Agilent PL-SAX Anion-Exchange Media for Amyloglucosidase Purification and Analysis

Technical Overview

Introduction

Agilent PL-SAX is a hydrophilic, strong anion-exchange chromatographic packing material. The open pore structure of PL-SAX 4000Å make it ideal for high resolution and high speed applications. In these examples we investigate the efficacy of the column for the purification of amyloglucosidase.

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High speed fractionation of amyloglucosidase

Using the 4000Å material it is possible to purify amyloglucosidase in less than two minutes. The capacity of the material was determined as 34 mg BSA/mL CV at 1.0 mL/min and 32 g BSA/mL CV at 4.0 mL/min with a minimal change in the shape of the breakthrough curve (Figure 1). Therefore, it is anticipated that high speed fractionations, where over 80% of the column bed can be utilized before valuable product is detected in the eluent stream, could be achieved.

The enzyme amyloglucosidase was fractionated from *Aspergillus niger* cell culture filtrate. The enzyme occurs in two forms with molecular weights of 99 kD and 112 kD (peaks 1 and 2, respectively, Figure 2), which have the same amino acid composition but different carbohydrate content. Using the PL-SAX 4000Å 8 µm, 50 x 4.6 mm column, it is possible to purify 3.6 mg of the isoenzymes or 20 mg of total enzyme in under two minutes[1].

Conditions

Column: PL-SAX 4000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1803)
Eluent A: 0.01M Tris HCl, pH 8
Eluent B: A + 0.5M NaCl, pH 8
Gradient: Linear 0-100% B in 2 min
Flow Rate: 4.0 mL/min
Detector: UV, 280nm

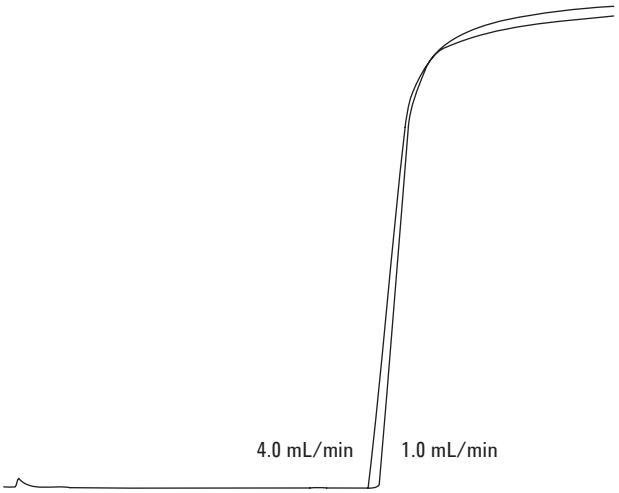

Figure 1. BSA front loading curves on an Agilent PL-SAX 4000Å 8 µm, 50 x 4.6 mm PEEK column.

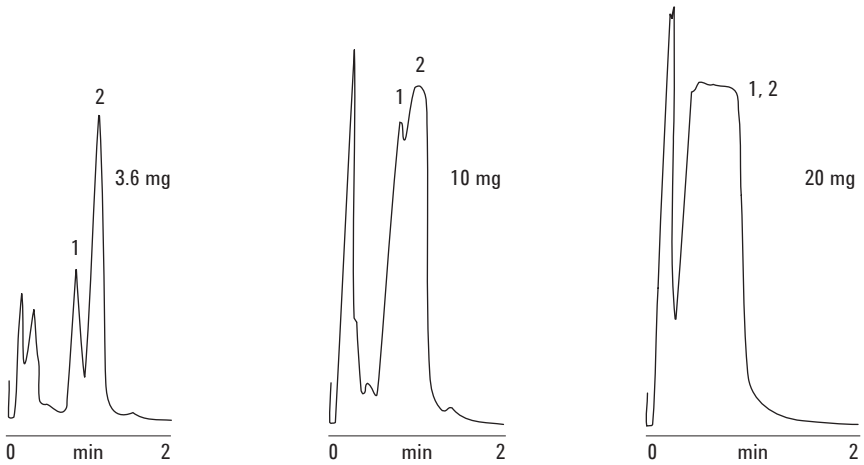

Figure 2. Purification of amyloglucosidase at different enzyme concentrations on Agilent PL-SAX 4000Å.

