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·评 论·

编者按：

从本期开始，《生物工程学报》新增设了“评论”栏目，邀请领域内的专家，就近期发表在著名权威期刊中的重要突破性成果或进展进行评论，希望为读者带来启发和参考。

本期邀请德国亚琛工业大学生物技术研究所季宇博士和 Ulrich Schwaneberg 教授团队，针对近期发表在期刊 *Chem Catalysis* 的一项关于塑料结合模块促进酶法降解塑料的研究成果进行了评论，以飨读者。

Ulrich Schwaneberg 教授 德国亚琛工业大学生物技术研究所所长，德国莱布尼兹交互材料研究所(DWI)成员，德国生物经济科学中心的董事会成员，SeSaM Biotech 和 Aachen Proteineers 公司联合创始人。主要从事蛋白质工程研究，在酶定向进化领域开发了多种独特方法。在 *Nature Catalysis*、*JACS*、*Angewandte Chemie* 等期刊发表文章 300 余篇，申请国际专利 20 余项。

季宇博士 德国亚琛工业大学生物技术研究所塑料降解小组组长。于 2020 年在德国亚琛工业大学获得博士学位(Summa Cum Laude)，师从 Ulrich Schwaneberg 教授。同年，进入德国亚琛工业大学生物技术研究所/德国莱布尼兹交互材料研究所进行博士后研究，并于 2020 年 4 月起担任项目负责人。目前的研究兴趣集中在发展酶的定向进化及高通量筛选技术用于生产高附加值产物。主持 EU Horizon/DFG/BMBF 等国际或国家级项目 11 项，在 *Angewandte Chemie*、*ACS Catalysis*、*Biotechnology Advances* 等期刊发表文章 20 余篇，担任 *Catalysis* “*High-Throughput Computational Design of Catalysts*”专刊编辑。指导 iGEM-Aachen2020 团队获得国际遗传工程机器设计竞赛亚军。

评论：塑料结合模块促进聚对苯二甲酸乙二醇酯的酶法降解

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LU Yi, HAN Ruizhi, SCHWANEBERG Ulrich, JI Yu. Commentary: polymer binding modules accelerate enzymatic degradation of poly(ethylene terephthalate)[J]. Chinese Journal of Biotechnology, 2023, 39(5): 1883-1888.

摘　要：塑料的大量生产和无节制的使用已造成严重的环境污染。为了减少塑料废物对环境的影响，近年来塑料酶法降解已成为国内外研究者关注的热点。例如，通过蛋白质工程策略提高塑料降解酶催化活性和热稳定性，进一步提高酶法降解的效率。另外，通过融合酶策略将塑料结合模块与塑料降解酶融合，也可以促进塑料降解。近期发表在期刊 *Chem Catalysis* 的一项研究表明，采用碳水化合物结合模块融合策略可以在低浓度(<10 wt%)的底物聚对苯二甲酸乙二醇酯[poly(ethylene terephthalate), PET]中提高塑料降解酶的活性。但是在高浓度底物(10 wt%–20 wt%)中，该策略无法提高 PET 的酶法降解。该项研究对于采用塑料结合模块促进酶法降解塑料具有重要的指导意义。
关键词：塑料降解酶；塑料结合模块；聚对苯二甲酸乙二醇酯

Commentary: polymer binding modules accelerate enzymatic degradation of poly(ethylene terephthalate)

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Abstract: The large scale production and indiscriminate use of plastics led to serious environmental pollution. To reduce the negative effects of plastics waste on the environment, an approach of enzymatic degradation was put forward to catalyze plastics degradation. Protein engineering strategies have been applied to improve the plastics degrading enzyme properties such as activity and thermal stability. In addition, polymer binding modules were found to accelerate the enzymatic degradation of plastics. In this article, we introduced a recent work published in *Chem Catalysis*, which studied the role of binding modules in enzymatic hydrolysis of poly(ethylene terephthalate) (PET) at high-solids loadings. Graham et al. found that binding modules accelerated PET enzymatic degradation at low PET loading (<10 wt%) and the enhanced degradation cannot be observed at high PET loading (10 wt%–20 wt%). This work is beneficial for the industrial application of polymer binding modules in plastics degradation.
Keywords: poly(ethylene terephthalate)-hydrolyzing enzymes; polymer binding modules; poly(ethylene terephthalate)

