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Unusual Enzymatic Glycoside Cleavage Mechanisms

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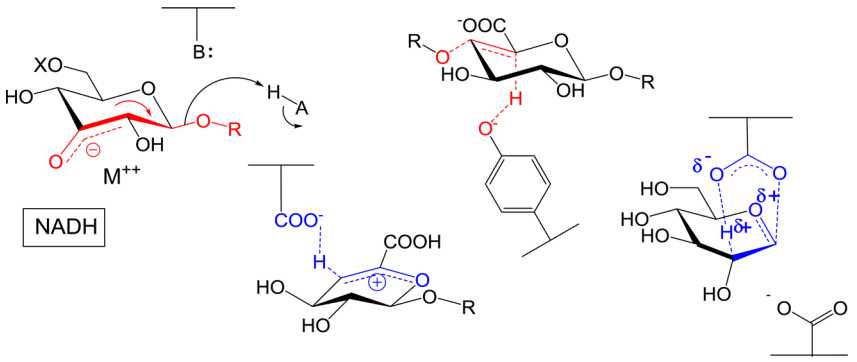
CONSPECTUS



Over the sixty years since Koshland initially formulated the classical mechanisms for retaining and inverting glycosidases, researchers have assembled a large body of supporting evidence and have documented variations of these mechanisms. Recently, however, researchers have uncovered a number of completely distinct mechanisms for enzymatic cleavage of glycosides involving elimination and/or hydration steps.

In family GH4 and GH109 glycosidases, the reaction proceeds via transient NAD^+-mediated oxidation at C3, thereby acidifying the proton at C2 and allowing for elimination across the C1−C2 bond. Subsequent Michael-type addition of water followed by reduction at C3 generates the hydrolyzed product. Enzymes employing this mechanism can hydrolyze thioglycosides as well as both anomers of activated substrates.

Sialidases employ a conventional retaining mechanism in which a tyrosine functions as the nucleophile, but in some cases researchers have observed off-path elimination end products. These reactions occur via the normal covalent intermediate, but instead of an attack by water on the anomeric center, the catalytic acid/base residue abstracts an adjacent proton. These enzymes can also catalyze hydration of the enol ether via the reverse pathway.

Reactions of α-(1,4)-glucan lyases also proceed through a covalent intermediate with subsequent abstraction of an adjacent proton to give elimination. However, in this case, the departing carboxylate "nucleophile" serves as the base in a concerted but asynchronous syn-elimination process. These enzymes perform only elimination reactions.

Polysaccharide lyases, which act on uronic acid-containing substrates, also catalyze only elimination reactions. Substrate binding neutralizes the charge on the carboxylate, which allows for abstraction of the proton on C5 and leads to an elimination reaction via an E1cb mechanism. These enzymes can also cleave thioglycosides, albeit slowly.

The unsaturated product of polysaccharide lyases can then serve as a substrate for a hydration reaction carried out by unsaturated glucuronyl hydrolases. This hydration is initiated by protonation at C4 and proceeds in a Markovnikov fashion rather than undergoing a Michael-type addition, giving a hemiketal at C5. This hemiketal then undergoes a rearrangement that results in cleavage of the anomeric bond. These enzymes can also hydrolyze thioglycosides efficiently and slowly turn over substrates with inverted anomeric configuration.

The mechanisms discussed in this Account proceed through transition states that involve either positive or negative charges, unlike the exclusively cationic transition states of the classical Koshland retaining and inverting glycosidases. In addition, the distribution of this charge throughout the substrate can vary substantially. The nature of these mechanisms and their transition states means that any inhibitors or inactivators of these unusual enzymes probably differ from those presently used for Koshland retaining or inverting glycosidases.

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Introduction

The enzymes responsible for hydrolysis of glycosides are termed glycoside hydrolases, or glycosidases. Many of these are very efficient catalysts, with rate enhancements on the order of 10^{17}-fold over the nonenzymatic case.[1] How these enzymes achieve this catalytic feat has been the topic of extensive work over many decades, with the latest insights capably covered in several recent reviews.[2−7] However, other studies have shed light on several newly uncovered mechanisms that are used by enzymes to cleave glycosides, and those making use of elimination or addition reactions are the topic of this Account.

Glycoside hydrolases, along with the related glycosyl transferases, polysaccharide lyases, carbohydrate esterases, and carbohydrate-binding modules, are classified into families on the basis of sequence homology in the Carbohydrate Active Enzymes (CaZY) Database (available online at http://www.cazy.org/).[8] For many of these families, the key mechanistic details have been elucidated, and representative crystal structures of members have been solved. This database thus provides a framework through which mechanistic similarities between these enzymes can be understood and reflects structural features and evolutionary relationships. Key mechanistic and structural features are summarized in a constantly updated Wiki site called CAZypedia (http://www.cazypedia.org).

Most glycoside hydrolases effect hydrolysis with either net retention or inversion of the stereochemistry at the anomeric center. The corresponding enzymes are called retaining and inverting glycosidases, and canonical mechanisms explaining these outcomes were outlined in a seminal paper by Koshland.[9] Other enzymes have recently been reported to cleave glycosides by very different mechanisms involving elimination, hydration, or both. The very different transition states involved mean that attempts to inhibit or repurpose these enzymes will likely require disparate strategies.

