Artificial plant cell walls as multi-catalyst systems for enzymatic cooperative asymmetric catalysis in non-aqueous media†

Luca Deiana,† Abdolrahim A. Rafi,† Veluru Ramesh Naidu,† Cheuk-Wai Tai,†,‡ Jan-E. Bäckvall†,§ and Armando Córdova*,§

The assembly of cellulose-based artificial plant cell wall (APCW) structures that contain different types of catalysts is a powerful strategy for the development of cascade reactions. Here we disclose an APCW catalytic system containing a lipase enzyme and nanopalladium particles that transform a racemic amine into the corresponding enantiomerically pure amide in high yield via a dynamic kinetic resolution.

The main structural building block of the plant cell wall is cellulose, which forms interwoven microfibril networks that are embedded into a polysaccharide and protein matrix to constitute a multilayer architecture (Fig. S1, ESI†).

Advances in biocatalysis are a requirement for addressing the increasing need for sustainable chemistry in the preparation of materials, fine-chemicals and pharmaceutical synthesis. The ability of biocatalysts to operate under unnatural conditions (e.g. in non-aqueous media and at high temperatures) and to catalyze non-natural chemical reactions are important factors for future advancements of the “enzyme universe.” To date, the primary strategy for obtaining biocatalyst stability in non-aqueous media is through immobilization within, or on the surface of, solid supports such as silica or fossil-based polymer resins. However, often increased stability leads to decreased in biocatalyst activity. Thus, there is a significant incentive for developing new (bio)technologies for the advancement of non-aqueous industrial biocatalysis. The development of homogeneous and heterogeneous multi-catalytic systems for cooperatively advancing chemical transformations by combining different types of catalysts (e.g. metal, enzyme or metal-free) is another elegant way of expanding chemical synthesis and biocatalysis.

† Department of Natural Sciences, Mid Sweden University, Holmgatan 10, 85 179 Sundsvall, Sweden. E-mail: Armando.Cordova@miun.se
‡ Department of Materials and Environmental Chemistry, Arrhenius Laboratory, Stockholm University, 10 691 Stockholm, Sweden
§ Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, SE-10 691 Stockholm, Sweden. E-mail: jeb@organ.su.se
* Electronic supplementary information (ESI) available. See DOI: 10.1039/d1cc02878b

The construction of protein-derived artificial metallo-enzymes for application in chemical synthesis in non-aqueous media is an important area of research that has attracted considerable attention. Here the state-of-the-art involves directed evolution and/or introducing a transition metal into the enzyme. In the latter approach, the metal of a heme enzyme may be replaced by a transition metal or a transition metal may be introduced into the active site of the enzyme. The development of heterogeneous artificial metalloenzyme systems based on dual enzyme/metal nanocatalysts was also recently disclosed. However, partial deactivation of the enzyme component led to a limited recycling of the artificial metalloenzyme. This can also be the case for other types of heterogeneous enzyme–metal nanohybrid catalyst systems.

To address this problem, we have focused on a multidisciplinary approach for the advancement of non-aqueous biocatalysis, which is based on a synergistically combined platform of cellulose chemistry, nanocatalysis, and heterogeneous catalysis. Based on our research on cooperative heterogeneous catalysis and construction/application of functional cellulose-based materials, we envisioned a novel approach for setting up nature-inspired catalyst systems that are guided by the intriguing assembly of the primary cell wall of plants. Specifically, self-assembled mixtures of cellulose, polysaccharides, enzymes, surfactants and metals can create an artificial plant cell wall (APCW)-like structure with an embedded multi catalyst system (1), which next could catalyze the stereoselective formation of product D from substrates A + B + C in non-aqueous media (Fig. 1). Herein, we disclose this concept by creation of an assembled PCW mimic, which is employed as a heterogeneous catalyst and a “deracemase” system for converting racemic mixtures of amines 2 to the corresponding chiral amides (R)-3 by dynamic kinetic resolution (DKR).

