Carbohydrate-active Enzymes

An increasing number of structurally related families of enzymes that degrade, modify, or create glycosidic bonds are being identified and characterized. Davies, et al., estimated that carbohydrate-active enzymes represent 1-3% of all proteins encoded by the genomes of most organisms, and identification of 12,000+ open reading frames for glycoside hydrolases and glycosyltransferases was projected for 2006. Since carbohydrate-active enzymes act on the most structurally diverse substrates in nature, the number of enzymes identified from gene sequences will continue to expand. The variety and specificity of carbohydrate-active enzymes reflects the large amount of information coded by the glycan moiety of glycosylated biomolecules and the impact those compounds have in the life cycle of complex cells.

The Carbohydrate-Active Enzymes Database (CAZy, www.cazy.org), maintained by Université de Provence/Université de la Méditerranée, Marseille, France contains information for over 200 families of glycoside hydrolases (glycosidases), glycosyltransferases, polysaccharide lyases, and carbohydrate esterases. Glycosidases, including endoglycosidases and exoglycosidases, are primarily used in analytical applications and deglycosylation techniques, but innovative preparative applications are emerging. Polysaccharide lyases and carbohydrate esterases are used to degrade glycosaminoglycans and prepare polysaccharide components. Glycosyltransferases are being studied for use in engineering selective glycosylation to improve the therapeutic properties of proteins and antibodies.

References:

Glycan Sequencing Using Exoglycosidases

Enzymatic analysis of oligosaccharides using highly specific exoglycosidases, either sequentially or in a matrix array, is a powerful technique in determining the sequence and structure of glycans. Exoglycosidases remove terminal carbohydrates from the non-reducing end of a glycan, but do not cleave internal bonds between carbohydrates. By using positionally specific exoglycosidases, the removed glycan residues can be identified by linkage as well as sugar. For example, β1(1→4) galactosidase will remove terminal β-galactose residues attached with a (1→4) linkage but not residues attached with a (1→3) or (1→6) linkage. In glycan sequencing, the glycan pool is separated into individual oligosaccharides, and each purified glycan is digested sequentially with various linkage-specific exoglycosidase enzymes. Figure 1 shows an example of an exoglycosidase digestion scheme for the structural analysis of a triantennary complex N-linked glycan.

Key to Monosaccharide Symbols

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-D-Glucose</td>
<td>Glc</td>
</tr>
<tr>
<td>β-D-Xylose</td>
<td>Xyl</td>
</tr>
<tr>
<td>β-D-Mannose</td>
<td>Man</td>
</tr>
<tr>
<td>α-N-Acetylgalactosaminic acid; Salt acid (NeuNAc)</td>
<td></td>
</tr>
<tr>
<td>β-D-Galactose</td>
<td>Gal</td>
</tr>
<tr>
<td>β-D-Glucuronic acid (GlcA)</td>
<td></td>
</tr>
<tr>
<td>β-N-Acetylglucosamine (GlcNAc)</td>
<td></td>
</tr>
<tr>
<td>α-L-Iduronic acid (IdoA)</td>
<td></td>
</tr>
<tr>
<td>β-D-N-Acetylgalactosamine (GalNAc)</td>
<td></td>
</tr>
<tr>
<td>α-L-Fucose (Fuc)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Exoglycosidase enzymes commonly used to determine the structure of N-linked glycans by sequential degradation.

Exoglycosidase digestion, when combined with high-performance separation techniques such as chromatography or electrophoresis, is a rapid and cost-effective method for oligosaccharide characterization. By incorporating enzymatic digestion with capillary gel electrophoresis, the sequence of each glycan in a released pool can be elucidated. When laser-induced fluorescence is the method of detection, picomolar amounts of purified glycoproteins can be sequenced. Exoglycosidases have been used in sequencing methods with fluorescent tags attached to the reducing-end of glycans and polynucleotide gel electrophoresis separations. Integral glycan sequencing (IGS) has been shown to identify complete sequence information in a single experiment. IGS integrates exoglycosidase digestion with a chemical cleavage step and permits rapid sequencing of glycosaminoglycans. Picomolar quantities of heparin decasaccharides have been sequenced by matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS) after integral glycan sequencing. MALDI-MS techniques incorporated with IGS are both sensitive and accurate, detecting as little as 100 femtomoles of oligosaccharides with a reported mass error of ±1 Dalton.
Glycan Sequencing Using Exoglycosidases

In addition to sequential degradation of glycans using exonucleases, newer methods have been reported that are capable of structural analysis of glycopeptides without deglycosylation. Temporini, et al., have developed an automated method incorporating on-line digestion of glycoproteins using immobilized pronase and subsequent multistage tandem mass spectrometry (MSn). This technique reduced analysis time from 3 days down to one hour.4 Deguchi, et al., have reported using a combination of negative-ion collision-induced dissociation (CID) and positive-ion electron-capture dissociation (ECD) mass spectrometry methods in both positive and negative ion mode. Multistage tandem mass spectrometry (MSn) results were used to assign O-glycan structures attached to peptides, peptide sequences, and location of glycosylation sites.5

