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Deep Sea Mining for Unique Biocatalysts

In this issue of *Chemistry & Biology*, Ferrer and coworkers describe the discovery of five esterases from a distinct deep sea hypersaline biotope [1]. Interestingly, one enzyme has two unique features—it contains three active centers mediating distinct esterolytic activities, and its tertiary/quaternary structure can be modulated by environmental changes.

The application of enzymes in biocatalysis is especially important for processes to synthesize building blocks for pharmaceuticals, pesticides, and fine chemicals [2–6]. The majority of enzymatic applications use hydrolases, and within this enzyme class lipases and esterases are the dominant biocatalysts. All processes require a priori the availability of a suitable enzyme. These enzymes have been traditionally identified by a screening approach, e.g., from soil samples or strain collections by enrichment culture [7, 8]. Once a suitable biocatalyst is identified, strain improvement as well as cloning and expression of the encoding gene enables production on a large scale. Unfortunately, only a tiny fraction (estimated to be 0.001%–1%) of the global biodiversity can be accessed using common cultivation technology [9, 10]. However, the metagenome approach has opened access to the vast wealth of information in previously inaccessible organisms. First demonstrated in 1980 for the extraction and digestion of genomic DNA from a soil sample [11], it soon became the technology of choice for biocatalyst discovery as gene libraries can be directly generated—without the need for cultivation—from environmental DNA. These DNA libraries are then expressed in suitable microbial hosts followed by screening or selection procedures to identify desired enzymatic activities [10, 12–16]. Most importantly, the methodology provides rather easy access to biotopes from extreme environments for which suitable cultivation technology mimicking conditions of the natural habitat (i.e., high pressure, high temperature, drastic pH, unknown nutrients) hardly exist.

Indeed, the contribution by Ferrer et al. [1] presents a clear application of this approach, as unique esterases were discovered from a deep-sea hypersaline anoxic basin (DHAB) of the Eastern Mediterranean created 5–6 million years ago. This habitat, which was physically isolated from other habitats for thousands of years, represents the most extreme conditions in our biosphere, as it is characterized by high salinity, density, and hydrostatic pressure, an absence of light, anoxia, and a sharp chemodecline (i.e., a drastic change in the chemical composition).

To date, thousands of enzymes have been identified from environmental DNA using the metagenome approach. One impressive example of the power of this methodology is the discovery of >130 novel nitrilases from more than 600 biotope-specific DNA libraries [17], compared to fewer than 20 nitrilases previously isolated by classical cultivation methods. Detailed characterization of nitrilase substrate specificity and enantioselectivity revealed not only a range of enzymes suitable for biocatalysis but also an interesting phylogenetic relationship between them and confirmed the broad evolutionary diversity expected from such an array of enzymes [18]. Similarly for lipases and esterases, several hundred novel biocatalysts have been found (for a recent overview see [12]). Thus, at a first glance, the discovery of only five new esterases by Ferrer et al. in the DHAB appears low [1]. However, one esterase shows rather surprising properties.

This enzyme (named O.16) exhibits several properties typical of most esterases but shows two highly unique characteristics: First, it efficiently resolves solketal acetate (Figure 1)—a chiral building block, for which no sufficiently selective enzyme has been identified—with very high enantioselectivity (E > 100), making it an interesting biocatalyst for organic synthesis. The much more striking second feature relates to its unique sequence and structural properties. Esterases, similar to lipases and many other hydrolases, contain *one* catalytic triad composed of Ser, His, and Asp with the active-site serine embedded in consensus sequence motifs (Gly-X-Ser-X-Gly or Gly-Asp-Ser-Leu; X denotes any amino acid). However, sequence analysis of O.16 revealed that this enzyme has no similarity to known esterases except for the consensus motifs, but surprisingly, it contains *three* of them. Indeed, site-directed mutagenesis and biochemical analysis strongly support the hypothesis that O.16 contains three catalytic serines, one conferring thioesterase activity and two mediating carboxyl esterase activity.

Moreover, the tertiary and quaternary structure of the isolated enzymes was shown to be pressure dependent. Experiments mimicking the pressure prevailing in the DHAB (around 40 MPa) showed that three esterases lost activity (54%–62% residual activity), one esterase was stable (O.21, 95%), and O.16 was even activated (1.9-fold more active at 20 MPa, 1.5-fold more active at 40 MPa). These results suggest that O.16 and O.21 evolved especially to withstand the high pressure in their habitat. O.16 appears to occur in several forms with differences not only in its multimeric forms but also in the molecular weights of the monomers. For instance, under standard conditions (atmospheric pressure, no salt), it is a monomer (104 kDa). Addition of the reducing agent DTT generates two polypeptides, a 85 kDa fragment hydrolyzing only propionyl-CoA, and a 21 kDa fragment active only toward *p*-nitrophenyl butyrate. Under pressure (40 MPa) and at high saline content (i.e., 2–4 M NaCl), it is a homotrimeric protein (325 kDa, the largest esterase reported until now) with up to 700-fold increased activity compared to standard conditions. This multimer can also be reversed to the 104 kDa monomer by either salt removal or at atmospheric pressure. Thus, O.16 has a substantially higher

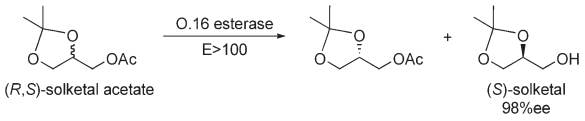

Figure 1. Kinetic Resolution of Racemic Solketal Acetate with O.16 Esterase Proceeded with the Highest Enantioselectivity Reported So Far in the Literature

level of structural and functional complexity than other known esterases.

In conclusion, the work by Ferrer and coworkers does not only add new enzymes to the number of available biocatalysts. It substantially expands our knowledge about enzyme functions and exemplifies Nature's ability to evolve remarkable biocatalysts with no similarity to known enzymes and bearing striking biochemical properties, which would have been rather impossible to discover without the metagenome approach.

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Selected Reading

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