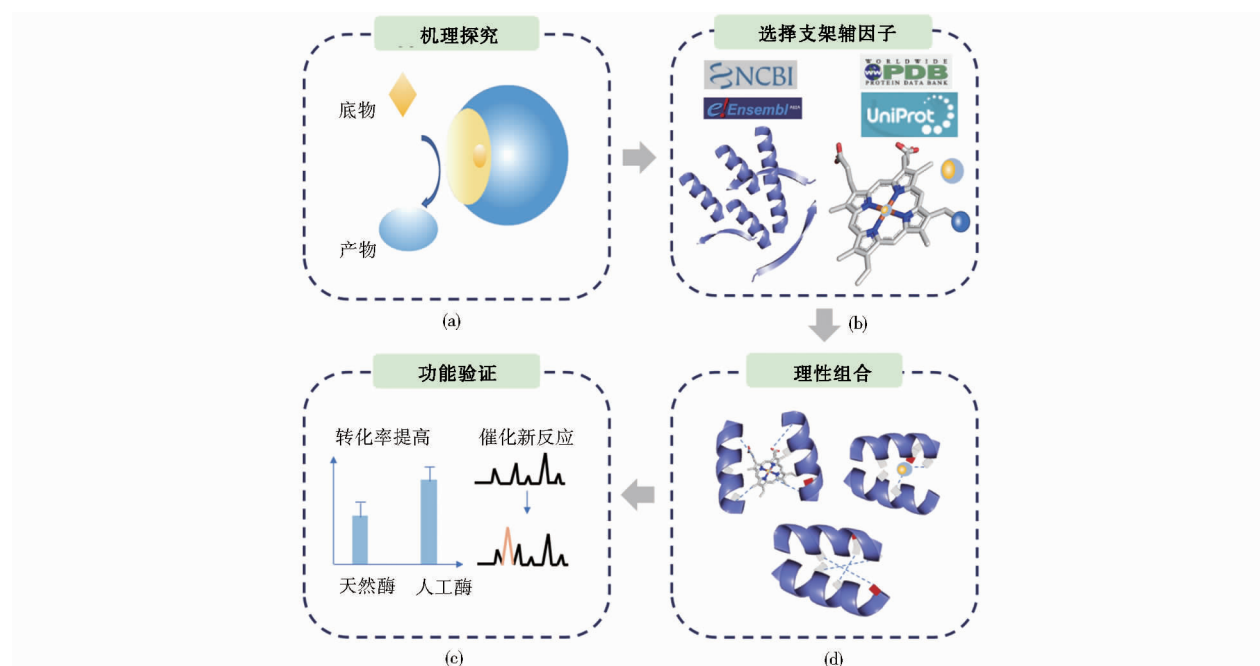


图 4 从头设计人工金属酶

Fig. 4 De novo design strategy of protein scaffolds

(a) Mechanism study of enzymatic reaction to find the key sites (b) Mining of stable protein structures and cofactors from databases (c) Rational design and assembly of the protein skeleton with cofactors (d) Screening of new artificial metalloenzymes. The protein structures are from PDB and edited with PyMOL

四螺旋束基序存在于大多数已表征的蛋白中,具有结合金属离子的能力,被广泛用于人工金属酶的设计^[79]。四螺旋束二铁蛋白(due ferri, DF)是从头设计金属蛋白较为成功的一个例子。Reig 等^[80]以单链四螺

旋束蛋白 DFsc 为模板,通过从头设计得到了对 4-氨基苯酚具有高活性的氢醌氧化酶 G4DFsc。Lombardi 等^[81]利用 DF 结合 di-Fe 和 di-Mn 构建了一系列人工金属酶,其中结构优化的 DF_{tet} 突变体催化 4-氨基苯酚转

化为醌单亚胺的反应速率提高了 1 000 倍。利用具有亲脂区域的人造四螺旋束, Joh 等^[66]将常见的天然螺旋堆积基序引入跨膜结构域创造了一种膜蛋白, 可实现 Zn(II) 的转运和质子的反向运输。Pirro 等^[82]模仿 Type 3(T3) 含铜多酚氧化酶结构, 在四螺旋束结构中分层设计了 di-Cu 结合位点, 使该酶具有催化邻苯二酚转化为邻醌的活性。