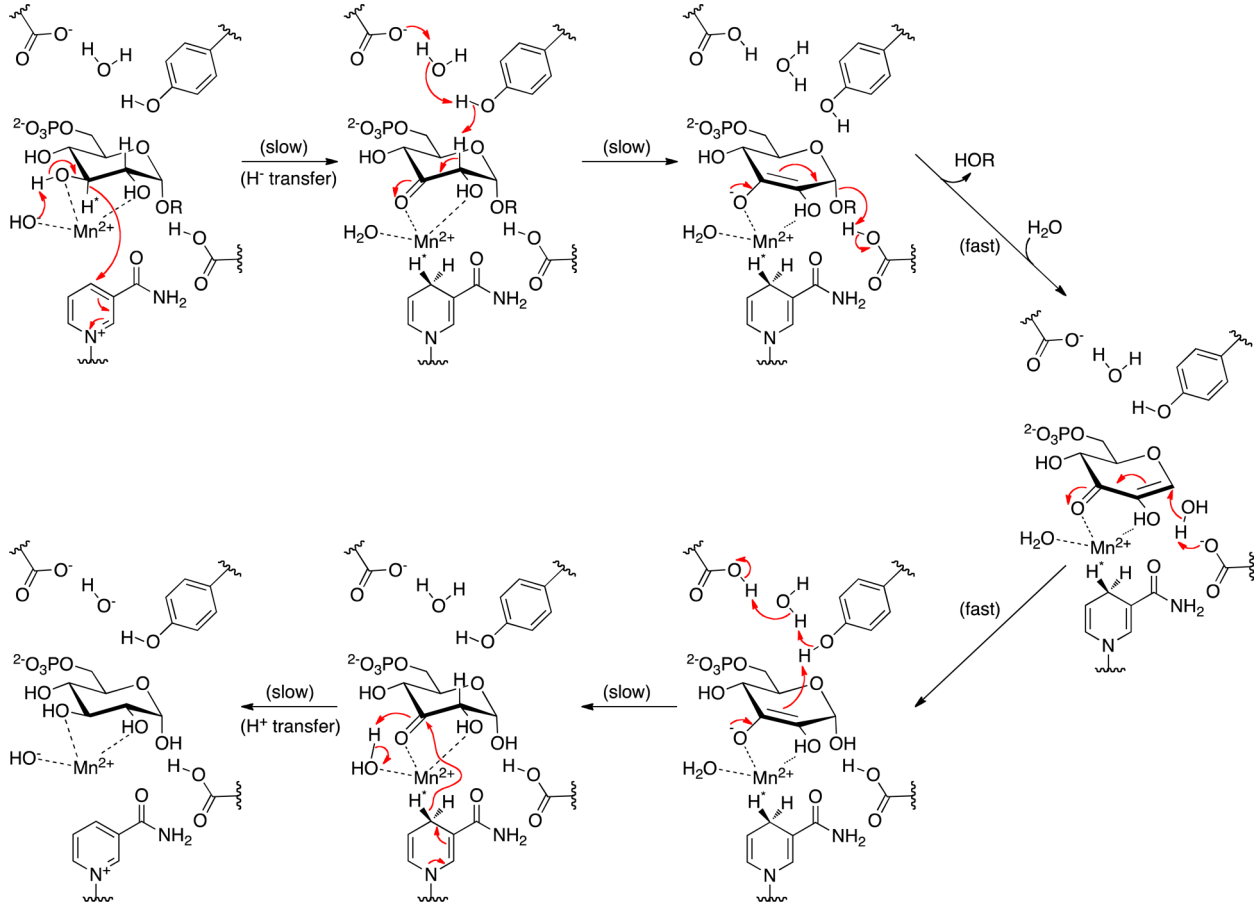
Family GH4 and GH109 Glycoside Hydrolases

Family GH4 glycoside hydrolases employ a fascinating mechanism involving both elimination and hydration reactions. In the first step, the C3 hydroxyl group is transiently oxidized, acidifying the adjacent hydrogen on C2 and allowing an elimination across the C2−C1 bond to generate a tightly bound α,β-unsaturated ketone intermediate. In a second half-reaction, water attacks the α/β-conjugated system, and this is followed by reduction of the C3 ketone, resulting in net hydrolysis (Scheme 1). Reaction proceeds through a series of anionic intermediates and transition states, in stark contrast to the cationic transition states of the Koshland mechanisms. Two cofactors are involved, a tightly bound NAD^+ to effect the transient oxidation and a divalent metal, usually manganese, to stabilize the negative charges that develop during reaction. Evidence for this mechanism, including crystal structures, kinetic isotope effects (KIEs), and linear free energy relationships, was reviewed by Yip and Withers;[10] this Account focuses on work since that publication.