塑料的无节制使用导致了严重的环境污染，极大地影响了自然生态系统[1]。因此，从环境、生态、经济和社会的角度出发，通过构建高酶解效率、环境友好型塑料降解酶来降解塑料废物和生产高价值的化学品有利于减少环境污染和建立循环经济。提高酶解效率主要表现在以下两方面，首先通过蛋白质工程策略提高酶的热稳定性、增强底物结合能力和减少产

物抑制等性质，进一步提高塑料降解酶的降解效率；另外，将塑料结合模块与塑料降解酶融合也是一种促进酶法降解塑料的策略。近年来，国内外研究者利用塑料结合模块来与多种固体材料结合，其中包括天然或者合成塑料[2]。塑料结合模块因其无与伦比的结合能力已被广泛使用在多个领域，如生物表面修饰(比如防污和抗菌)[3-4]、酶的固定化[5]、材料组装[6]、植物保护[7]和塑料降解[8]等。塑料结合模块尤其在塑料降解方面发挥着重要的作用[9]。

天然的碳水化合物结合模块(carbohydrate-binding modules, CBM)能够增强纤维素酶降解纤维素能力。纤维素酶含有单结构域或多结构域，其中催化结构域与 CBM 连接[10]，可将纤维素等天然聚合物降解为糖单体。由此启发，融合塑料结合模块策略将在提高聚合物降解酶的活性方面具有巨大潜力。例如，与角质酶 Tcur1278 野生型相比，将锚定肽 Tachystatin A2 与角质酶 Tcur1278 融合可将聚酯-聚氨酯纳米颗粒的降解提高 6.6 倍[8]。

聚对苯二甲酸乙二醇酯 [poly(ethylene terephthalate), PET]酶促降解的广泛研究表明，塑料结合模块可以增强酶促 PET 水解[11-12]。因此，我们介绍了最近的一项工作，该工作研究了塑料结合模块策略在PET高固含量下酶促PET水解中的作用[13]。基于工业应用考虑，Graham 等[13]研究了 PET 水解酶在与结合模块融合后，分别在低固体负载和高固体负载下的催化效率，这有助于快速部署切实可行的方法来解决塑料污染问题。

Graham 等[13]首先构建了叶堆肥角质酶(leaf compost cutinase, LCC)的 1 种新的热稳定变体(LCC^{YCCG})，并将其与 5 种 A 型 CBM 融合(LCC^{YCCG}-CBM) (图 1)。通过比较 LCC^{YCCG}- CBM 融合酶与 LCC^{YCCG} 性质(包括酯酶活性、PET 结合活性和热稳定性)，结果表明 LCC^{YCCG}- CBM 融合酶酯酶活性[即水解双(2-羟乙基)对苯二甲酸二酯(BHET)的活性]与 LCC^{YCCG} 保持不变。但是，在 LCC^{YCCG}-*Bs*CBM3 的基础上，其他 4 种 LCC^{YCCG}-CBMs (LCC^{YCCG}-*Tr*CBM1、LCC^{YCCG}-*Tt*CBM10、LCC^{YCCG}-*St*CBM64 和 LCC^{YCCG}-*Tf*CBM2a)显示出比 LCC^{YCCG} 更高的 PET 结合能力。另外，通过差示扫描量热法(differential scanning calorimetry, DSC)测量 LCC^{YCCG} 和 LCC^{YCCG}-CBMs 的热变性温度，结果显示 LCC^{YCCG} 和 LCC^{YCCG}-CBMs 具有相似的热变性温度，表明 CBMs 对 LCC^{YCCG} 的性能影响有限。因此，将 LCC^{YCCG} 与 CBMs 融合可以改善 PET 的结合，而 CBMs 并不影响 LCC^{YCCG} 的酯酶活性和热稳定性。