References:

Proteomics Grade Exoglycosidases

β-N-Acetylglucosaminidase

Synonyms: β-N-acetylgalactosaminidase; NAGASE

β-N-Acetylglucosaminidase is an exoglycosidase thought to be involved in the turnover of N-linked glycans. This enzyme exhibits a broad specificity, cleaving terminal β(1→2,3,4,6) linked N-acetylglucosamine (GlcNAc) and N-acetylgalactosamine (GalNAc) residues from the non-reducing ends of oligosaccharides or those present on the glycan moiety of glycoproteins. This specificity has proved to be useful in the determination of linkage and anomeric configuration of carbohydrate units in isolated glycans, glycolipids, and glycoproteins, in combination with other glycosidase enzymes.

Proteomics Grade β-N-Acetylglucosaminidase has been purified to near homogeneity by several chromatographic techniques. The enzyme is tested and the absence of contaminating protease and endoglycosidase activities is confirmed.

α-Mannosidase

Synonyms: α-α-Mannosidase mannohydrolase

α-Mannosidase is an exoglycosidase thought to be involved in the turnover of N-linked glycans. The enzyme cleaves terminal α-1-mannosyl residues which are α(1→2,3,6) linked to the non-reducing ends of oligosaccharides or those present on the glycan moiety of glycoproteins. α(1→3) Linked mannose residues are reported to be hydrolyzed at a lower rate than α(1→2) and α(1→6) linked residues. This property of the enzyme has been exploited in the linkage determination of mannose in glycoproteins. The enzyme has been shown to cleave mannose from the glycans of oxomucoid, orosomucoid, ovalbumin, and other glycoproteins.

Proteomics Grade α-Mannosidase has been purified to near homogeneity by several chromatographic techniques. The enzyme is tested and the absence of contaminating protease and exoglycosidase activities is confirmed.

β-Mannosidase

Synonyms: β-Mannosidase mannohydrolase; β-α-Mannosidase

β-Mannosidase is an exoglycosidase that cleaves single terminal α-mannosyl residues, which are β(1→4) linked to the non-reducing ends of oligosaccharides or those present on the glycan moiety of glycoproteins with relative specificity. Other mannose residues linked β(1→3) and β(1→6) are reported to be hydrolyzed at much lower rates. This property of the enzyme has been exploited in:

- The determination of the sequence and structure of N-linked and O-linked glycans (together with other exoglycosidases).
- The characterization of α-galactose containing mannoooligosaccharides that are produced by acid or enzymatic hydrolysis of galactomannans, allowing location of α-galactosyl residues on such glycans.

Proteomics Grade β-Mannosidase has been purified to near homogeneity by several chromatographic techniques. The enzyme is tested and the absence of contaminating protease and exoglycosidase activities is confirmed.

α(2→3,6,8,9) Neuraminidase

Synonyms: Sialidase; Acylneuraminyl hydrolase

α(2→3,6,8,9) Neuraminidase is a highly purified enzyme from Arthrobacter ureafaciens that releases α(2→3), α(2→6), α(2→8), and α(2→9) linked sialic acids. The relative rates of cleavage are reported to be α(2→6) > α(2→3) > α(2→8) and α(2→9), however, these rates make little practical difference as sufficient enzyme is used to ensure cleavage of all sialic acid residues. This wide spectrum of activity makes it ideal for complete non-specific removal of sialic acid groups prior to analysis.

Proteomics Grade α(2→3,6,8,9) Neuraminidase is lyophilized from 10 mM sodium/potassium phosphate buffer without any added stabilizers. The low levels of buffer salts make it compatible with subsequent analysis by MALDI-TOF MS or HPLC. The enzyme is tested and the absence of contaminating exo- and endoglycosidase activities is confirmed.

Reference:
Glycosyltransferases were initially considered to be specific for a single linkage position of the glycoside bond formed \([\text{e.g. } \alpha(1\rightarrow3) \text{ or } \beta(1\rightarrow4)]\). Subsequent observations have refuted the theory of absolute enzymatic specificity by describing the transfer of analogs of some nucleoside mono- or diphosphate sugar donors. \[3,8\]

Glycosyltransferases can tolerate modifications to the acceptor sugar, as long as the acceptor meets specific structural requirements, e.g., appropriate stereochemistry and availability of the reactive hydroxyl group involved in the glycosidic bond.