The passive role of the anomeric center in this mechanism allows this family to contain both α- and β-glycosidases, with both classes using the same general mechanism but slightly different active-site residues. Intriguingly, the 6-phospho-α-glucosidase GlvA from *Bacillus subtilis* has been demonstrated to cleave both α- and β-glucosides within the same active site of the same enzyme with similar kinetic parameters,[11] provided that the substrate contains a sufficiently activated aglycone. This anomerically blind reactivity is possible because the ability to bind substrate adjacent to the NAD^+ is the primary determinant of hydrolysis, and aglycone activation is less important. For less activated substrates, where aglycone protonation becomes more important, the enzyme shows more specificity for its natural α-configured substrates, consistent with the elimination chemistry involved. GH4 enzymes have also been shown to cleave thio-linked substrates. For example, unactivated 4-deoxy-4-thio-D-cellobiose-6′-phosphate is cleaved by the β-glucosidase BglT from *Thermotoga maritima* with kinetic parameters similar to those for the analogous *O*-glycoside.[12] The incorporation of deuterium at C2 of the glucose-6-phosphate product during the course of enzymatic hydrolysis and the determination that K_i is equal to K_m provided evidence that this hydrolysis proceeds through the same active site and with the same mechanism as for hydrolysis of *O*-glycosides.

Much recent work has focused on clarifying the rate-determining step(s) of family GH4 through the determination of further KIEs and linear free energy relationships as well as density functional theory (DFT) calculations.[13] KIEs measured on GH4 α- and β-glycosidases[11,14−17] generally agree well and reveal that oxidation at C3 and deprotonation at C2 are both partially rate-limiting. Indeed, the glycosidic bond cleavage step is shown to be kinetically unimportant for activated aryl glycosides by the lack of rate dependence upon leaving group ability and the lack of a substantial KIE from deuteration at C1. In MelA, an α-galactosidase from *Citrobacter freundii*, KIEs on k_{cat}/K_m were measured for substrates with deuterium substituted at C2 and C3 individually as well as for the

SCHEME 1. General Mechanism of Family GH4 6-Phospho-α-glucosidases[a]

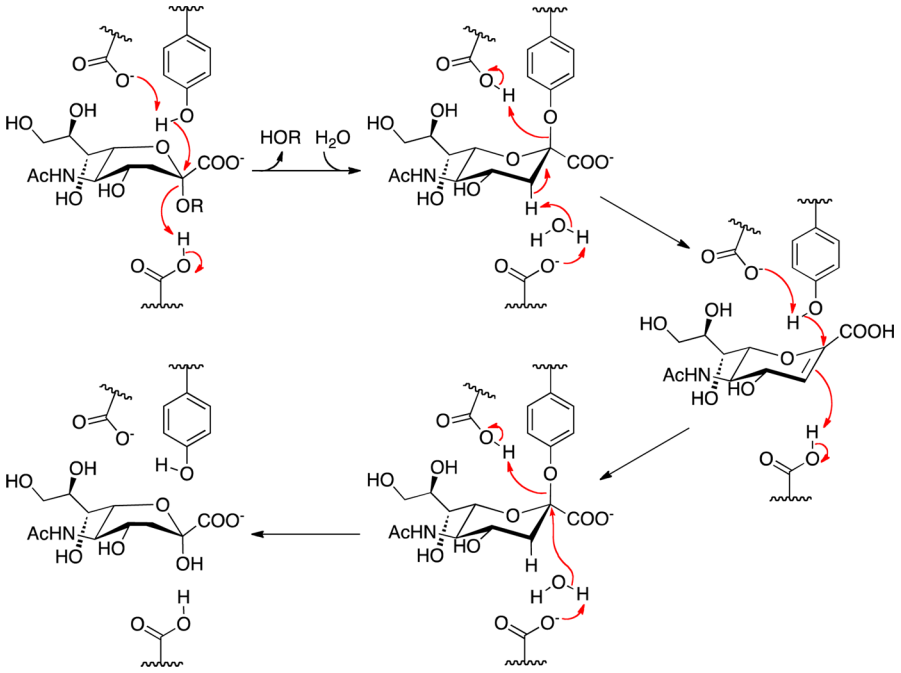


[a]Partially rate-limiting steps are indicated as "slow," and the hydride transferred in the redox steps is indicated by H*.

disubstituted species. Comparison of the rates for the dideuterated and monodeuterated isotopologues showed the same KIE magnitude as did a comparison of the rates for the monodeuterated and nondeuterated isotopologues, suggesting that these isotopes influence the same transition state. This result agrees with concerted oxidation at C3 and deprotonation at C2 with no formation of a discrete ketone intermediate.[16] Interestingly, the KIEs on k_{cat} for this same enzyme suggested a shift in the overall rate-determining step to a step after cleavage of the glycosidic bond. On the basis of the calculated reaction coordinate (which showed an overall flat profile with no single step strongly rate-limiting),[13] this step may be reprotonation at C2. The change in hybridization from sp^2 to sp^3 upon reprotonation is consistent with the observed small inverse KIE at this center.[12]