QC of an HPLC fractionation of amyloglucosidase

An HPLC fractionation of *Aspergillus niger* cell culture filtrate containing amyloglucosidase was quality controlled using the high speed PL-SAX 4000Å column (Figure 4). Greater than 98% purity was obtained for both fractions 1 and 2 when a 20 mg total protein load was used on a 50 x 50 mm column[2] (Figure 3).

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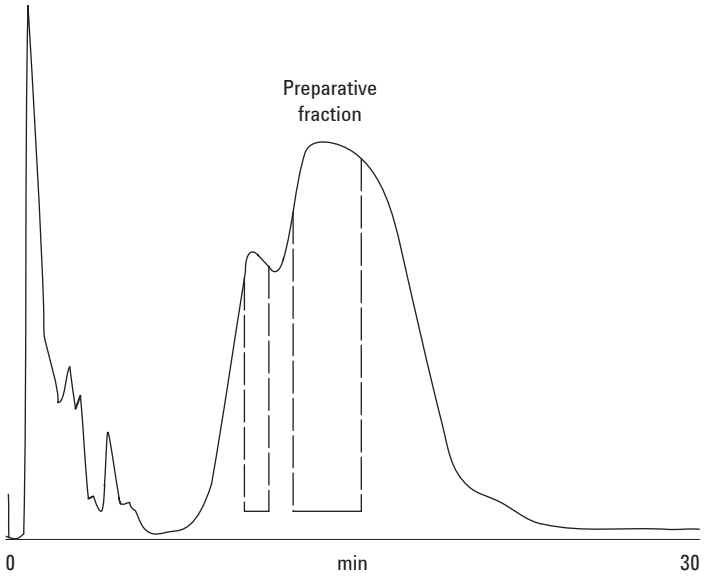

Figure 3. Preparative fractionation of a culture filtrate containing amyloglucosidases on Agilent PL-SAX 4000Å.

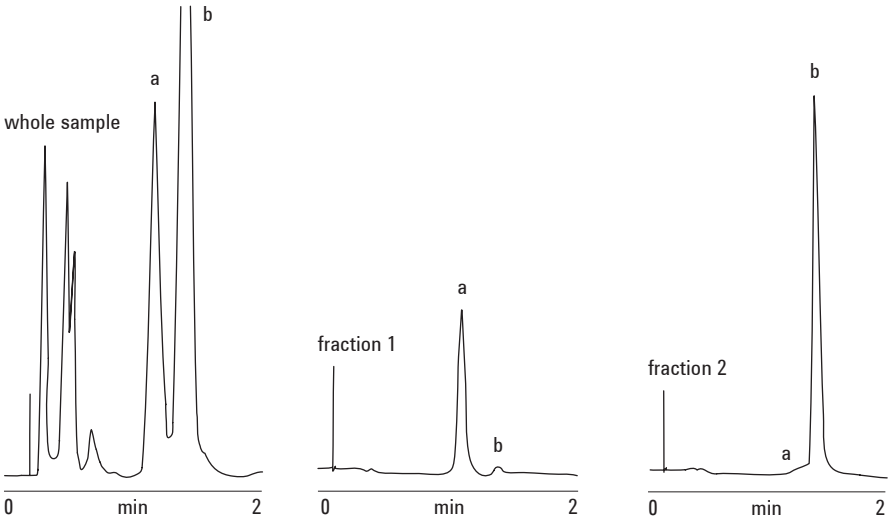

Figure 4. High speed profiles of amyloglucosidase fractions at 20 mg total protein loading on Agilent PL-SAX 4000Å.

References

[1] Linda L. Lloyd and Frank P. Warner (1990) Preparative high performance liquid chromatography on a unique high speed macroporous resin. *J. Chrom.*, 512, 365-376.

[2] Linda L. Lloyd and Frank P. Warner (1991) High speed analytical and preparative separation of biological macromolecules. In: D L Pyle (Ed.) *Separations for Biotechnology.* Elsevier Applied Science.

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