A family of \(\alpha\)-mannosidases uses a catalytic mechanism akin to that of the uncatalyzed base-promoted aqueous reaction in which an epoxide intermediate is transiently formed.

One of the remarkable features in the evolution of biological systems is their ability to experiment and sample, over time, many solutions to a problem. In the end, nature agnostically picks any solutions that work to support life. With regard to enzymes, this process of divergent evolution often leads to different families of functionally related enzymes that use differing catalytic mechanisms. Nevertheless, a feature commonly seen is that one of these functionally related enzyme families will exploit and improve upon the existing lowest energy pathway for the corresponding spontaneous aqueous phase reaction. In this issue of ACS Central Science, Sobala et al. provide the first convincing data to demonstrate such a case for an unusual family of glycoside hydrolases (GHs).

The GHs form a vast superfamily of enzymes found in every kingdom of life. These biotechnologically relevant enzymes have been categorized into over 100 distinct GH families (http://www.cazy.org/), yet they use a relatively limited repertoire of catalytic mechanisms. Indeed, stabilization of a cationic transition state by the endocyclic ring oxygen is simply too favorable a feature for nature to ignore. Accordingly, GHs nearly always exploit reaction coordinates that involve oxocarbenium ion-like transition states.

Among these GHs, six families of \(\alpha\)-mannosidases (GH 38, 47, 76, 92, 99, and 125) are found that cleave off \(\alpha\)-linked mannose from various conjugates. The \(\alpha\)-mannosidases from GH families 47, 92, and 125 use a one-step catalytic mechanism that involves water attacking the anomeric center to yield a hemiacetal product with inverted stereochemistry (Figure 1, Path A). \(\alpha\)-Mannosidases from GH families 38 and 76 use a two-step catalytic mechanism, commonly used by many other glycosidase families that cleave glycosides to produce a hemiacetal, that retains the stereochemistry at the anomeric center. The catalytic mechanism of these two families of \(\alpha\)-mannosidases involves transient formation of a covalent glycosyl enzyme intermediate in which the mannose unit is attached to an enzymic nucleophile (Figure 1, Path B).

Of course, it is hard for the corresponding reaction in aqueous solution to benefit from a catalytic nucleophile. Instead, a process similar to the mechanism used by the one-step \(\alpha\)-mannosidases operates in acidic conditions (Figure 1, Path A), whereas in basic aqueous conditions a distinctive mechanism functions to exploit the trans-diaxial relationship of the 2-hydroxyl group of the mannose unit and the oxygen of the leaving group. In this two-step base-promoted reaction, the deprotonated 2-alkoxide acts as an intramolecular nucleophile that attacks the anomeric center and kicks out the leaving group oxygen (Figure 1, Path C). The resulting epoxide is then opened by the attack of water at the anomeric center—a near microscopic reverse of the first step.

An often-repeated mantra in the field of mechanistic enzymology is that one cannot truly “prove” a mechanism but can really only “disprove” competing mechanisms. Nevertheless, as experimental observations become more precise over time, the ability to provide compelling support for a catalytic mechanism becomes increasingly feasible.
paper, an impressive suite of studies shows, in a nearly unambiguous manner, that GH99 mannosidases use this substrate-assisted catalytic mechanism wherein an enzymic general base promotes participation of the 2-hydroxyl group (Figure 1, Path C).

Building from previous studies that speculatively proposed such a mechanism based on limited X-ray structural data,7,8 the authors perform a series of kinetic isotope effect (KIE) experiments using an elegant competitive nuclear magnetic resonance-based approach.9 The high similarity of these KIE values to those of the base-promoted hydrolysis of related synthetic substrates,6 and especially a nonunity 18O-2 KIE, allows the authors to propose the involvement of the 2-hydroxyl group in the transition state (Figure 2A). These data are bolstered by a series of X-ray structural studies of these enzymes in complex with substrates, products, and epoxide intermediate analogues that together reveal the complete reaction coordinate for this GH99 family of α-mannosidases.

Figure 1. Catalytic mechanisms used by α-mannosidases. (Path A) The one-step inverting mechanism is akin to the spontaneous acid-promoted hydrolysis process that occurs in acidic solutions. (Path B) Two-step retaining mechanism involving an enzymic nucleophile and formation of a covalent intermediate. (Path C) The catalytic mechanism shown to operate for GH 99 α-mannosidases is akin to the spontaneous base-promoted hydrolysis mechanism that occurs in basic solutions. The 2-hydroxyl oxygen is shown in red and the mannose residue in blue.

Figure 2. Some of the key experimental evidence supporting Path C of Figure 1 being operative for GH 99 α-mannosidases. (A) The proposed transition state for the enzyme catalyzed process reveals a large distinctive 18O-2 KIE supporting substrate-assisted catalysis from the 2-hydroxyl group. (B) A synthetic cyclohexane epoxide mimic of a mannoside substrate in complex with a GH 99 α-mannosidase is seen to engage the catalytic machinery within the active site and, in the presence of WT enzyme, is turned over to form the expected trans-1,2-dihydroxy product. The 2-hydroxyl oxygen is shown in red and the mannose residue in blue. Adapted from ref 1 with permission. Copyright 2020, American Chemical Society.
High-level modeling brings together these data and enables proposing a detailed reaction coordinate in which participation of the 2-hydroxyl group figures prominently in the two transition states that flank an epoxide intermediate (Figure 1, Path C).

One notable line of experimental support stems from the concise synthesis and use of a cyclohexane epoxide substrate analogue to observe an epoxide bound within the active site of a GH99 α-mannosidase (Figure 2B). The team shows that this species is hydrolyzed by the wild-type enzyme to give the trans-dihydroxy product. With a turnover rate of just 5 min	extsuperscript{-1} under the conditions used, this substrate is clearly much less reactive owing to the absence of the endocyclic ring oxygen. Nevertheless, these data show that such epoxides are catalytically competent intermediates, fulfilling a key expectation for catalytic mechanisms involving intermediates. This also hints at the potential to engineer GH99 α-mannosidases to use synthetic intermediates such as epoxides or thiiranes in combination with synthetic acceptors including thiols or amines to create entirely new glycoconjugates.

At a more fundamental level, this paper highlights the ability of nature to exploit and improve upon favorable uncatalyzed reaction pathways. Indeed, based on crystallographic observations alone, a similar mechanism was hypothesized for the α-L-rhamnosidases from GH145.10 Accordingly, it is reasonable to expect that this epoxide mechanism will likely be found in new families of GHs as they continue to be discovered. Thinking beyond the glycoside hydrolases, when this work is considered in the context of molecular evolutionary pathways, one is inspired to look to the large body of synthetic and physical organic chemistry on spontaneous reaction pathways. Perhaps in this literature one may find examples of reaction coordinates for uncatalyzed reactions that are likely to be exploited by enzymes that have not had their catalytic mechanisms defined.

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