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Nature's many mechanisms for the degradation of oligosaccharides

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Recent work on the mechanistic elucidation of the polysaccharide lyases, the α-1,4-glucan lyases, and the Family 4 glycosidases have demonstrated that nature has evolved to use elimination steps for the degradation of oligosaccharides. The polysaccharide lyases (E.C. 4.2.2.-) have been shown to cleave uronic acid-containing polysaccharides via a stepwise E1cB mechanism. The mechanism of the α-1,4-glucan lyases (E.C. 4.2.2.13) is similar to the Family 31 glycosidases, forming a covalent glycosyl-enzyme intermediate, which is subsequently cleaved by an E1-like E2 mechanism. Meanwhile, the Family 4 glycosidases (E.C. 3.2.1.6) are suggested to undergo an oxidation-elimination-addition-reduction sequence. These three groups of enzymes are examples of stark contrast to the vast number of well-characterized glycosidases (E.C. 3.2.1.-), which utilize either the direct or double displacement mechanisms as proposed by Koshland over 50 years ago.

1 Introduction

Carbohydrates are amongst the most crucial molecules for life, accounting for as much as two-thirds of the carbon found in the biosphere.[1] Therefore, the biological significance of carbohydrates should not be underestimated, although it has been greatly overshadowed in the past by interest in nucleic acids and proteins. Traditionally, carbohydrates were thought of only as reservoirs for metabolic energy or as inert polymers for providing structural support. However, carbohydrates, along with glycoproteins and glycolipids, are now known to serve much more diverse and vital roles in biological processes, including roles in signal recognition systems that regulate intercellular communication and cellular activity.[2,3] Carbohydrates are amongst the most ideal media for information transfer, their ability to encode information and act as signalling devices being a direct consequence of their structural diversity. Indeed, it is estimated that a simple reducing hexasaccharide can have 10^{12} isomeric forms,[4] this large number of isomers arising from the many different potential stereoisomers, side branching as well as the possibility of different ring sizes. Although the study of glycobiology is over a century old,[5] only in recent years, with a burst of recent reviews and influential publications,[2,3,6–8] has it really enjoyed such attention. Due to the vast number of biological functions of oligosaccharides, glycoproteins, and glycolipids, there are potential applications in biochemistry, medicine and biotechnology for molecules that interfere with their processing. These include potential cures for infections and diseases, such as diabetes, cancer, HIV, and influenza.[2,8]

1.1 Carbohydrate degradation and glycosidases

The glycosidic linkage is one of the most stable bonds found in natural polymers, being 100 times more stable than the phosphodiester bond in DNA,[9] which is in turn 1,000 and 100,000 times more stable than the peptide bond of proteins and the phosphodiester bond of RNA respectively.[10–12] The estimated half-life for spontaneous hydrolysis of a single glycosidic bond of polysaccharides such as cellulose is approximately 5 million years.[13] In spite of this, nature has evolved a class of enzymes, the glycoside hydrolases (E.C. 3.2.1.-), that catalyse the hydrolysis of the glycosidic bond by factors of up to 10^{17} times.[13] With this kind of rate acceleration, glycosidases are amongst the most proficient of enzymes.[13] This class of enzymes has been the subject of extensive studies, and the interested reader is referred to a number of excellent recent reviews.[1,14–22]

Glycoside hydrolases are classified into different families based on sequence similarity, and this information can be found on the frequently updated CAZY Web site (http://afmb.cnrs-mrs.fr/~cazy/CAZY/index.html).[23,24] Glycoside hydrolases can also be grouped into two major mechanistic classes. Hydrolysis of the glycosidic linkage can proceed with two distinct stereochemical outcomes: either with retention or inversion of the anomeric configuration. Thus, there are two general and now widely accepted mechanisms (see Figs. 1a and 1b) that glycosidases utilize for catalysis, both involving oxocarbenium ion-like transition states.[1,14–16,18,20] Numerous studies on glycosidase mechanisms have provided significant support for these two mechanisms, which were first proposed by Koshland in 1953.[25] In both mechanisms, two carboxylic acid functionalities (either glutamate or aspartate residues) are generally found in the enzyme active site. For inverting glycosidases, the two carboxylic

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acids acting as general acid and base catalysts are separated by 6–12 Å, and the glycosides are hydrolysed *via* direct displacement of the aglycone moiety.[1,14–16,18,20,26,27] Meanwhile, the retaining glycosidases utilize a double-displacement mechanism involving a covalent glycosyl–enzyme intermediate in which one carboxylic acid group acts as the catalytic nucleophile, and the other carboxylic acid, 5 Å away, as the general acid/base catalyst. The general acid/base carboxyl group therefore promotes the acid-catalysed formation of the covalent glycosyl–enzyme intermediate with the nucleophile. The intermediate subsequently undergoes hydrolysis *via* base-assisted catalysis by the same amino acid residue.[1,14–16,18,20,26–28] As expected, not all glycosidases conform exactly to these two mechanisms. However, there only seem to be slight variations upon the same theme. For example, some *N*-acetyl-β-hexosaminidases belonging to Families 18 and 20 as well as hyaluronidases in Family 56 utilize the oxygen atom on the *N*-acetyl group of the substrate as an intramolecular nucleophile in favor of carboxylic acid-containing amino acid residues.[29–31] Furthermore, a tyrosine residue, as opposed to an aspartate or glutamate, has been implicated as the catalytic nucleophile in a *Trypanosoma cruzi trans*-sialidase as well as sialidases from *Trypanosoma rangeli* and *Micromonospora viridifaciens*.[32–34] Moreover, for the Family 1 myrosinase from *Sinapis alba*, the catalytic acid/base is replaced by a glutamine residue and exogenous ascorbic acid functions in this role.[35,36]