In contrast to organic chemical synthesis, enzymatic glycosylation has potential for application use within biological systems, where the modification of glycosylation sites may be used to investigate the regulation of cell signaling processes. Various application strategies for glycosyltransferases have employed an assortment of glycosyl donors and reaction conditions for the synthesis of carbohydrates and the glycosylation of natural products. \[9,10\]

A major limitation to enzyme-catalyzed glycosylation reactions is the glycosyltransferase inhibition caused by nucleotide diphosphates generated during the reaction. Two strategies have been identified to prevent enzymatic inhibition (see Figure 2):

1. Phosphatase is added to the reaction to degrade the nucleotide diphosphates by removal of the phosphate group (see Figure 2A). \[11\]

2. Nucleotide diphosphates are recycled to the appropriate nucleotide triphosphates by employing multi-enzyme regeneration schemes. Although several different enzymes and cofactors are involved in these in situ regeneration schemes, the method avoids the use of stoichiometric amounts of sugar nucleotides (see Figure 2B). \[12-14\]

### Table: Endoglycosidases

<table>
<thead>
<tr>
<th>Name</th>
<th>Unit Definition</th>
<th>Form</th>
<th>Packaging</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-M-Acylglucosaminidase from Canavalia ensiformis (Jack bean), Proteomics Grade</td>
<td>One unit will hydrolyze 1.0 μmole of p-nitrophenyl-α-D-glucosaminide to p-nitrophenol and N-acetyl-α-glucosamine per min at pH 9.0 at 37 °C.</td>
<td>Solution in 20 mM sodium citrate buffer, pH 6.0, containing 0.25 mg/ml BSA and 0.02% azide. Provided with 5x concentrate reaction buffer.</td>
<td>PP0600-1KT</td>
<td></td>
</tr>
<tr>
<td>e-Mannosidase from Canavalia ensiformis (Jack bean), Proteomics Grade</td>
<td>One unit will hydrolyze 1.0 μmole of p-nitrophenyl-α-D-mannopyranoside to p-nitrophenol and D-mannose per min at pH 4.5 at 37 °C.</td>
<td>Solution in 20 mM Tris HCl, pH 7.5, containing 25 mM NaCl. Provided with 5x concentrate reaction buffer.</td>
<td>M7944-10UN</td>
<td></td>
</tr>
<tr>
<td>β-Mannosidase from Helix pomatia, Proteomics Grade</td>
<td>One unit will hydrolyze 1 μmole of p-nitrophenol (measured at 400 nm) and D-mannose per minute at pH 4.0 at 37 °C.</td>
<td>Enzyme is lyophilized from 10 mM sodium acetate buffer, pH 4.0, containing BSA and sodium chloride. Provided with 5x concentrate reaction buffer.</td>
<td>M7819-1UN</td>
<td></td>
</tr>
<tr>
<td>α(2→3,6,8,9) Neuraminidase from Arthrobacter ureafaciens, Proteomics Grade</td>
<td>One unit will release 1 nmole of 4-methylumbelliferone from 2-(4-methylumbelliferyl)-α-N-acetylneuraminic acid per minute at pH 5.5 at 37°C.</td>
<td>Lyophilized powder Provided with 5x concentrate reaction buffer.</td>
<td>N3786-15ET</td>
<td></td>
</tr>
</tbody>
</table>

### Glycosyltransferases

Glycosyltransferases are specific for the type of linkage (α or β), and the linkage position of the glycoside bond formed \([\text{e.g. } \alpha(1\rightarrow3) \text{ or } \beta(1\rightarrow4)]\). Glycosyltransferases were initially considered to be specific for a single glycosyl donor and acceptor, which led to the “one enzyme-one linkage” concept. \[1,2\] Subsequent observations have refuted the theory of absolute enzymatic specificity by describing the transfer of analogs of some nucleoside mono- or diphosphate sugar donors. \[3,8\]

Glycosyltransferases can tolerate modifications to the acceptor sugar, as long as the acceptor meets specific structural requirements, e.g., appropriate stereochemistry and availability of the reactive hydroxyl group involved in the glycosidic bond.

In contrast to organic chemical synthesis, enzymatic glycosylation has potential for application use within biological systems, where the modification of glycosylation sites may be used to investigate the regulation of cell signaling processes. Various application strategies for glycosyltransferases have employed an assortment of glycosyl donors and reaction conditions for the synthesis of carbohydrates and the glycosylation of natural products. \[9,10\]

A major limitation to enzyme-catalyzed glycosylation reactions is the glycosyltransferase inhibition caused by nucleotide diphosphates generated during the reaction. Two strategies have been identified to prevent enzymatic inhibition (see Figure 2):

1. Phosphatase is added to the reaction to degrade the nucleotide diphosphates by removal of the phosphate group (see Figure 2A). \[11\]

2. Nucleotide diphosphates are recycled to the appropriate nucleotide triphosphates by employing multi-enzyme regeneration schemes. Although several different enzymes and cofactors are involved in these in situ regeneration schemes, the method avoids the use of stoichiometric amounts of sugar nucleotides (see Figure 2B). \[12-14\]