The catalytic base responsible for deprotonation of C2 in GH4 hydrolases acting on phosphorylated substrates, as predicted on the basis of X-ray crystal structures, has been experimentally confirmed as tyrosine 241 (BglT numbering) by careful measurement of KIEs on k_{cat} for mutants modified at this position.[17] In a valiant set of KIE measurements on deuterated substrates with several mutants at varied pH, it was found that mutation of Tyr241 to Ala ablated the KIE seen from substrates deuterated at C3, while the primary KIE from deuterium at C2 was strengthened. This demonstrated that proton abstraction from C2 becomes completely rate-limiting as a result of the retardation of this step by the removal of its catalytic base. At higher pH, the KIE from deuterium at C2 decreased while deuterium substitution at C3 remained silent, suggesting that in the presence of adequate exogenous base a later step in the reaction becomes partially rate-limiting. Again, DFT calculations[13] suggested that this step may be the reprotonation at C2 in the final step. An alternative explanation is that the Michael-type addition of water becomes rate-limiting, which would be consistent with the small inverse KIE that was observed for the Y241A mutant of BglT at its optimal pH. Unfortunately, limited supplies of labeled compound prevented the determination of C1 deuterium effects at other pH values. Mutation of this residue to Phe, however, did not give such clear results. This was suggested to result because the role of

SCHEME 2. General Mechanism of Elimination and Hydration by Sialidases



the phenolate oxygen of Tyr was played by a water molecule binding adjacent to the phenyl group of Phe.

The GH109 family of glycoside hydrolases, which appear to follow a mechanism very similar to that of the GH4 family, was founded on the basis of an α-*N*-acetylgalactosaminidase discovered in *Elizabethkingia meningosepticum*[18] and remains poorly characterized. This enzyme showed a dependence on NAD^+ for its catalytic activity, similar to that of GH4 enzymes, but did not require a metal cofactor. Since in GH4 enzymes this metal coordinates to hydroxyls on C2 and C3, one of which is substituted by an *N*-acetyl group in α-*N*-acetylgalactosamine, it is likely that another means of activating the hydroxyl proton on C3 is employed in GH109. In contrast to the 6-phospho-α-glucosidase GlvA, this enzyme only very slowly hydrolyzes activated substrates of nonoptimal anomeric configuration or lacking an *N*-acetyl moiety at C2. Furthermore, the crystal structure revealed no clear candidate for the acid/base residue that activates the aglycone for departure and water as a nucleophile.

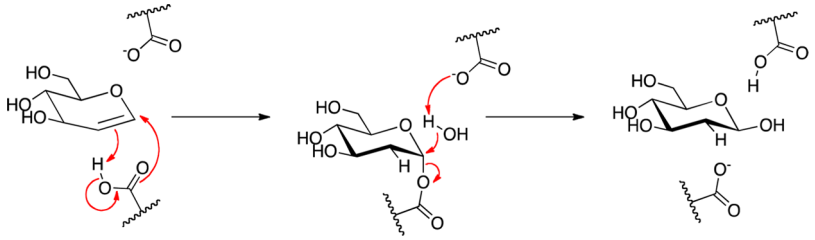
Elimination and Hydration in Sialidases

Several sialidases from bacterial and viral sources have been observed to catalyze elimination[19−22] and/or hydration[20−23] reactions of sialosides, thereby forming or degrading the sialidase competitive inhibitor 2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid (Neu5Ac2en, also known as DANA). Elimination proceeds through the same glycosyl−enzyme intermediate as that formed in the hydrolysis reactions (Scheme 2). However, proton abstraction from C3 to trigger elimination competes with water attack at C2 to varying degrees. A similar mechanism is also responsible for hydration of Neu5Ac2en, with formation of the same glycosyl−enzyme intermediate preceding hydrolysis to give overall hydration. This reaction is similar to the established mechanism for hydration of other glycals by retaining glycosidases, which results in a net trans addition of water (Scheme 3).[24−27] Notably, however, in sialidases the tyrosine is unable to undergo a concerted syn addition across the double bond, as seems to be the case for aspartate/glutamate in other glycosidases. In most cases, these elimination and hydration reactions represent minor pathways for hydrolases and are correspondingly slow, but in one case it is the primary reaction catalyzed and reasonably efficient (elimination $k_{cat} = 19\ min^{-1}$ in NanC from *Streptococcus pneumoniae*).[22] Evidence for this mechanism came from the reaction in D_2O, which showed an overall syn addition of water; catalytic competence of synthetic Neu5Ac2en for hydration; and a lack of reaction with thiosialosides, arguing against direct elimination without a covalent intermediate.[22] In view of the fact that Neu5Ac2en is a reasonably potent inhibitor of sialidases, its formation and decomposition has been proposed to be a regulatory system for sialidase activity.[22,28]

α-1,4-Glucan Lyase

Some GH31 enzymes that are predominantly involved in the metabolism of polysaccharides such as glycogen and

SCHEME 3. Mechanism for Hydration of Glucal by a Retaining β-Glucosidase



starch, the α-1,4-glucan lyases, carry out an elimination reaction, but with no hydration.[29] The first step in their mechanism is identical to that of a retaining α-glycosidase. However, the glycosyl−enzyme intermediate thus formed then undergoes syn elimination of an adjacent proton and the nucleophile to form anhydrofructose. Upon release from the enzyme active site, this tautomerizes to its keto form and then is hydrated to give a C2 geminal diol (Scheme 4).[30] The product of these lyases can be used directly in energy metabolism[29] or alternatively can be hydrated by α-glucosidases from GH13 or GH31 to yield glucose in what is proposed to be an anhydrofructose scavenging system.[31]