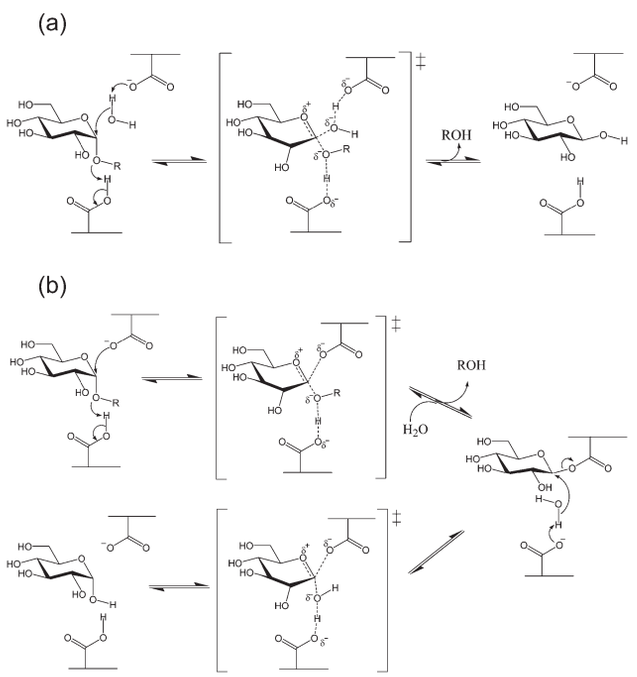


Fig. 1 (a) General mechanism of inverting glycosidases and (b) general mechanism of retaining glycosidases.[1,14–16,18,20,26,27]

2 Elimination reactions and their roles in oligosaccharide degradation

Interestingly, glycosidases are not the only enzymes that have evolved to degrade glycosides. Other classes of enzymes exist that proceed *via* mechanisms vastly different from those of the two common types of glycosidases. The following discussion will focus on three different groups of such enzymes: the polysaccharide lyases, the α-1,4-glucan lyases, and the Family 4 glycosidases. The reactions carried out by these enzymes are briefly outlined in Fig. 2. A common feature of these three groups of enzymes is that an elimination step is involved in each case. This review highlights the importance of elimination mechanisms in carbohydrate-degrading enzymes.

2.1 The polysaccharide lyases

The polysaccharide lyases (E.C. 4.2.2.-) are enzymes involved in the degradation of glycosaminoglycans and pectin. These

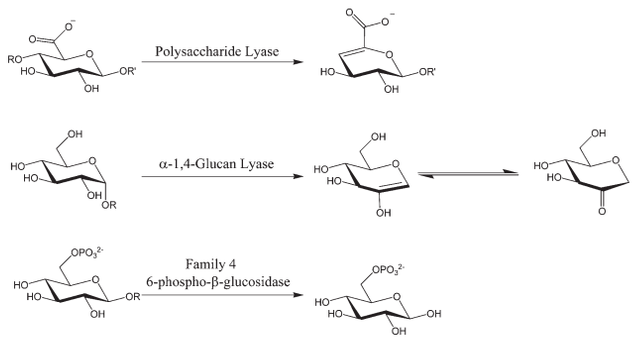
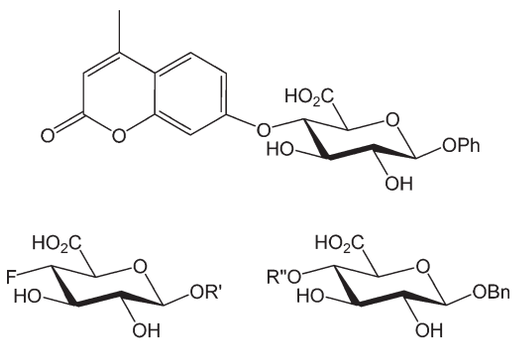


Fig. 2 Representative reactions catalysed by the polysaccharide lyase, α-1,4-glucan lyase, and Family 4 phospho-β-glucosidase classes of enzymes.[45,51,52,77,78]

enzymes have been known for many years and sequences for many such enzymes have been determined. Indeed, the CAZY Web site also maintains an up-to-date inventory of these lyases, classifying them into different families based on primary sequence similarity.[37] Since the early 1960s, it has been recognized that, even though both the bacterial and testicular hyaluronidases degrade hyaluronic acid, these two "hyaluronidases" are mechanistically distinct.[38–41] The testicular hyaluronidase behaves as a typical glycosidase, hydrolysing the substrate to give two saturated saccharides as the products and incorporating an ^{18}O label into C1′ of the uronic acid-containing polymer when the enzymatic reaction is carried out in $H_2{}^{18}O$, a technique used for identifying the scissile bond for other glycosidases.[39,42] Meanwhile, the products of the bacterial hyaluronidase reaction were a saturated reducing sugar and an α,β-unsaturated carboxylic acid-containing sugar.[41] Furthermore, when the reaction was carried out in $H_2{}^{18}O$, ^{18}O was not incorporated into either of the sugar products. The bond cleaved therefore is that between C4 and the glycosidic oxygen rather than that between C1′ and the glycosidic oxygen, with reaction involving an elimination process. As such, it is evident that these bacterial "hyaluronidases" are markedly distinct from glycosidases and have, in fact, eventually been given their own classification, along with enzymes involved in polyuronic acid polymer degradation, as the polysaccharide lyases. The detailed mechanism proposed by Gacesa in 1987 is now largely accepted, and involves neutralization of the negative charge on the carboxylic acid functionality, followed by abstraction of the C5 proton, and subsequent β-elimination of the 4-*O*-glycosidic bond.[43] The following discussion will focus on the chondroitin AC lyase from *Flavobacterium heparinum*, which is probably the best-studied mechanistically amongst this class of enzymes.

