Waters Lab Highlights

An Internal Communication of Applications and Techniques

LAH 0311 2/86
AN,SP/LS/MD/PR/EZ

NO. 564 ISOLATION AND PURIFICATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE FROM YEAST ENZYME CONCENTRATE USING A NEW ION EXCHANGE MEDIA

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The isolation and purification of proteins and enzymes is of central importance in the fields of life science, biochemistry, and biotechnology. Ion exchange chromatography has been widely used as a method for the separation of biologically significant molecules. Traditional methods have been laborious using salt fractionation, electrophoresis, or gel permeation and ion exchange chromatography on soft gels. Waters has recently introduced ACCELL™ media, a new ion exchange material specifically designed for the separation of proteins and enzymes. The media has a base of rigid non-compressible 40 micron silica, coated with a polymeric layer containing either the anion or cation exchange functionality. The rigid nature of the material allows higher flow rates which enable a more extensive methods development to be explored within a greatly reduced time period.

We will present the isolation and purification of glucose-6-phosphate dehydrogenase (G6P-DH) from yeast enzyme concentrate using ACCELL™ QMA anion exchange material packed in glass columns and fitted to an HPLC chromatographic system. The purification and recovery of biological activity were investigated at a loading of 10% of the maximum binding capacity of a 9mm ID X 10cm column. The 10% sample load of 22mg protein was chromatographed in 20 mM Tris pH 8.0 at 1.5 ml/min using a linear 25 minute gradient from 0.00 to 0.38 M NaCl.

The pool of active fractions contained 95% of the units applied and resulted in a 4-fold increase in specific activity. A smaller fraction resulted in a 7-fold increase in specific activity. The fractions from the separation were also assayed by re-chromatography on a gel filtration column and an analytical ion exchange column.

The Accell™ QMA separation was incorporated with other procedures at various positions within a multi-step isolation scheme. Different buffers and pH's were investigated as well as gradient shapes and counter-ions to optimize conditions which best accommodate the purification protocol.

The effect of sample load on resolution was also investigated using a preparation of partially purified G6P-DH. The total binding capacity of the Accell™ QMA material (mg of protein/ml of column volume) was determined using a reduced column volume and the value obtained was used to determine the loading on larger capacity columns.

The G6P-DH was loaded at 1% and 10% of the maximum binding capacity for a 9mm X 16cm column and the resolution and recoveries determined. The 1% loading gave a purification of 6.2-fold for 100% of the units recovered and a 9.6-fold purification for a selectively smaller fraction of 27% of the recovered units. The 10% loading gave a purification of 5.0-fold for 100% of the units recovered and an 8.3-fold purification for a smaller fraction of 29% of the recovered units. The total recovery for both loadings approached 100%. Contamination of a closely eluting peak resulted in a decrease of 15-20% in resolution at the higher loading.

A scale-up to a column with 8 times more capacity was loaded to 8% of the maximum binding capacity. A purification of 3.0-fold was achieved for 100% of the units recovered and a 6.2-fold purification was seen for a smaller fraction of 26% of the recovered units.

An open column configuration was also run using a peristaltic pump and a glass gradient generator. A longer column of 9mm X 22cm dimensions was utilized at a reduced flow rate and extended gradient time. The column was loaded to 10% of its capacity and a higher purification was seen but this was accompanied by a reduction in recovery possibly due to the longer gradient time period at room temperature.

With preparative-type samples, the column can rapidly become contaminated with sample components. The use of brief, short-term acid and base washes resulted in regeneration of the column with little degradation of column performance.