Building a Porous Molecular Machine That Replicates Natural Enzymes

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An artificial enzyme with record catalytic activity for organophosphorus degradation has been created from a crown-ether threaded covalent organic framework.

Enzymes are the biological molecules that speed up the rate of chemical reactions in all living organisms from bacteria to the human body through processes called biocatalysis. Naturally occurring organophosphorus hydrolase (OPH) is one example of an enzyme found in several bacteria, a few fungi and cyanobacteria, that has the job of hydrolyzing organophosphates (OPs). Some 40% of the pesticides used worldwide, along with plasticizers, petroleum additives, and chemical warfare agents, are organophosphates. While multiple biotechnological applications across industry such as bioremediation are reliant on OPH, natural enzymes are not sufficiently stable for practical use. In this issue of ACS Central Science, Yuan, Zhu, and co-workers demonstrate, for the first time, a new strategy for an industrially applicable “mimic” for OPH capable of degrading OPs.

While nature has evolved over millennia and has optimized enzymatic processes such as those involving OPH, chemists have been busy designing artificial molecules that replicate nature’s exquisite machinery. This “molecular engineering” of sorts is limited only by the creativity and ingenuity of the chemist and their skill in executing synthetic reactions to achieve a desired product. In their work, Yuan, Zhu, and co-workers propose a new strategy for achieving a robust porous artificial enzyme that mimics OPH function. The thermal stability of the artificial enzyme is a key parameter for its practical usage; most natural-microorganism enzymes lose their activity as the temperature increases. Central to their design philosophy is replicating the active domain of OPH, which involves two zinc fragments within a 3.3–4.6 Å distance, corresponding to the transition-state barrier crossing and the product-release steps, as depicted in Figure 1. These next-generation artificial enzymes are affectionately called nanozymes.

To mimic the function of the binuclear zinc active site in OPHs such as that shown in Figure 1, the authors exploited the concept of mechanically interlocked molecules (MIMs). These MIMs function like a simple machine with a wheel and an axle (Figure 2): in the present case, the “wheel” is a cyclic molecule called a crown-ether, and the “axle” is a linear 2,2′-bipyridine molecule. The molecule formed has the special name of pseudorotaxane. The two molecular components of the MIM are linked through a mechanical bond that allows them to be separated and combined while maintaining structural integrity. Specifically, when zinc(II) ions are added to the MIM, the wheel moves along the axle, creating dynamic motion. The excellent properties of the crown-ether ring threaded COF solid, such as its high activity, substantial stability, and excellent...
As a platform for the MIMs, a covalent organic framework (COF) was used to host the dynamic molecules. The advantage of a porous scaffold, based on a COF, is that the pore sizes, chemical functionality, and network topology can be tuned. The authors also leveraged recent evidence showing that COFs incorporating porphyrin active sites are good biomimetic catalysts that resemble natural enzymes. One significant issue with COF architectures, however, is their rigidity that can limit catalytic performance. Through the incorporation of the MIM, the COF was conferred with greater structural flexibility, thus enabling the nanozyme to mimic the conformational transformation in OPH biocatalysis.

The elegance of the chemical design deserves some further attention as it underpins the structure–function relationship leading to biocatalysis. It is well-known that nanomaterials such as COFs can be treated postsynthetically to incorporate additional functionality by regarding the material somewhat like a molecular flask. Since there are

Figure 1. An example of the active site of the OPH enzyme called phosphotriesterase which can hydrolyze OPs. (A) Three pockets (large pocket, small pocket, leaving pocket) of the substrate-binding region. (B) Coordination of the binuclear zinc center. Reprinted with permission from ref 3. Copyright 2019. The Authors. Published by American Chemical Society.

Figure 2. Schematic showing the design strategy for the crown-ether threaded mechanically intercalated COF network that mimics the active site of OPH enzymes.
two zinc active sites, one coordinated to the crown-ether “wheel” and the other to the bipyridine “axle”, the coordination for the zinc(II) ions can change as the wheel moves along the axle. It is this dynamic behavior, and the variation of the geometry that confers the structure with the excellent flexibility that enables the structural transformation of OP during hydrolysis, akin to the natural enzyme in Figure 1. This mechanism is in line with the authors design philosophy, where the interlocked crown-ether ring slides along the COF skeleton to simulate the behavior of natural hydrolase.²

One challenge in working with COFs is their semi-crystallinity, which necessitates the use of a wide array of structural and physical characterization methods. Here, the authors used a plethora of experimental techniques including nuclear magnetic resonance (NMR), infrared (IR) and X-ray photoelectron spectroscopy (XPS), and gas sorption analysis, coupled with computation (Materials Studio 7.0), to gain insight into the structure of the COF and its function for OP degradation. Careful analysis of the NMR and IR spectroscopic data coupled with molecular dynamics calculations to explore the mechanism demonstrated that the material was thermally stable and recyclable over 10 cycles. The degradation performance of the COF for OPs (e.g., paraoxon-ethyl and paraoxon-methyl) was the highest of a range of nanomaterials, including metal–organic frameworks (MOFs).⁶

While the concept of molecular machines performing useful tasks dates back some 60 years to a lecture by physicist Richard Feynman titled “There’s Plenty of Room at the Bottom,”³⁹ the current paper demonstrates that artificial molecular machines with a pathway to industrial implementation are exceedingly rare. This paper presents a stand-out performing nanozyme with exceptional catalytic performance, even surpassing the rates for some natural OPHs by over 30 times.

In future, synthesis protocols for COFs with remarkable performance characteristics such as this will be sought. For example, the batch-to-batch variability of COFs (and for that matter MOFs) presents a hurdle that can be overcome using continuous synthesis protocols that could enable the scale-up required to reach industrial quantities of materials. From a fundamental perspective, significant further attention is required to elucidate the mechanism for the biocatalytic reaction. In the present case, computation has been used in concert with experimentation to provide mechanistic insights. It is also interesting to consider how further improvements could generate even more efficient and effective biocatalysts (“nanozymes”) that are applicable across the field of biotechnology from biosensing to immunooassays, theranostics, and removal of environmental pollutants, among others.

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