除了四螺旋束外, 还有许多其他的多肽聚集体用于人工酶的设计, 如 β -sheet 蛋白^[83-84]、三螺旋线圈 3SCC^[84]、 α 螺旋桶 α HB^[85-86] 等。 β -sheet 蛋白是蛋白设计的有力支架, 但由于其结构存在非局部相互作用和 β 链边缘聚集现象, 导致从头设计全 β -sheet 蛋白十分困难。Marcos 等^[83]通过将未配对 β 链中的 loop 环进行连接, 得到了由氢键和 β 折叠堆积约束引发的结构规则。利用这些规则, 研究人员从头设计了利用由 8 条反向平行 β 链形成的双链 β -螺旋构建的 Jellyroll 拓扑结构, 这种结构接近于天然蛋白结构, 可用于结合金属和配体及构建活性位点。3SCC 支架包含三个组氨酸残基, 可模拟亚硝酸还原酶催化位点的三个氮供体。通过将 Cu 嵌入 3SCCs 的疏水内腔, 创建了具有氧化还原活性的一系列 $\text{Cu}(\text{NO}_2)_2$ 还原酶, 相较于以往的人造亚硝酸还原酶, 该系列酶的活性有了显著提升^[87]。

近年来, 迅速发展的人工智能技术辅助了酶结构的预测与设计, 加速了酶的进化筛选过程, 极大缩减了研究的时间成本。基于酶结构的计算机辅助设计, 可

使酶获得更高的催化活性和底物耐受性, 甚至新的催化活性^[88-89]。计算机技术的发展使得人工金属酶的设计方法更加便捷, 构建过程更加高效。

3 纳米技术在人工金属酶构建中的应用

近年来, 纳米技术发展迅速并被应用到多个领域, 包括生物探测器、药物递送以及基因传递等^[90]。同时, 利用纳米技术修饰人工金属酶也使其具有明确、稳定、可调控的物化结构和多类酶活性。酶固定化是提高酶适用性的一种常用策略, 利用纳米技术可以实现金属酶的固定化(图 5)。Zambrano 等^[91]通过将人工血红素酶与硫辛酸(lipoic acid, LA)形成的复合物与金纳米颗粒(nanoparticles, NP)结合或将其固定在金电极表面上, 可使其表现出可逆的氧化还原特性和过氧化物酶活性。除固定在纳米颗粒表面, 将金属酶包裹在由纳米材料组成的框架中也能使其性质得到改善^[92]。通过共沉淀法把中空纳米颗粒和葡萄糖氧化酶(glucose oxidase, GOx)固定在金属有机框架(metal-organic frameworks, MOFs)沸石咪唑酸酯骨架 8(ZIF-8)上制备的人工金属酶, 在保持 GOx 氧化活性的同时也显示了良好的过氧化物酶活性^[93]。Wu 等^[94]将非晶态 MOF(amorphous MOF, aMOF)用于酶的原位包装, 通过在水溶液中混合酶、金属离子和有机配体, 所得的 GOx 显示出比晶体 MOF 高 20 倍的活性, 说明 aMOF 对包封的酶起到了保护作用, 具有比天然酶更高的稳定性。