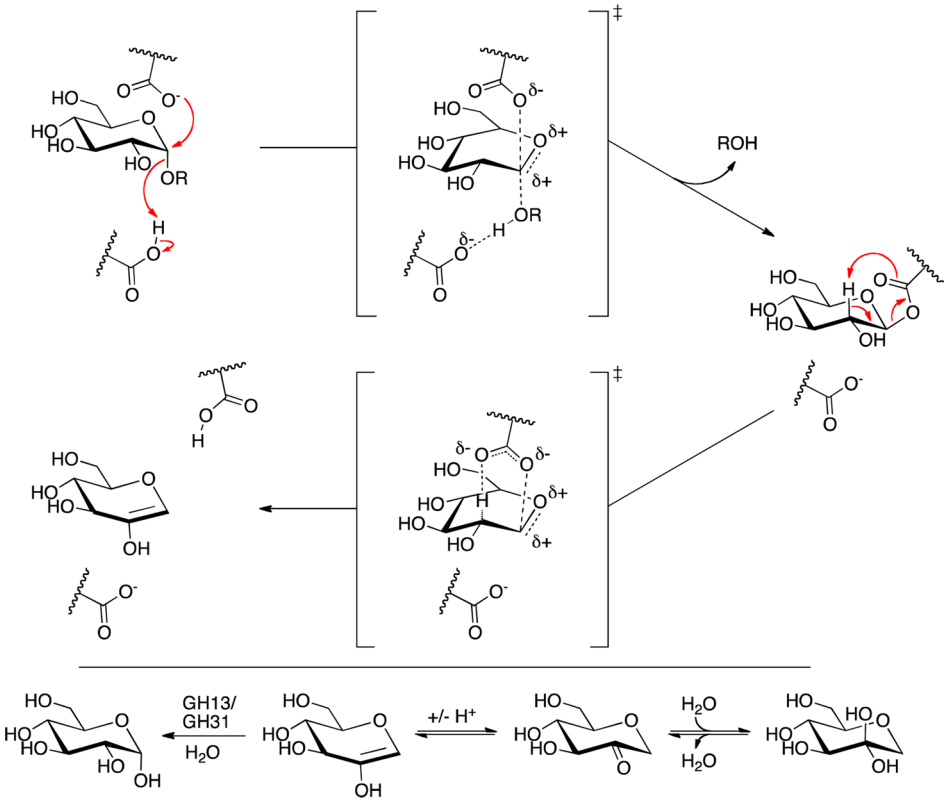
This α-glucan lyase mechanism uses the same basic catalytic machinery, and indeed the same first step, as the α-glucosidases of the same family. The formation of the covalent glycosyl−enzyme intermediate was observed by trapping with 5-fluoro-β-L-idosyl fluoride and subsequent detection and sequencing of the labeled peptide by mass spectrometry.[32] Interestingly, trapping could not be observed with 1-, 2,- or 5-fluoro-α-D-glucosyl fluorides, which instead acted as slow substrates.[33] The formation of this covalent glycosyl−enzyme intermediate was seen to be rate-limiting, with a robust α-secondary KIE from 1-{^{2}H} substrates showing a dissociative transition state and shallow negative slopes in the linear free energy plots for both k_{cat} and k_{cat}/K_m showing substantial proton donation to the leaving group.[33] Consistent with this, substitution with deuterium at C2 revealed a small β-secondary effect, whose small size suggested a conformation in which the H−C2 bond is not fully aligned with the empty p orbital of C1. For the particularly slow substrate 5-fluoro-α-D-glucosyl fluoride, the deglycosylation/elimination step was found to be rate-limiting and a small primary KIE from a 2-{^{2}H} substrate was seen, while a 1-{^{2}H} substitution showed a fairly large α-secondary KIE.[33] Together these results suggest that the proton abstraction step occurs in a concerted but asynchronous manner. In other words, proton abstraction occurs only after substantial bond cleavage at the anomeric carbon to generate an oxocarbenium ion-like transition state, the charge of which increases the acidity of the proton on C2.

The catalytic base for this proton abstraction has been suggested to be the catalytic nucleophile itself. The asynchronous nature of this elimination makes this plausible, as at the transition state the glycosyl−enzyme bond has largely broken to give a carboxylate in close proximity to a proton, which is acidified by an adjacent carbocation. Indeed, the placement of the carbonyl oxygen in GH31 α-glucosidases has been shown by X-ray crystallography to be over the proton on C2 of glucose.[34] The very recently published structure of an alpha glucan lyase trapped as a 5-fluoroglycosyl enzyme shows that indeed the carbonyl oxygen of the nucleophile is positioned over the C2 proton.[35] Such a syn elimination is the reverse of the syn addition of the catalytic nucleophile in glucal hydration. A similar elimination has been observed in the formation and subsequent hydration of a minor product, D-ribal, by the 2-deoxyribosyltransferase from *Lactobacillus leishmanii*.[36] Likewise, 1,5-anhydro-D-arabinohex-1-enitol, an isomer of anhydrofructose, has been observed in the active site of an X-ray crystal structure of glycogen synthase from *Escherichia coli*.[37] These are both formed by glycoside-synthesizing enzymes in the absence of an appropriate acceptor. In the first case, the same enzyme is responsible for both the elimination and hydration reactions, emphasizing the implied relationship between the mechanisms of α-(1,4)-glucan lyases and glucal hydration by glycoside hydrolases and the elimination and hydration seen in some sialidases.