A major obstacle in obtaining kinetic data has been the lack of easily assayed, defined substrates. The difficulty lies within the fact that the natural substrates are heterogeneous polymers. Mechanistic studies on the polysaccharide lyases were difficult until recently, when chromophore-, fluorophore-, and fluoride-releasing substrates were synthesized for chondroitin AC lyase.[44] Some such substrates are shown in Fig. 3,[44] and the proposed elimination mechanism is shown in Fig. 4.[45] A logarithmic plot of k_{cat}/K_m values for the elimination of a series of aryl leaving groups *versus* the leaving group pK_a value gives a flat linear free energy relationship.[45] This most likely indicates that cleavage of the 4-*O*-glycosidic linkage is not the rate-limiting step, as changes in leaving group ability have no significant effect on the overall reaction rate. Furthermore, a primary deuterium kinetic isotope effect of $k_H/k_D = 1.67 \pm 0.07$ was measured when the C5 proton was substituted by a deuterium atom in the substrate,[45] showing that cleavage of the C5–H5 bond occurs in the rate-limiting step. The small primary kinetic isotope effect is consistent with the formation of an enolic intermediate, which is known to have a transition state that is late on the reaction coordinate.[46,47] Meanwhile, deuterium substitution at C4 of the substrate allowed the measurement of a secondary kinetic isotope effect on the reaction.[45] The very small k_H/k_D value of 1.01 ± 0.03 shows that

there is no significant rehybridization of C4 from an sp^3 to an sp^2 center at the transition state, again showing that expulsion of the C4 leaving group is not part of the rate-limiting step.[45–47] The kinetic isotope effect data, along with the flat linear free energy relationship, indicate that the rate-limiting step involves abstraction of the C5 proton and that cleavage of the C4–oxygen bond is clearly a separate step. Therefore, the chondroitin AC lyase does not undergo a concerted *syn*-elimination or an E1 mechanism, but rather a stepwise E1cB mechanism.[45]



Where R' = Ph or Bn
R" = 2,4-dinitrophenyl,
4-chloro-2-nitrophenyl
3,4-dinitrophenyl
2-nitrophenyl

Fig. 3 Synthetic substrates for chondroitin AC lyase.[44]

From X-ray crystallographic data and mutagenesis studies, a tyrosine residue has been identified as the potential catalytic base in the abstraction of the C5 proton by chondroitin AC lyase.[48] The pH/activity profile revealed an apparent pK_a value of 7.2 which could correspond to that of a tyrosine residue in the enzyme active site.[45] It is worthwhile to note that the proposed catalytic base for pectate lyase from *Fusarium moniliformae* is a lysine residue.[49] It is therefore likely that the catalytic machinery across the different lyase families is not as uniform as that for the glycosidases.

2.2 α-1,4-Glucan lyases

A new class of polysaccharide lyases, the α-1,4-glucan lyases (E.C. 4.2.2.13), was recently discovered.[50] As illustrated in Fig. 5, the reaction carried out by α-1,4-glucan lyases is clearly unlike that of other polysaccharide lyases, as is evident by noting the differences between the substrates and the reaction products.[50–53] The substrates for the currently known 13 families of polysaccharide lyases are acidic polysaccharides, and abstraction of the C5 proton is only possible due to activation by the adjacent carboxylic acid functionality.[43] Upon cleavage, a double bond between C4 and C5 is formed on the resulting non-reducing end sugar.[45] On the other hand, since the natural substrates for the α-1,4-glucan lyase are starch and glycogen, no protons are activated for abstraction.[50–53] Furthermore, the α-1,4-glucan lyase reaction produces 1,5-anhydrofructose, which in its enol form contains a double bond between C1′ and C2′.[50–53]

It is worth noting that one of the major accomplishments of the glycosidase classification system developed by Henrissat is that primary sequence identity has shown itself to be a remarkably useful tool in the prediction of tertiary structure, and catalytic mechanism and machinery.[54] Therefore, based on the high primary sequence identity to Family 31 retaining α-glucosidases,[53,55] the mechanism of α-1,4-glucan lyases is expected to share similarities with that of the well-studied retaining α-glucosidases. This presumption is supported by various studies including inhibition experiments with acarbose,[52,55] 1-deoxynojirimycin,[52,55] carbodiimides,[56] and 5-fluoro-β-L-idopyranosyl fluoride,[51] all of which are inhibitors of retaining α-glucosidases.[57–61] Inhibition by carbodiimides, which selectively react with aspartic and glutamic acids, suggests that carboxylic acid groups are an important part of the catalytic machinery, similar to other glycosidases.[56] Meanwhile 1-deoxynojirimycin and acarbose are known to inhibit α-glucosidases by mimicking the oxocarbenium ion-like transition state structure.[57–59] Inactivation by the mechanism-based inactivator, 5-fluoro-β-L-idopyranosyl fluoride, provided evidence for formation of a covalent glycosyl–enzyme intermediate.[51] Indeed when the labelled *Gracilariopsis* α-1,4-glucan lyase was subjected to proteolysis followed by purification and tandem mass spectrometric analysis of the labelled peptide sequence, the catalytic nucleophile was identified as Asp 553.[51] This aspartate residue is in fact completely conserved in the Family 31 glycosidases, and had previously been shown to be the catalytic nucleophile for one such α-glucosidase, from *Aspergillus niger*.[51,62] Values of k_{cat} for cleavage of a series of aryl glucosides of different reactivity by the α-1,4-glucan lyase revealed a small, but significant dependence of rate on leaving group ability (β_{lg} for the plot of log k_{cat} *vs.* pK_a = −0.32), showing that bond cleavage is rate-limiting.[52] A large α-secondary kinetic isotope effect (k_H/k_D = 1.23) measured with the 1-deutero-substrate demonstrates a highly oxocarbenium ion-like transition state for this glycosylation step; thus, glycosidic bond cleavage is largely complete at the transition state.[51,52] The small β_{lg} value therefore shows that substantial proton transfer has occurred at the transition state, reducing the charge on the glycosidic oxygen. Together, these two experiments indicate that the first step is the acid-catalysed formation of a glycosyl–enzyme intermediate *via* an oxocarbenium ion-like transition state.[51,52]