对 LCC^{YCCG}-CBMs 的性能进行表征后，在 PET 薄膜的低负荷(2 wt%)、40–90 °C 的温度下检测 LCC^{YCCG} 和 LCC^{YCCG}-CBMs 的无定形 PET 水解活性。结果表明，在 40−70 °C 的温度范围内，酶的活性随着温度的增加而增强，随后在 70−90 °C 的温度范围内酶的活性随着温度的增加而下降。LCC^{YCCG}-*Tr*CBM1 和 *Tt*CBM10 比 LCC^{YCCG} 和其他 LCC^{YCCG}-CBM 表现出较高的活性。然而，LCC^{YCCG} 与 *Tr*CBM1 的等摩尔混合物不能提高 PET 的降解活性，表明只有通过共价连接 LCC^{YCCG} 和 CBM 才能提高 PET 的降解活性。

此外，在 1 wt%–20 wt%的 PET 负载条件下，检测了 LCC^{YCCG}、LCC^{YCCG}-*Tr*CBM1、LCC^{YCCG}-*Tt*CBM10 和 LCC^{YCCG}-*St*CBM64 的 PET 降解活性。结果表明，在 PET 负载低于 5 wt%时，与 LCC^{YCCG} 相比，LCC^{YCCG}-*Tr*CBM1、LCC^{YCCG}-*Tt*CBM10 和 LCC^{YCCG}-*St*CBM64 从 PET降解中产生了更多的单体。然而，对于10 wt%–20 wt%的PET负载，即使酶浓度提高至1 mmol/L，LCC^{YCCG}-CBMs 的活性也并未提高。为了评估塑料结合模块在工业相关条件下的作用，将反应体系扩大至 1 L，并将 PET 负载设定为 20 wt%。在此反应条件下，LCC*YCCG*-*Tr*CBM1 并没有显示出比 LCC^{YCCG} 更好的活性。

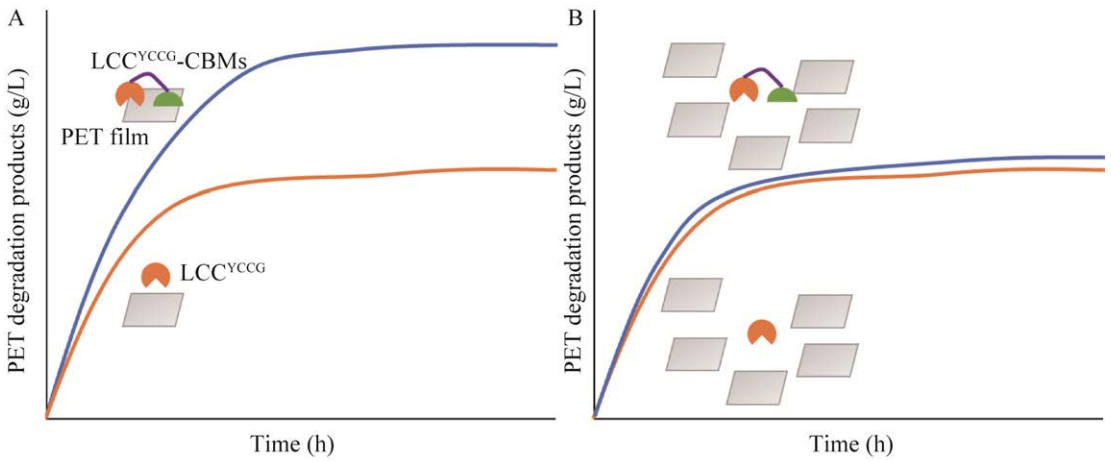

图 1　在 PET 低负载(A)和高负载(B)的情况下，LCC^{YCCG} 和 LCC^{YCCG}-CBM 降解 PET 的比较

Figure 1　The PET degradation performance of LCC^{YCCG} and LCC^{YCCG}-CBM with low PET loading (A) and high PET loading (B).