Polysaccharide Lyases

Polysaccharide lyases are a class of enzymes that catalyze an elimination reaction that is superficially similar to that of the α-(1,4)-glucan lyases. However, the mechanism by which this is achieved is very different and depends upon the presence of uronic acids within the polymer substrate. These enzymes are classified separately in the CaZY database, and substantially fewer of these families are known in comparison with the glycoside hydrolases (22 compared with 131, respectively, as of March 2013). The general mechanism of this class of enzymes involves three key components.[38] The

SCHEME 4. (top) General Mechanism of α-(1,4)-Glucan Lyases; (bottom) Rearrangements and Further Reaction of the Product in Water

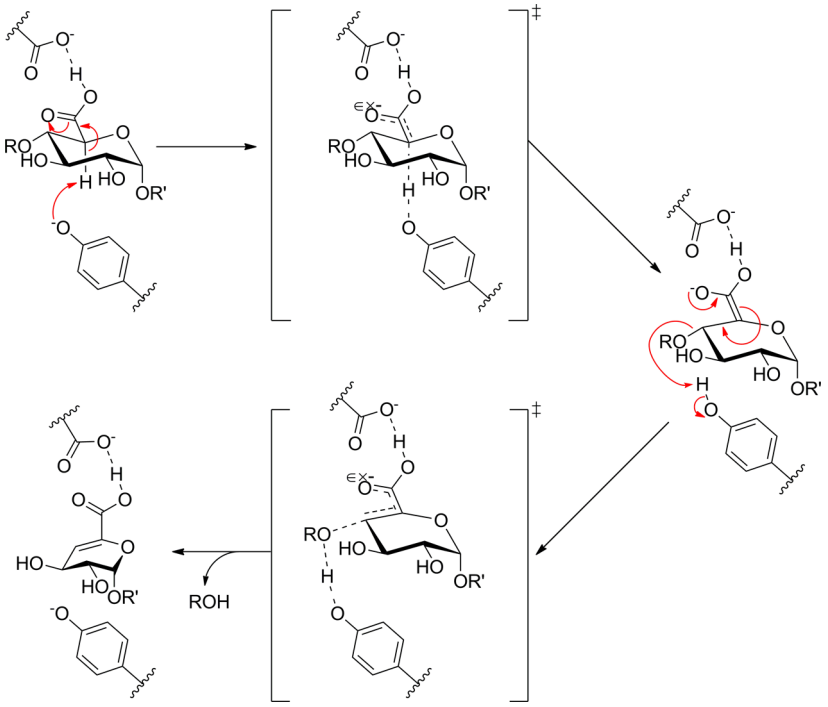


first is neutralization of the charge on the carboxylate at C6 upon binding of the substrate to the enzyme active site, followed by abstraction of the proton at C5 to form a carbanion intermediate with resonance stabilization by the adjacent carboxylic acid. Finally, the free electron pair forms a double bond between C4 and C5, expelling the leaving group at C4 in the process and giving an unsaturated glucuronide (ΔGlcA) as the product. This overall E1cb reaction mechanism is outlined in Scheme 5. C5-epimerases acting on the same substrates follow a very similar mechanism; the two mechanisms differ only in the nature of the final step, which involves protonation on the C4 oxygen to give elimination or on C5 from the opposite face to give epimerization, respectively.[38] For some polyanionic saccharides, degradation by lyases is the only known path for catabolism, while others are also cleaved by glycosidases.[39] In general, bacteria employ lyases to degrade uronic acid-containing glycosaminoglycans, while eukaryotes rely upon standard glycoside hydrolases.

On the basis of a set of experiments using chemically defined short substrates to probe chondroitin AC lyase from *Flavobacterium heparinum*, the rate-determining step for this mechanism was found to be abstraction of the proton from C5 of the substrate,[40] which is carried out by a tyrosine residue.[41] When this proton was substituted with deuterium, a small primary KIE was observed, while variation of the leaving group at C4 (R in Scheme 5) was found to have no effect on the rate of reaction for a set of activated aryl substrates. Consistent with this, deuterium substitution at C4 resulted in no significant KIE ($k_H/k_D = 1.0$). Deuterium exchange at C5 was not observed in a partially cleaved sample, suggesting that deprotonation is irreversible. However, this may also indicate solvent inaccessibility of the active site during catalysis, in which case elimination would be faster than reprotonation or solvent exchange. A corollary of this E1cb mechanism is that unactivated thio-linked substrates are cleaved by these enzymes, but only relatively poorly.[42]