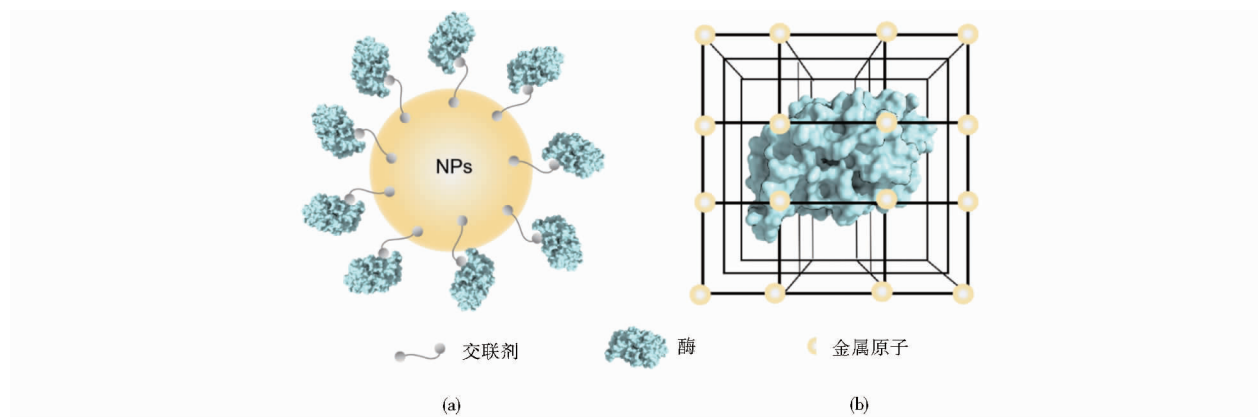


图 5 利用纳米技术辅助修饰人工金属酶

Fig. 5 Strategies of artificial metalloenzymes modification assisted by nanotechnology

(a) Immobilization of artificial metalloenzymes on the NPs surface using crosslinking agents (b) Immobilization of artificial metalloenzymes into the MOFs

目前,研究人员也发现了许多纳米颗粒可以模拟酶的作用。例如,利用手性配体修饰非手性纳米酶催化中心的表面可获得手性纳米酶,将具有超分子手性微环境的生物手性物质与纳米酶结合可构建具有对映选择性的纳米酶^[95]。由不含任何手性分子的 M-聚苯胺(M-polyaniline, M-PANI)扭曲纳米带和 AuNPs 组成的超分子纳米复合材料具有模拟手性过氧化物酶的活性^[96]。Cu₂O 纳米颗粒(Cu₂O nanoparticles, Cu₂O NPs)具有类似细胞色素 c 氧化酶的活性,在有氧条件下,可以催化 Fe(II) 转变为 Fe(III)^[97]。纳米酶固有的物理化学性质使其成为天然酶的良好补充,同时纳米酶还具有更加稳定、经济的特性,从而在生物医学领域具有突出的优势和广阔的应用前景。

4 总结与展望

人工金属酶近年来取得了丰硕的研究成果。通过对天然辅因子进行化学修饰和将非天然辅因子引入蛋白骨架,可改善人工金属酶的催化性质,使其具有催化非天然底物反应的能力;通过选用稳定或优化的天然蛋白骨架和从头设计更简单的新蛋白骨架,可提高人工金属酶的活性和稳定性,赋予其新的催化功能;通过将纳米技术引入人工金属酶,可获得具有良好物理化学性质和催化能力的酶纳米颗粒。随着蛋白结构不断被解析和新技术、新策略的不断涌现,对人工金属酶的研究也更加深入。对具有 22 个残基的锌指水解酶进行研究时发现,小的合成肽可以为金属活性位点提供良好的反应微环境^[98];通过对蛋白质界面进行修饰,可实现蛋白质之间相互作用的微调^[99];利用蛋白融合技术,可得到具有新活性的人工金属酶^[100-101];应用双功能小分子(dual-functional small molecule, DFSM)策略使得细胞色素 P450 酶催化不饱和烃的直接硝化成为可能,构建的人工 P450 过加氧酶催化体系突破了天然硝化酶催化效率低、底物谱窄和产物区域选择性差等局限^[102]。人工金属酶作为生物合成的催化剂和药物设计的化学生物学工具,在有机合成、生物传感器和生物医药等领域具有巨大的应用潜力。另外,将人工金属酶整合到生物体代谢网络中实现利用人工细胞工厂生产非天然产物,为绿色生物制造提供了更加丰富的可选酶元件,有望扩展现有生物合成产物库。Huang 等^[62]将带有 Ir(Me)MPIX 的 CYP119 引入大肠杆菌生产非天然产物,利用 HUG 转运系统取代血红素转运系统 ChuA,在细胞质中可以催化左旋香芹酮的环丙烷

化,非对映选择性达到 80%,环丙烷化产物的产量提高了 13 倍以上,同时突变体 CYP119-P/R256W/V254A 使环丙基柠檬烯产量提高了 3 倍,非对映选择性达到了 54%。然而,由于生物体的复杂性以及金属辅因子对生物体的毒性,将人工金属酶引入微生物仍具有挑战性。