The second step of the proposed mechanism is a 1,2-elimination with the release of 2-hydroxyglucal as the product.[51,52]

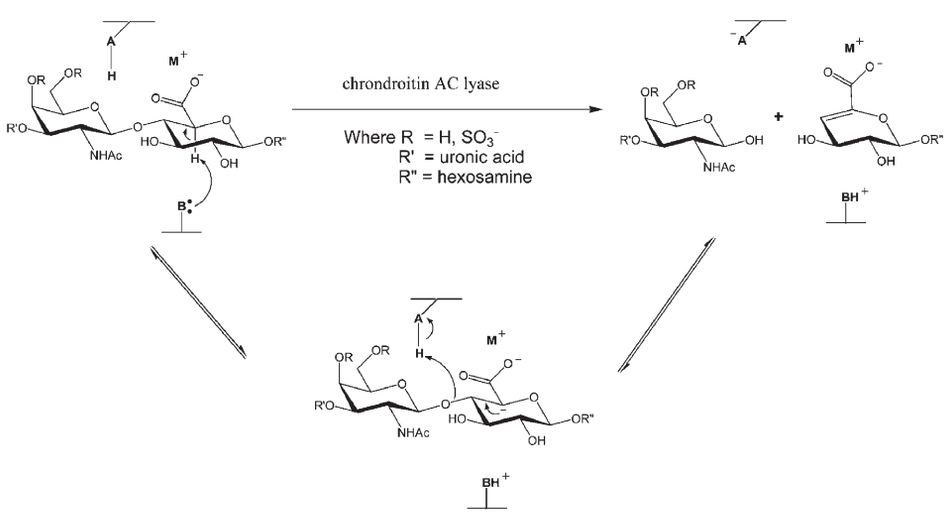


Fig. 4 Proposed elimination mechanism of chondroitin AC lyase, where $M^+ = Ca^{2+}$ or H^+.[45]

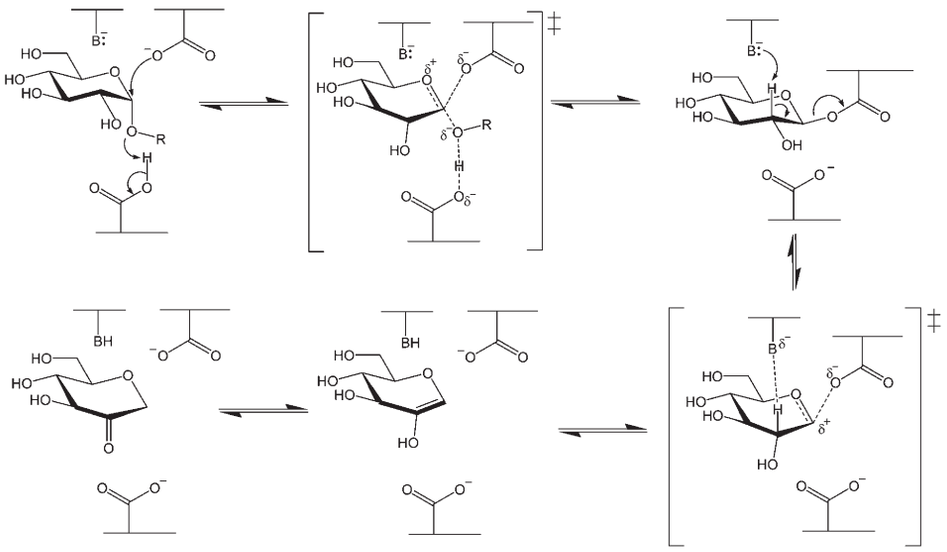


Fig. 5 Proposed mechanism of the α-1,4-glucan lyase.[51,52]

Deuterium kinetic isotope effects were also measured for the second step, namely the elimination step, of the enzyme-catalysed reaction. Using fluoride-releasing substrates for which the second step is rate-limiting, with deuterium substitutions at C1 and C2, a large α-secondary deuterium kinetic isotope effect at C1 (k_H/k_D = 1.23) and a small primary kinetic isotope effect at C2 (k_H/k_D = 1.92) were measured for the respective substrates.[52] These kinetic isotope effect measurements imply that, following the formation of the covalent glycosyl–enzyme intermediate, the second step proceeds *via* an E2 elimination mechanism with substantial E1 character.[52] Proton abstraction in this step largely occurs from the oxocarbenium ion-like species formed as the transition state approaches. Activation of the C2 proton is therefore a consequence of cation formation in the transition state, rather than any in-built acidifying group.[51,52] Based on the above mechanistic studies, the proposed mechanism of the α-1,4-glucan lyases includes the acid-catalysed formation of a covalent glycosyl–enzyme intermediate *via* the nucleophilic aspartic acid residue, followed by an E1-like E2 elimination of the enzyme carboxylate to generate 1,5-anhydrofructose.[51,52] This process has been suggested to occur in a somewhat concerted manner, with the departing nucleophile itself also acting as the base catalyst.[51,52] X-ray crystallographic data on Family 13 α-glycosidases have shown that the carbonyl oxygen of the nucleophilic amino acid residue is located near the endocyclic ring oxygen of the substrate.[63] Rotation about the C–O bond would therefore place the oxygen adjacent to H2 to act in the proposed role.[51,52] Furthermore, retaining glycosidases catalyse the hydration of glycal substrates *via* a covalent glycosyl–enzyme intermediate through the *syn*-addition of the C2 proton in a somewhat concerted process much like the one proposed for α-1,4-glucan lyase.[51,52,64]