The various structural features of polysaccharide lyases have been thoroughly reviewed by Garron and Cygler.[43] These enzymes can generally be classified into two groups depending on the residues responsible for the catalytic functions of charge neutralization, base catalysis, and acid catalysis. One group, containing almost exclusively pectate and pectin lyases, employs a divalent metal for charge neutralization, an arginine or a lysine as a catalytic base, and water as a catalytic acid. An example is the pectin lyase from *Cellvibrio japonicus*, which contains a single calcium bridging the carboxylates of the +1 and −1 subsites, an

SCHEME 5. General Mechanism of Polysaccharide Lyases Acting on Pectate



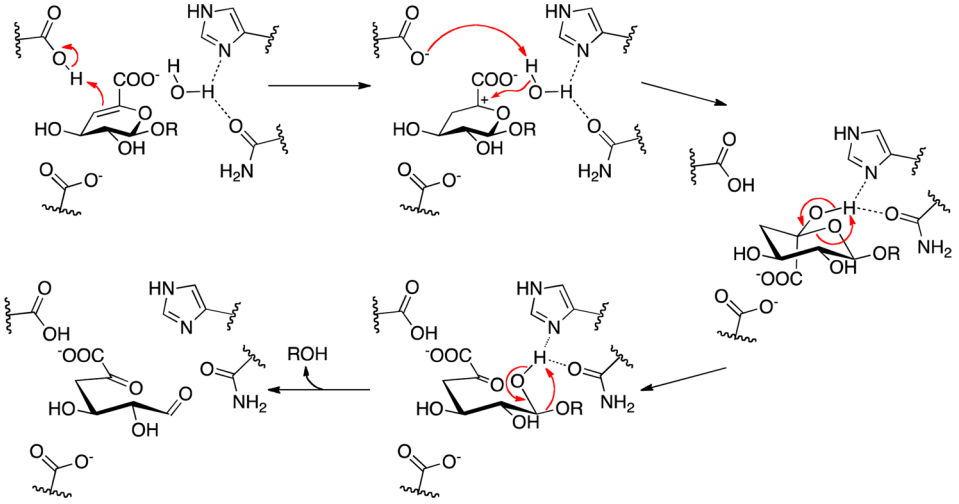
arginine catalytic base, and no clear acid residue, although a water ligand of the calcium ion or an aspartate acting via the C3 hydroxyl have been proposed as candidates for this role.[44] Because of the high charge density of pectate, a polygalacturonide, these enzymes may contain as many as four divalent metals.[45] The other group of polysaccharide lyases is more diverse but generally employs an amide or acid side chain to neutralize the substrate carboxylate, a histidine or tyrosine as the catalytic base, and a tyrosine as the catalytic acid. In syn-eliminating enzymes, a single tyrosine can act as both the acid and the base.[41,46] An interesting case within this class is exemplified by the chondroitin ABC lyases from *Bacteroides thetaiotaomicron* and *Proteus vulgaris*, which are able to catalyze both syn and anti elimination in the same active site.[47] It appears that binding of each substrate type (glucuronides or iduronides) recruits the appropriate catalytic machinery for its degradation through conformational shifts, with a single substrate binding domain forming a part of two partially overlapping active sites. For syn elimination, a single tyrosine acts as both the base and the acid, as has been seen in the related chondroitin AC lyases.[41] For the anti elimination, the same tyrosine still acts as a catalytic acid, while mutagenesis suggested a catalytic role for a pair of histidine residues that are located 12 Å away in the enzyme's resting state, possibly as a base.

Unsaturated Glucuronyl and Galacturonyl Hydrolases

Unsaturated glucuronyl and galacturonyl hydrolases (UGLs and UGHs, respectively) degrade the products released by polysaccharide lyases through a hydration reaction. These enzymes were identified in *Bacillus* sp. GL1 in a pathway for total degradation of xanthan[48] and have since been cloned and expressed from a variety of bacteria.[49−52] These were initially assumed to be a specialized group of hydrolases operating by a Koshland mechanism,[53] but structural and biochemical evidence has suggested otherwise.[54,55] The proposed mechanism comprises an initial hydration of the double bond between C4 and C5 followed by rearrangement of the hemiketal product, possibly through an intermediate hemiacetal, to cleave the glycosidic bond and afford a free unsaturated uronic acid (an α-keto acid) (Scheme 6). This free unsaturated glucuronic acid is further catabolized in a pathway that yields two common metabolites, pyruvate and D-glyceraldehyde-3-phosphate.[56] The expression of an unsaturated glucuronyl hydrolase and polysaccharide lyase pair, along with a phosphoenolpyruvate-dependent phosphotransferase system for import of unsaturated glucuronic acid disaccharides, is necessary for growth of *S. pneumoniae* on hyaluronic acid as a sole carbon source.[57]

Unequivocal evidence for the hydration reaction was provided by a series of experiments wherein the UGL

SCHEME 6. General Mechanism of Unsaturated Glucuronyl Hydrolases



reaction was carried out in D_2O and in 10% methanol, resulting in products in which the locations of proton addition and nucleophilic attack could be clearly identified.[55] UGL from *Clostridium perfringens* was shown to cleave an activated thioglycoside with efficiency similar to that of its oxygen analogue as well as a substrate with inverted anomeric configuration and also to hydrate a *C*-glycoside substrate analogue.