We began our search for catalytically active and synthetically useful APCW catalyst systems by a thorough study of the kinetic resolution of racemic amine 2a using Candida antarctica Lipase B (CALB) as the enzyme of choice (Table S1 and Fig. S2, ESI†).
The assembly of various APCWs containing this serine hydro-lase revealed a significant enhancement in activity of the enzyme by the different self-assembled polysaccharide-based systems. In fact, CALB is nearly inactive without some type of activation. We also investigated the role of all components used when the APCW contained microcrystalline cellulose (MCC) as the component. The use of cellulose as a support material led to a significant activation as compared to just the use of CALB. Thus, the cellulose immobilization improved the performance of the enzyme in the organic solvent. This is a beneficial effect of immobilization.34 The use of buffer35 within the assembled network was also beneficial but is not necessary. Modifying CALB with a surfactant increased the rate of amidation, but this catalyst system cannot be recycled and used for multiple reactions.36–40 The best self-assembly combination turned out to be non-covalent modification of CALB with surfactant polyethylene glycol hexadecyl ether (Brij) in phosphate buffer together with MCC forming APCW system 3 (APCW3).41 We also successfully created assembled APCWs containing nanofibrillated cellulose (NFC, APCW5), chitosan (APCW6) and nanocrystalline cellulose (CNC, APCW7), respectively. However, in the latter cases (APCW5-7) the E-value (enantiomeric ratio)42 for 2a was lower than that obtained when MCC was used as the polysaccharide component. We next decided to further explore APCWs containing aminopropylsilane modified MCC (MCC-Amp) and Pd(0) nanoparticles (MCC-Amp-Pd(0)-NPs) for the kinetic resolution of rac-2a. Thus, self-assembled APCW8 and APCW9 (Fig. 2a) were evaluated as heterogeneous catalysts. The AmP surface modification as well as the presence of Pd(0)-NPs did not decrease the excellent enantioselectivity of the enzyme (E > 400). We also prepared and evaluated a hybrid catalyst containing CALB/Brij/Pd(0)/buffer salts. This catalyst did not exhibit good selectivity (E = 21). Thus, the presence of the MCC component is essential for reaching a high E-value under the evaluated reaction conditions. With these results in hand, the novel APCW9 structure was chosen and further investigated as the nature-inspired multi-catalyst system. The assembled APCW9 multi-catalyst structure (Fig. 2a) had a narrow distribution of Pd nanoparticles (main distribution of 1.6 to 2.8 nm in size, Fig. 2f) as confirmed by TEM analyses (Fig. 2b and c). The distribution between Pd(0) and Pd(II) in the Pd NPs of APCW9 was difficult to determine due to the presence of the salts of the phosphate buffer; however, it was similar to that of its MCC-AmP-Pd(0) component as determined by XPS (Fig. 2d and e). Thus, we had accomplished the assembly of an APCW structure containing Pd-nanoparticles and enzyme units as well-defined catalysts. Next, the APCW-catalyzed deracemization of amine 2a was investigated (Table 1). We found that APCW9 is an excellent catalyst system, which is able to catalyze the formation of amide 3a under DKR
The heterogeneous APCW9 multi-catalyst system catalyzed the deracemization of chiral amines (2a) in high enantioselectivity and the corresponding amide (R)-3a was isolated in 48% yield (50% is the maximum theoretical yield) in >99% ee even after 9 recycles (Fig. S2a, ESI†). It is remarkable that the sustainable APCW9 multi-catalyst system can catalyze the DKR of 2a over several cycles (Fig. S2b, ESI†) since most of the previous recycling studies of artificial “deracemases” and hybrid enzyme catalyst systems containing CALB displayed a limited recycling due to significant deactivation of the enzyme catalyst.22 Thus, the disclosed artificial plant-cell wall concept enables several important features of accomplishing effective biocatalysis in non-aqueous media such as maintaining activity and stability in organic solvents, high stereoselectivity, recyclability, asymmetric synthesis, high temperature tolerance (90 °C), and efficient cooperation with chemical catalysts. No leaching of the metal catalyst was observed during the recycling studies.

We have demonstrated that sustainable artificial plant cell-wall (APCW) structures, which contain a multi-catalytic system of both enzyme and metal nanoparticle catalysts, can be used to accomplish asymmetric catalysis and cascade synthesis in non-aqueous media. A key feature of the disclosed APCW concept is its intrinsic ability to activate the enzyme protein for catalysis in organic solvents. The heterogeneous polysaccharide-based APCW network having both enzyme and metal nanoparticles in its structure, catalyzed DKR of primary amines by cooperative catalysis. Thus, the assembled sustainable APCW structure acts as a “deracemase”. It is also possible to reuse the bioinspired APCW structures in multiple-reaction cycles. In comparison, previous attempts of constructing artificial “deracemases” using silica-based or cross-linked enzyme aggregate systems had limitations in their recycling due to deactivation of the enzyme.
Conflicts of interest

There are no conflicts to declare.