Interestingly, the discovery of the α-1,4-glucan lyase is one of the first indications that lyases are in some way linked to glycosidases. As dictated by the International Union of Biochemistry and Molecular Biology (IUBMB), the reaction catalysed by this enzyme classifies it as a lyase, and the enzyme is given the E.C. number E.C. 4.2.2.13.[65] Conversely, the classification system of glycosidases and lyases based upon sequence alignment, which has been shown to be a useful tool in structural and mechanistic elucidation, groups the α-1,4-glucan lyases under Family 31 of the glycosidases.[54] Indeed, the α-1,4-glucan lyase mechanism shares similarities, namely the formation of the covalent glycosyl–enzyme intermediate, with the retaining α-glucosidases. Beyond the sequence similarities, no observations can be made concerning structural resemblances, since no structural data are available for either Family 31 glycosidases or the α-1,4-glucan lyases. A fascinating notion is that perhaps the α-1,4-glucan lyases and the retaining α-glucosidases result from divergent evolution from a common ancestor.[53,55] Further evidence of the connection between these two seemingly different classes of enzymes is that 1,5-anhydrofructose was reported as a side product from maltose hydrolysis by the Family 31 mammalian glucosidase II, suggesting the presence of minor lyase activity in this glycosidase.[66] Likewise, the nucleoside 2-deoxyribosyltransferase from *Lactobacillus leishmanii* was shown to also produce D-ribal, in addition to its normal transfer product.[67] Moreover, the crystal structure of a recently discovered Family 82 ι-carrageenase was found to have a rare protein fold.[68] The inverting glycosidase contains a right-handed parallel β-helix, which is known in some Family 1 polysaccharide lyases but is completely novel to the glycosidases.[68,69] These findings highlight the fact that assignment of function to enzymes based solely upon sequence analysis can be hazardous. Confirmation of function requires demonstration of the actual reaction catalysed using purified proteins. Equally intriguing in this regard are the Family 4 glycosidases, which are the focus of the remaining discussion.

2.3 Family 4 glycosidases

The Family 4 glycoside hydrolases are an unusual group of enzymes. These glycosidases are unique in their requirement for both NAD and a divalent metal cation, such as Mn^{2+} or Ni^{2+}, for activity. However, the NAD cofactor is not consumed during catalysis.[70–72] Furthermore, unlike other families of glycosidases, which exclusively hydrolyse either α-glycosidic linkages or β-glycosidic linkages, Family 4 includes both α-glycosidases, such as *Bacillus subtilis* 6-phospho-α-glucosidase GlvA[70] and the *B. subtilis* α-galactosidase,[73] and β-glycosidases, such as the *Escherichia coli* 6-phospho-β-glucosidase, CelF.[71] Furthermore, like Family 1 glycosidases,[23,24] members of this family can hydrolyse both the natural phosphorylated and non-phosphorylated disaccharide substrates.[70,71,74–77] Additionally, Family 4 glycosidases should probably be classified as retainers, as both BglT (a 6-phospho-β-glucosidase)[77] and GlvA (a 6-phospho-α-glucosidase),[78] have been shown to be retaining glycosidases through ^{1}H NMR analysis of the methyl glycoside products formed in the presence of methanol. However, some Family 4 glycosidases, such as the 6-phospho-α-glucosidase MalH,[79] are also exceptional in that they can hydrolyse both α- and β-linkages for substrates with good leaving groups such as the *p*-nitrophenolate anion. Since product stereochemistries in these cases have not been analysed, it is currently unclear whether all Family 4 members should be classified as retaining glycosidases until more work is performed. Recently, X-ray crystal structures for three Family 4 glycosidases, AglA (an α-galactosidase),[80] BglT,[77]

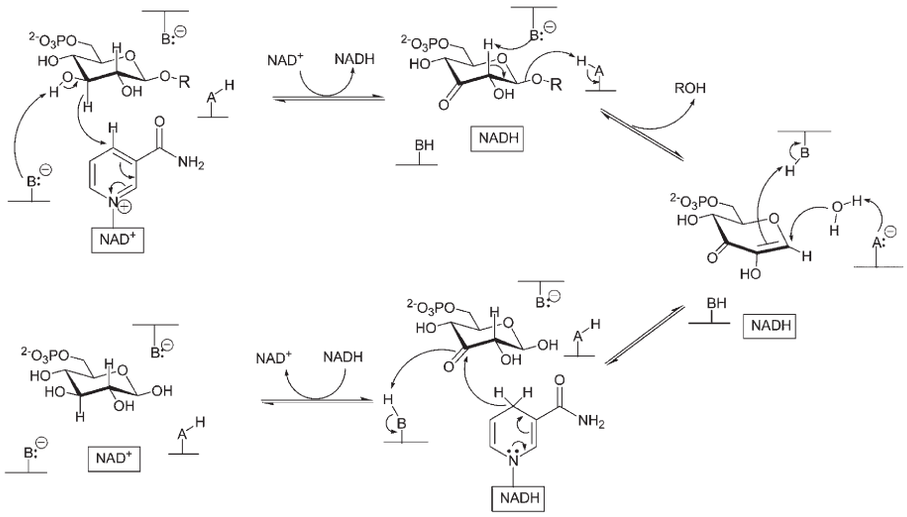


Fig. 6 Proposed mechanism of BglT, a 6-phospho-β-glucosidase belonging to glycoside hydrolase Family 4.[77]

and GlvA[78,81] have been solved. All three structures show high similarity to lactate/malate dehydrogenases, despite relatively low primary sequence identity to these otherwise unrelated enzymes.[77,78,80] The similarity to the dehydrogenases, particularly the retention of the same active-site architecture, in conjunction with the lack of structural similarity to any known structures of glycosidases suggests the involvement of the NAD cofactor in some redox chemistry.[77,78,80] Furthermore, the crystal structures of BglT and GlvA show that C4 of the nicotinamide ring is located 4.16 Å and 3.56 Å respectively from C3 of the enzyme-bound product, glucose 6-phosphate, suggesting that oxidation may occur at this position.[77,78] Additionally, NMR experiments show that the C2 proton of the substrate exchanges with solvent water during reaction as a direct consequence of the enzymatic reaction.[77,78] This is an important clue to the mechanism, as it implies abstraction of the C2 proton, reminiscent of events occurring during the α-1,4-glucan lyase reaction in which a 1,2-unsaturated hydroxyglucal product is formed *via* an elimination reaction.[51,52] However, in this case the final product is a normal sugar rather than an elimination product.[70] For BglT, primary kinetic isotope effects of $k_H/k_D = 1.84 \pm 0.02$ were measured when the C2 proton was substituted by a deuterium atom in the substrate, and a value of $k_H/k_D = 1.63 \pm 0.01$ was measured when the C3 proton was replaced by a deuterium atom.[77] The primary kinetic isotope effect measurements prove that both of the C2–H2 and C3–H3 linkages are cleaved in the (partially) rate-limiting steps.[77]