Two related GH families that operate by this same general mechanism have been identified: GH88 containing UGLs [50] and GH105 containing UGHs [also known as unsaturated rhamnogalacturonyl hydrolases (URHs)].[51] These names refer to the source polysaccharides of the substrates, with UGL substrates being derived from polymers containing β-glucuronide monomers in the repeating unit, usually glycosaminoglycans, and UGH substrates being derived from polymers containing α-galacturonide monomers in the repeating unit, usually pectins.[58] Because of this specificity, enzymes from mammalian pathogens and symbiotes predominate in GH88 and enzymes from plant pathogens and symbiotes predominate in GH105, but many cases of both are found in soil bacteria for decomposition of dead organic matter.[58]

A large proportion of the work carried out on UGLs and UGHs has focused on X-ray crystallography, with an excellent series of structures being determined by Murata and coworkers at Kyoto University. Structures have been determined for a UGL from a nonpathogenic *Bacillus* strain with a bound inhibitor (glycine),[59,60] with bound substrates,[54,61] and in apo form[61] and also for a UGL from a pathogenic *Streptococcus* species both in apo form[52] and with bound substrate.[62] Similarly, UGH structures have been determined in apo form, with bound inhibitor (UGL substrate in the +1 subsite),[51] and with bound substrate.[63] The active sites of these two families are very similar, with catalytic residues overlaying well. No catalytically important residues are positioned close to the anomeric oxygen, as is the case in Koshland glycoside hydrolases. Strong interactions are seen with the ΔGlcA in the -1 subsite, while fewer interactions take place in the $+1$ subsite. This gives UGLs and UGHs fairly wide substrate specificities, the strongest requirement being for a ΔGlcA moiety.

On the basis of the published structures and the placement of residues within them, one aspartate (D149 in *Bacillus* sp. GL1 numbering) has been proposed to act as the proton donor in the initial hydration step and also as the base in the subsequent addition of water.[52,54,62,63] Another catalytically important aspartate, D88, was proposed to form hydrogen bonds with the hydroxyl groups on C2 and C3, preventing their interference with the role of D149 by hydrogen bonding, and to stabilize the oxocarbenium ion-like transition state for the hydration reaction. D88 was also proposed to modulate the acidity of D149, ensuring that it is protonated through the hydrogen bond between these two residues seen in the apo form.[61] However, this role seems inadequate to explain the >30000-fold reduction of k_{cat}/K_m seen when D88 is mutated to asparagine, especially since mutation of D149 to asparagine results in only a 1000-fold reduction. If the role of D88 is to aid D149, then mutation of D88 to an amide should not have a larger effect on turnover than the same mutation in D149, especially since an amide at this position should still be able to form hydrogen bonds. Furthermore, D88 is located on the plane of the pyranose ring adjacent to C2 and C3 and not C5, at a distance of around 5.5 Å from the site of charge development, making

any direct transition-state stabilization of this charge implausible. The role of this residue thus remains unclear.

Conclusions

In addition to the vast number of glycosidases that operate by the classical Koshland retaining and inverting mechanisms, a growing body of enzymes that cleave glycosides by mechanisms involving either elimination or hydration reactions or both is now coming to light. As a result of these unusual mechanisms, these enzymes are often able to cleave substrates such as thioglycosides that are recalcitrant to other glycosidases. Their transition states can involve the development of either negative or positive charge, in contrast to the exclusively cationic transition states of the classical Koshland retaining and inverting glycosidases, and the distribution of this charge throughout the substrate can vary substantially. The nature of these mechanisms and their transition states means that any inhibitors or inactivators of these unusual enzymes likely must be very different from those presently used for Koshland retaining or inverting glycosidases. As a corollary of this, however, any inhibition or inactivation of these enzymes that takes advantage of their unique mechanisms should allow for a high degree of specificity and, in view of the fact that many of these enzymes are sourced from pathogenic microorganisms, may allow for effective treatments of their related diseases with minimized side effects.

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BIOGRAPHICAL INFORMATION

Seino A. K. Jongkees was born in New Plymouth, New Zealand, in 1983. He received a B.Sc. (Hons.) and a B.A. from the University of Otago, New Zealand, in 2007 and is currently working towards a Ph.D. in chemistry at the University of British Columbia under the supervision of Prof. Stephen Withers. His primary research interest is in the study of enzyme mechanisms.

Stephen G. Withers was born in Somerset, England, in 1953 and obtained his undergraduate and Ph.D. degrees at the University of Bristol under Dr. Michael Sinnott. After postdoctoral research at the University of Alberta with Neil Madsen and Brian Sykes, he moved to the University of British Columbia in 1982, where he holds the Khorana Chair of Biological Chemistry and a Tier One Canada Research Chair in Chemical Biology.

FOOTNOTES

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The authors declare no competing financial interest.

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