人工金属酶拓展了天然酶的催化功能^[103],但仍然面临着一些挑战,如部分人工金属酶的催化效率有待提高^[10];人工金属酶的金属辅因子和蛋白骨架之间的结合力较天然酶普遍偏弱,影响金属酶的稳定性^[13];由于缺乏有效的筛选方法,酶工程面临耗时、费力的问题。随着计算模拟和人工智能技术的发展,“从头设计”使得定制结构新颖的蛋白骨架及新型金属辅因子-蛋白结合模式成为可能^[104]。通过参数优化改变现有结构模块之间的几何形状来设计螺旋或通过组装已知结构的肽段等,可构建新的蛋白骨架^[105-107]。但新的蛋白结构往往依赖于有限的可选模板,使得从头设计蛋白结构缺乏丰富性和独特性,过于偏好理想化局部结构等,从而限制了从头设计蛋白的功能多样性。深度学习(deep learning, DL)正在助力蛋白结构设计突破这一限制。基于深度学习, Huang 等^[108]开发了一种从头设计氨基酸序列的算法 ABACUS-R,并利用该算法对三个天然主链结构重新设计,经实验验证,其中 86% 的序列可溶表达并能折叠为稳定单体。该方法克服了模板依赖的限制,扩展了从头设计蛋白的结构和功能多样性。深度学习神经网络算法 NLP^[109]、AlphaFold^[110]、AlphaFold 2^[111]等的出现使得模拟蛋白质的三维结构成为可能,从而进一步辅助人工金属酶的设计^[112]。深度学习也可以应用于设计新的蛋白结构, Wang 等^[113]提出一种基于深度学习构建蛋白质功能位点的方法,通过在整个 PDB 上训练 RoseTTAFold,可以生成多样的新结构和构建任何所需的功能残基。算法“受限幻觉(constrained hallucination)”和“修复(inpainting)”在设计金属配位蛋白等方面展示了巨大的潜能。人工智能技术和分子建模技术的发展为解析金属辅因子与配体之间的反应机理提供了有力工具,促进了对反应过程的预测与了解,助推了人工金属酶的理性设计。未来,合成生物学策略和计算机辅助设计工具将进一步助力人工金属酶的设计和构建。

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Research Progress of Artificial Metalloenzymes

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Abstract Enzymes with high efficiency and specificity have attracted much attention from researchers. Among them, metalloenzymes account for about 1/3 of natural enzymes. Metalloenzymes are generally composed of metal cofactors and corresponding protein scaffolds, in which the metal cofactors provide the active center. The protein scaffolds provide the chiral environment and attachment sites for metal cofactors. Existing studies have revealed that metalloenzymes fail to work without metal cofactors. The metal cofactors mainly exist in the form of metal ions or metal ligands. Among the natural metalloenzymes discovered so far, the metal elements in metal cofactors are mainly Fe, Cu, and Zn. Besides, there are also Mn and other metal elements. Metalloenzymes play an important role in organisms, including signal transduction and immune regulation. Various metalloenzymes can catalyze different reactions, such as hydroxylation and epoxidation. However, it is difficult for natural metalloenzymes to catalyze nonnatural substrates. Some metalloenzymes have low catalytic efficiency and poor stability *in vitro*, making them unable to be widely used. Recently, rapidly developed biotechnology has accelerated the development of metalloenzymes. By simulating natural metalloenzymes, artificial metalloenzymes (ArMs) have been constructed continuously. The appearance of ArMs has expanded reaction types. In summary, three main strategies have been applied in designing ArMs, including the reconstruction of cofactors, design of protein scaffolds, and modification of nanoparticles. The reconstruction of cofactors is mainly achieved by chemical modification and replacement. Design of protein scaffolds is achieved by selecting some stable structures and utilizing computer-aided methods. Notably, the development of nanotechnology has also provided good ideas for redesigning ArMs. The enzyme property can be improved by binding metalloenzymes to the surface of nanometers or being embedded in nanoparticles. Herein, we summarize some achievements of ArMs in recent years. A brief introduction about the challenges and opportunities faced by ArMs is provided, which is helpful for the design and application of ArMs.

Key words Artificial metalloenzyme Cofactor Protein scaffold Rational design Nanotechnology