Based on the above evidence, a detailed mechanism that is consistent with the unusual observations on this family of enzymes was proposed for BglT and GlvA.[77,78] The proposed mechanism is shown for the 6-phospho-β-glucosidase (BglT) in Fig. 6, and involves, as a first key step, the partially rate-limiting abstraction of a hydride from C3 *via* reduction of NAD, thereby generating a ketone. As a consequence, the C2 proton is acidified, allowing for deprotonation by a suitably positioned base, thence a 2,1-elimination of the aglycone moiety. The Mn^{2+} polarizes the carbonyl and stabilizes the intermediate, facilitating proton abstraction at C2. Subsequently, a water molecule attacks the intermediate species, an α,β-unsaturated Michael acceptor, with attack from the same face as the departed aglycone likely being favored by the location of the acid/base catalyst on that side. Finally, reduction of the C3 ketone by NADH returns the enzyme to its starting state, resulting in overall hydrolysis. In both the BglT and GlvA structures, a tyrosine residue is in close proximity to C2 of the glucose 6-phosphate product.[77,78] This tyrosine residue is the proposed catalytic base responsible for deprotonating the C2 proton.[77,78] Indeed, it is interesting to find a tyrosine in this role since, as noted earlier, a tyrosine apparently plays that role in a polysaccharide lyase also.[45,48,77,78] Possibly tyrosine is a good candidate as the catalytic base, since its pK_a more closely matches that of the proton to be removed.

The Family 4 glycosidase mechanism constitutes the first completely novel mechanism of enzymatic glycoside hydrolysis since Koshland's proposals for inverting and retaining glycosidases over 50 years ago.[25,78] As is supported by structural data, this mechanism also satisfies the varied substrate anomeric stereochemistry seen in reactions catalysed by Family 4 glycosidases, since the key catalytic components are the NAD cofactor and the catalytic base, which are located in the same spatial arrangement for all substrates (see Fig. 7).[77,78] While this mechanism appears completely foreign to any known mechanisms of glycosidases, it resembles those of dehydratases such as dTDP-D-glucose 4,6-dehydratase (RmlB), which catalyses the dehydration of dTDP-D-glucose to form dTDP-4-keto-6-deoxy-D-glucose as part of the L-rhamnose biosynthetic pathway.[82,83]

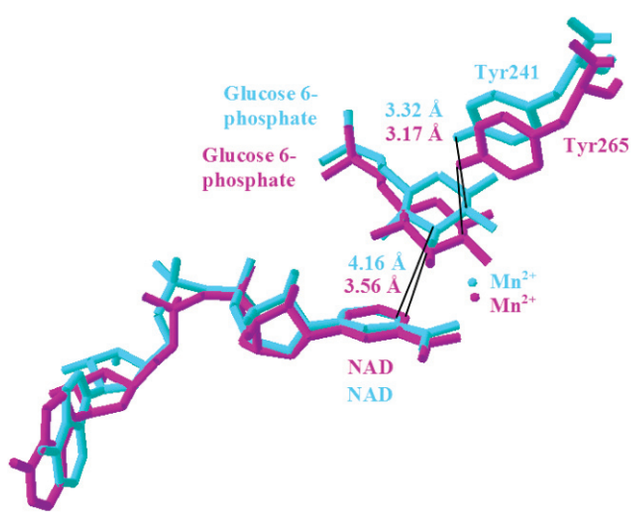


Fig. 7 Enzyme active sites of BglT (cyan), 6-phospho-β-glucosidase, and GlvA (magenta), 6-phospho-α-glucosidase.[77,78]

Interest in Family 4 glycosidases stems from their uniqueness amongst their glycosidase cousins. The proposed mechanism has an elimination step similar to those of the polysaccharide lyases and the α-1,4-glucan lyases, and the transient redox chemistry involving the NAD cofactor is reminiscent of dehydrogenases and dehydratases. Furthermore, structural similarity with oxy-acid dehydrogenases suggests that these enzymes are descended from a common, ancient precursor. Many details of this mechanism remain to be elucidated. In the process we should gain some fascinating insights into alternative mechanistic paradigms to achieve a previously well-understood reaction—enzyme-catalysed glycoside degradation.

4 Conclusion

The most common way that nature has evolved to cleave the glycosidic bond is through hydrolysis, which is usually carried out by a class of well-characterized enzymes, the glycosidases. In addition, it has been known for some time that nature has evolved to use an elimination mechanism for uronic acid-containing polysaccharide degradation in the polysaccharide lyases. The more recent discovery and mechanistic analysis of the α-glucan lyases and Family 4 glycosidases, which employ elimination mechanisms, highlights the inherent plasticity of enzyme active sites and provides fascinating glimpses into how enzyme mechanisms themselves can evolve.