综上所述，Graham 等[13]的报道表明，当 PET 负载较低(15 wt%)时，CBMs 可以发挥促进 PET 降解的作用，而当 PET 负载较高(10 wt%–20 wt%)时，CBMs 的作用变得很弱。因此，本团队提出了一些策略来发挥塑料结合模块在促进 PET 降解方面的作用。首先，塑料结合模块对高负载 PET 的降解作用降低的原因尚不清楚。由于塑料结合模块加速 PET 降解与 PET 结合强度有关，了解塑料结合模块的 PET 结合机制将有助于解决这一问题。其次，不同的 PET 应在降解过程中进行测试，如不同的类型、结晶度、化学纯度和商业的 PET 塑料瓶。第三，许多研究人员已经研究了塑料结合模块的改造以提高塑料结合强度[14-15]，应用具有更高的塑料结合强度的塑料结合模块突变体将会在更高的 PET 负载下促进塑料的降解。相信经过深入地研究，可以实现 PET 或其他种类的聚酯在较高塑料负载下的加速降解。

The indiscriminate use of plastics leads to serious environmental hazards including increased land and water pollution, which has largely affected the natural ecosystem[1]. Therefore, there are significant environmental, ecological, economic, and social necessity for the development of novel environmentally friendly enzymatic technologies to degrade plastic wastes and generate added-value chemicals that will mitigate the present detrimental effects of plastics pollution and at the same time establish a new source of raw materials, in conformity to the notion of a sustainable and circular economy. To improve the degradation efficiency of plastic degradation enzymes, protein engineering strategies were utilized to modify enzymes and ultimately improve their catalytic performance. Protein engineering can be used to tailor different enzyme properties, including improving enzyme thermostability, enhancing the binding of enzyme to substrate, and reducing product inhibition effects. Fusing polymer degradation enzymes with polymer binding modules is an alternative approach to advance polymer enzymatic degradation. In the past decades, polymer binding modules have been utilized to bind with a variety of solid materials including natural and synthetic polymers[2]. With unparalleled binding affinity, polymer binding modules have been widely applied in biological surface functionalization (e.g., antifouling and antimicrobial properties)[3-4], enzyme immobilization[5], material assembling[6], plant protection[7], and plastic degradation[8]. Especially, polymer binding modules display significant roles in the acceleration of polymer enzymatic degradation[9].

Nature generates carbohydrate binding modules (CBM) to accelerate cellulose degradation by cellulases. In general, cellulases, the carbohydrate-active enzymes used to degrade natural polymers like cellulose into sugar monomers, contain a single domain or multiple domains and the catalytic domain is connected with CBM through a linker[10]. Inspired by nature, polymer binding modules have enormous potential in accelerating the activity of polymer degrading enzymes. For instance, fusing anchor peptide Tachystatin A2 with cutinase Tcur1278 enhanced the degradation of polyester-polyurethane nanoparticles by a factor of 6.6 compared with the Tcur1278 wild-type[8].

In the last decades, extensive work in poly(ethylene terephthalate) (PET) enzymatic degradation has revealed that the polymer binding modules could enhance enzymatic PET hydrolysis[11-12]. Here, we introduced a recent work, which studied the role of binding modules in enzymatic PET hydrolysis at high-solids loadings[13]. Taking industrial application into consideration, Graham et al.[13] investigated the catalytic efficiency of PET-hydrolyzing enzymes at low and high-solids loading after fusing with binding modules, which facilitates the rapid deployment of feasible approaches to realistically address plastics pollution.