References

1 (a) D. J. Cosgrove, *Nat. Rev. Mol. Cell Biol.*, 2005, 6, 850; (b) B. B. Buchanan, W. Grussiem and R. L. Jones, *Biochemistry & Molecular Biology of Plants*, American Society of Plant Physiologists, 2000; (c) E. Sjöström and Wood Chemistry, *Fundamentals and Applications*, Elsevier, 1992; (d) D. Klemm, B. Heublein, H.-P. Fink and A. Bohn, *Angew. Chem., Int. Ed.*, 2005, 44, 3358.

2 T. Li, C. Chen, A. H. Brozena, J. Y. Zhu, L. Xu, C. Driemeier, J. Dai, O. J. Rojas, A. Isogai, L. Wägberg and L. Hu, *Nature*, 2021, 590, 47.

3 (a) A. J. Ragauskas, et al., *Science*, 2006, 311, 484; (b) R. A. Sheldon and P. C. Pereira, *Chem. Soc. Rev.*, 2017, 46, 2678; (c) C. Wohlgemuth, *Curr. Opin. Biotechnol.*, 2010, 21, 713.

4 A. M. Klíbanov, *Nature*, 2001, 409, 241.

5 A. Zaks and A. M. Klíbanov, *Science*, 1984, 224, 1249.

6 P. S. Brogan, L. Bui-Le and J. P. Hallett, *Nat. Chem.*, 2018, 10, 859.

7 H. Renata, H. Z. Wang and F. H. Arnold, *Angew. Chem., Int. Ed.*, 2015, 54, 3351.

8 R. A. Sheldon and S. van Pelt, *Chem. Soc. Rev.*, 2013, 42, 6223.

9 (a) C. A. Denard, J. F. Hartwig and H. Zhao, *ACS Catal.*, 2013, 3, 2856; (b) R. C. Wende and P. R. Schreiner, *Green Chem.*, 2012, 14, 1821; (c) O. Pámies and J.-E. Bäckvall, *Chem. Rev.*, 2010, 103, 3247; (d) F. Rudroff, et al., *Nat. Catal.*, 2018, 1, 12; (e) R. A. Sheldon and D. Frides, *Chem. Commun.*, 2018, 54, 6088; (f) O. Verho and J.-E. Bäckvall, *J. Am. Chem. Soc.*, 2015, 137, 3996; (g) S. Afwerki and A. Córdova, *Chem. Rev.*, 2016, 116, 13512; (h) D. Parmar, E. Sugiono, S. Raja and M. Rüepe, *Chem. Rev.*, 2014, 114, 9047; (i) Z. C. Litman, Y. Wang, H. Zhao and J. F. Hartwig, *Nature*, 2018, 560, 355.

10 (a) M. E. Wilson and G. M. Whitesides, *J. Am. Chem. Soc.*, 1978, 100, 100; (b) M. Diéguez, J.-E. Bäckvall and O. Pámies, ed., *Artificial Metalloenzymes and MetalloDNAzymes in Catalysis*, Wiley-VCH, 2018. ISBN: 978-3-527-34178-8.

11 S. B. J. Kan, R. D. Lewis, K. Chen and F. H. Arnold, *Science*, 2016, 354, 1048.

12 S. C. Hammer, G. Kuhl, E. Watkins, S. Huang, H. Minges and F. H. Arnold, *Science*, 2017, 358, 215.

13 J. B. S. Kan, X. Huang, Y. Gumulya, K. Chen and F. H. Arnold, *Nature*, 2017, 552, 132.

14 K. Chen, X. Huang, S. B. J. Kan, J. Kan, R. Zhang and F. H. Arnold, *Science*, 2018, 360, 6384.

15 R. K. Zhang, K. Chen, X. Huang, L. Wohlschlag, H. Renata and F. H. Arnold, *Nature*, 2019, 565, 67.

16 H. M. Key, P. Dydio, D. S. Clark and J. F. Hartwig, *Nature*, 2016, 534, 534.