Acknowledgements

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References

1 M. L. Sinnott, *Chem. Rev.*, 1990, **90**, 1171–1202.
2 S. Roseman, *J. Biol. Chem.*, 2001, **276**, 41527–41542.
3 H. Schachter, *J. Clin. Invest.*, 2001, **108**, 1579–1582.
4 R. A. Laine, *Glycobiology*, 1994, **4**, 759–767.
5 J. Montreuil, in *Glycoproteins*, ed. J. Montreuil, J. F. G. Vliegenthart and H. Schachter, Elsevier Science, Amsterdam, 1995, vol. 29a, pp. 1–12.
6 C. R. Bertozzi and L. L. Kiessling, *Science*, 2001, **291**, 2357–2364.
7 J. B. Lowe and J. D. Marth, *Annu. Rev. Biochem.*, 2003, **72**, 643–691.
8 J. Alper, *Science*, 2003, **301**, 159–160.
9 R. Wolfenden, C. Ridgway and G. Young, *J. Am. Chem. Soc.*, 1998, **120**, 833–834.
10 N. Carmi and R. R. Breaker, *Bioorg. Med. Chem.*, 2001, **9**, 2589–2600.
11 R. R. Breaker, *Nat. Biotechnol.*, 1999, **17**, 422–423.
12 Y. Li and R. R. Breaker, *J. Am. Chem. Soc.*, 1999, **121**, 5364–5372.
13 R. Wolfenden, X. D. Lu and G. Young, *J. Am. Chem. Soc.*, 1998, **120**, 6814–6815.
14 D. L. Zechel and S. G. Withers, *Acc. Chem. Res.*, 2000, **33**, 11–18.
15 A. Vasella, G. J. Davies and M. Bohm, *Curr. Opin. Chem. Biol.*, 2002, **6**, 619–629.
16 H. D. Ly and S. G. Withers, *Annu. Rev. Biochem.*, 1999, **68**, 487–522.
17 V. H. Lillelund, H. H. Jensen, X. F. Liang and M. Bols, *Chem. Rev.*, 2002, **102**, 515–553.
18 D. L. Zechel and S. G. Withers, *Curr. Opin. Chem. Biol.*, 2001, **5**, 643–649.
19 S. J. Williams and G. J. Davies, *Trends Biotechnol.*, 2001, **19**, 356–362.
20 C. S. Rye and S. G. Withers, *Curr. Opin. Chem. Biol.*, 2000, **4**, 573–580.
21 S. G. Withers, *Can. J. Chem.*, 1999, **77**, 1–11.
22 S. G. Withers, *Carbohydr. Polym.*, 2001, **44**, 325–337.
23 B. Henrissat and G. Davies, *Curr. Opin. Struct. Biol.*, 1997, **7**, 637–644.
24 B. Henrissat and A. Bairoch, *Biochem. J.*, 1996, **316**, 695–696.
25 D. E. Koshland, *Biol. Rev.*, 1953, **28**, 416.
26 J. D. McCarter and S. G. Withers, *Curr. Opin. Struct. Biol.*, 1994, **4**, 885–892.
27 Q. Wang, R. W. Graham, D. Trimbur, R. A. J. Warren and S. G. Withers, *J. Am. Chem. Soc.*, 1994, **116**, 11594–11595.
28 S. L. Lawson, W. W. Wakarchuk and S. G. Withers, *Biochemistry*, 1997, **36**, 2257–2265.
29 B. L. Mark, D. J. Vocadlo, S. Knapp, B. L. Triggs-Raine, S. G. Withers and M. N. G. James, *J. Biol. Chem.*, 2001, **276**, 10330–10337.
30 D. Piszkiewicz and T. C. Bruice, *J. Am. Chem. Soc.*, 1968, **90**, 2156–2163.
31 S. Drouillard, S. Armand, G. J. Davies, C. E. Vorgias and B. Henrissat, *Biochem. J.*, 1997, **328**, 945–949.
32 J. N. Watson, V. Dookhun, T. J. Borgford and A. J. Bennet, *Biochemistry*, 2003, **42**, 12682–12690.
33 A. Buschiazzo, O. Campetella and A. C. C. Frasch, *Glycobiology*, 1997, **7**, 1167–1173.
34 A. G. Watts, I. Damager, M. L. Amaya, A. Buschiazzo, P. Alzari, A. Frasch and S. G. Withers, *J. Am. Chem. Soc.*, 2003, **125**, 7532–7533.
35 W. P. Burmeister, S. Cottaz, H. Driguez, R. Iori, S. Palmieri and B. Henrissat, *Structure*, 1997, **5**, 663–675.
36 M. Shikita, J. W. Fahey, T. R. Golden, W. D. Holtzclaw and P. Talalay, *Biochem. J.*, 1999, **341**, 725–732.
37 P. M. Coutinho and B. Henrissat, in *Genetics, Biochemistry and Ecology of Cellulose Degradation*, ed. K. Ohmiya, K. Hayashi, K. Sakka, Y. Kobayashi, S. Karita and T. Kimura, Uni Publishers Co., Tokyo, 1999, pp. 15–23.
38 S. Hasegawa and C. W. Nagel, *J. Biol. Chem.*, 1962, **237**, 619–621.
39 J. Preiss and G. Ashwell, *J. Biol. Chem.*, 1962, **237**, 309–316.
40 J. Ludowieg, B. Vennesland and A. Dorfman, *J. Biol. Chem.*, 1961, **236**, 333–339.
41 A. Linker, K. Meyer and P. Hoffman, *J. Biol. Chem.*, 1956, **219**, 13–25.
42 D. E. Koshland and S. S. Stein, *J. Biol. Chem.*, 1954, **208**, 139–148.
43 P. Gacesa, *FEBS Lett.*, 1987, **212**, 199–202.
44 C. S. Rye and S. G. Withers, *Anal. Biochem.*, 2002, **308**, 77–82.
45 C. S. Rye and S. G. Withers, *J. Am. Chem. Soc.*, 2002, **124**, 9756–9767.
46 W. C. Alston, K. Haley, R. Kanski, C. J. Murray and J. Pranata, *J. Am. Chem. Soc.*, 1996, **118**, 6562–6569.
47 W. C. Alston, M. Kanska and C. J. Murray, *Biochemistry*, 1996, **35**, 12873–12881.
48 W. Huang, L. Boju, L. Tkalec, H. Su, H. O. Yang, N. S. Gunay, R. J. Linhardt, Y. S. Kim, A. Matte and M. Cygler, *Biochemistry*, 2001, **40**, 2359–2372.
49 M. N. Rao, A. A. Kembhavi and A. Pant, *Biochem. J.*, 1996, **319**, 159–164.
50 S. Yu, L. Kenne and M. Pedersen, *Biochim. Biophys. Acta*, 1993, **1156**, 313–320.
51 S. S. Lee, S. K. Yu and S. G. Withers, *J. Am. Chem. Soc.*, 2002, **124**, 4948–4949.
52 S. S. Lee, S. Yu and S. G. Withers, *Biochemistry*, 2003, **42**, 13081–13090.
53 S. Yu, *Trends Glycosci. Glycotechnol.*, 2000, **12**, 381–388.
54 B. Henrissat, I. Callebaut, S. Fabrega, P. Lehn, J. P. Mornon and G. Davies, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 7090–7094.
55 S. K. Yu, K. Bojsen, B. Svensson and J. Marcussen, *Biochim. Biophys. Acta*, 1999, **1433**, 1–15.
56 P. Nyvall, M. Pedersen, L. Kenne and P. Gacesa, *Phytochemistry*, 2000, **54**, 139–145.
57 I. Przylas, Y. Terada, K. Fujii, T. Takaha, W. Saenger and N. Strater, *Eur. J. Biochem.*, 2000, **267**, 6903–6913.
58 M. J. Papandreou, R. Barbouche, R. Guieu, M. P. Kieny and E. Fenouillet, *Mol. Pharmacol.*, 2002, **61**, 186–193.
59 K. A. Lee and M. S. Kim, *Can. J. Microbiol.*, 2000, **46**, 1077–1081.
60 J. D. McCarter and S. G. Withers, *J. Am. Chem. Soc.*, 1996, **118**, 241–242.
61 J. D. McCarter and S. G. Withers, *J. Biol. Chem.*, 1996, **271**, 6889–6894.
62 S. S. Lee, S. M. He and S. G. Withers, *Biochem. J.*, 2001, **359**, 381–386.
63 J. C. M. Uitdehaag, R. Mosi, K. H. Kalk, B. A. van der Veen, L. Dijkhuizen, S. G. Withers and B. W. Dijkstra, *Nat. Struct. Biol.*, 1999, **6**, 432–436.
64 G. Legler, *Adv. Carbohydr. Chem. Biochem.*, 1990, **48**, 319–384.
65 E. C. Webb, *Enzyme Nomenclature*, Academic Press, Inc., San Diego, 1992.
66 K. Hirano, M. Ziak, K. Kamoshita, Y. Sukenaga, S. Kametani, Y. Shiga, J. Roth and H. Akanuma, *Glycobiology*, 2000, **10**, 1283–1289.
67 M. Smar, S. A. Short and R. Wolfenden, *Biochemistry*, 1991, **30**, 7908–7912.
68 G. Michel, L. Chantalat, E. Fanchon, B. Henrissat, B. Kloareg and O. Dideberg, *J. Biol. Chem.*, 2001, **276**, 40202–40209.
69 M. D. Yoder, N. T. Keen and F. Jurnak, *Science*, 1993, **260**, 1503–1507.
70 J. Thompson, A. Pikis, S. B. Ruvinov, B. Henrissat, H. Yamamoto and J. Sekiguchi, *J. Biol. Chem.*, 1998, **273**, 27347–27356.
71 J. Thompson, S. B. Ruvinov, D. I. Freedberg and B. G. Hall, *J. Bacteriol.*, 1999, **181**, 7339–7345.
72 C. Raasch, M. Armbrecht, W. Streit, B. Hocker, N. Strater and W. Liebl, *FEBS Lett.*, 2002, **517**, 267–271.
73 A. Lapidus, N. Galleron, A. Sorokin and S. D. Ehrlich, *Microbiology*, 1997, **143**, 3431–3441.
74 J. Thompson, S. A. Robrish, A. Pikis, A. Brust and F. W. Lichtenthaler, *Carbohydr. Res.*, 2001, **331**, 149–161.
75 J. Thompson, S. A. Robrish, S. Immel, F. W. Lichtenthaler, B. G. Hall and A. Pikis, *J. Biol. Chem.*, 2001, **276**, 37415–37425.