Rosie Graham and coworkers[13] first generated a new thermostable variant (LCC^{YCCG}) of leaf compost cutinase (LCC) and fused it with five type A CBMs (LCC^{YCCG}-CBM) (Figure 1). Several properties of LCC^{YCCG}-CBM fusion proteins, including esterase activity, PET binding activity, and thermal stability, were compared with that of LCC^{YCCG}. The esterase activity was measured from the hydrolysis of the diester bis(2-hydroxyethyl) terephthalate (BHET), and the LCC^{YCCG} esterase activity remained unchanged after fusing with CBMs. In addition, on top of LCC^{YCCG}-*Bs*CBM3, other four LCC^{YCCG}-CBMs showed similar PET binding and all of them displayed higher PET binding than LCC^{YCCG}. Among them, LCC^{YCCG}-*Tr*CBM1 displayed the highest PET binding affinity. The temperatures of thermal unfolding transition for LCC^{YCCG} and LCC^{YCCG}-CBMs were measured by differential scanning calorimetry (DSC). Results showed that LCC^{YCCG} and LCC^{YCCG}-CBMs had similar temperatures of thermal unfolding transition, indicating that CBMs posed limited effects on the properties of LCC^{YCCG}. Thus, fusing LCC^{YCCG} with CBMs could improve the PET binding while CBMs did not affect the esterase activity and thermal stability of LCC^{YCCG}.

After characterization of LCC^{YCCG}-CBMs properties, amorphous PET hydrolysis activity of LCC^{YCCG} and LCC^{YCCG}-CBMs was detected from 40 °C to 90 °C at low loadings of PET films (2 wt%). Generally, the enzyme activity was increased from 40 °C to 70 °C and later decreased until the temperature reached 90 °C. LCC^{YCCG}-*Tr*CBM1 and *Tt*CBM10 exhibited the highest activity than LCC^{YCCG} and other LCC^{YCCG}-CBMs. However, the activity cannot be improved for the PET degradation by equimolar mixtures of LCC^{YCCG} with *Tr*CBM1, indicating the enhanced PET degradation activity can only be achieved by covalently linked LCC^{YCCG} and CBMs.

Moreover, the PET degradation activity of LCC^{YCCG}, LCC^{YCCG}-*Tr*CBM1, LCC^{YCCG}-*Tt*CBM10, and LCC^{YCCG}-*St*CBM64 were measured at 1 wt%–20 wt% PET loading. In comparison to LCC^{YCCG}, LCC^{YCCG}-*Tr*CBM1, LCC^{YCCG}-*Tt*CBM10, and LCC^{YCCG}-*St*CBM64 generated a higher number of monomers from PET degradation when the PET loading is lower than 5 wt%. However, no improved activity of LCC^{YCCG}-CBMs can be observed for 10 to 20 wt% PET loading even when the enzyme concentration was elevated to 1 mmol/L. In order to evaluate the role of polymer binding modules in industrially relevant conditions, the reaction system was enlarged into 1 L and the PET loading was set as 20 wt%. Under these reaction conditions, LCC^{YCCG}-TrCBM1 did not show improved activity than LCC^{YCCG}.

In summary, Graham *et al*.[13] reported that CBMs could exert the role of facilitating PET degradation when the PET loading was low (1 wt%–5 wt%) and the effects of CBMs became weak when the PET loading was high (10 wt%–

20 wt%). To exert the role of polymer binding modules in advancing PET degradation, we put forward some approaches. First, the reasons underlying the reduced effects of polymer binding modules on the degradation of high loading PET are not clear. As the accelerated PET degradation of polymer binding modules is associated with the PET binding strength, understanding the binding mechanism behind will be helpful for solving this problem. Second, different PET substrates such as different types, crystallinity, chemical purity, and commercial bottles, should be tested during degradation. Third, the engineering of polymer binding modules to improve polymer binding strength has been studied by many researchers[14-15]. Applying polymer binding module variants with higher polymer binding strength would be a strategy to improve polymer degradation with higher PET loading. We believed that the accelerated degradation of PET or other kinds of polyesters with higher polymer loading can be realized after extensive investigation.

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