76 J. Thompson, C. R. Gentry-weeks, N. Y. Nguyen, J. E. Folk and S. A. Robrish, *J. Bacteriol.*, 1995, **177**, 2505–2512.
77 V. L. Y. Yip, A. Varrot, G. J. Davies, S. S. Rajan, X. Yang, J. Thompson, W. F. Anderson and S. G. Withers, *J. Am. Chem. Soc.*, 2004, **126**, 8354–8355.
78 S. S. Rajan, X. Yang, F. Collart, V. L. Y. Yip, S. G. Withers, A. Varrot, J. Thompson, G. J. Davies and W. F. Anderson, *Structure*, 2004, in press.
79 A. Pikis, S. Immel, S. A. Robrish and J. Thompson, *Microbiology*, 2002, **148**, 843–852.
80 J. A. Lodge, T. Maier, W. Liebl, V. Hoffmann and N. Strater, *J. Biol. Chem.*, 2003, **278**, 19151–19158.
81 A. Varrot, H. Yamamoto, J. Sekiguchi, J. Thompson and G. J. Davies, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 1999, **55**, 1212–1214.
82 S. T. M. Allard, K. Beis, M. F. Giraud, A. D. Hegeman, J. W. Gross, R. C. Wilmouth, C. Whitfield, M. Graninger, P. Messner, A. G. Allen, D. J. Maskell and J. H. Naismith, *Structure*, 2002, **10**, 81–92.
83 S. T. M. Allard, M. F. Giraud, C. Whitfield, M. Graninger, P. Messner and J. H. Naismith, *J. Mol. Biol.*, 2001, **307**, 283–295.