UNO

Use of an UNO Q1 Column Step in the Purification of Phosphoinositide 3-Kinase from Rat Liver.

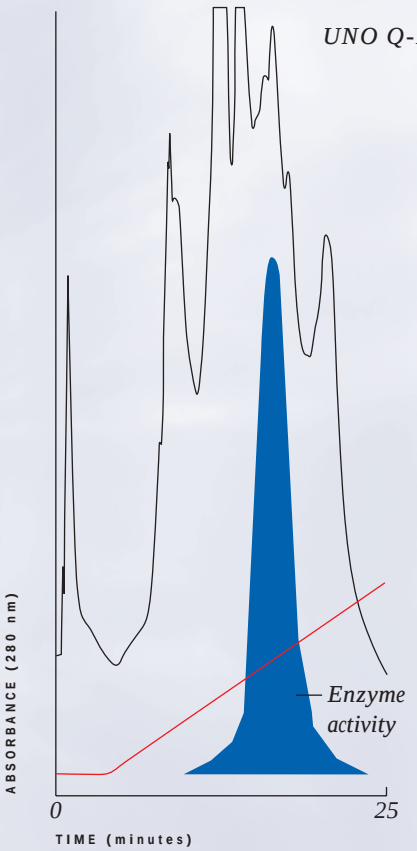
The homogeneous, nonporous, Continuous Bed matrix allows:

- Extremely fast mass transfer of proteins
- Minimal band-broadening during elution
- Unsurpassed resolution, even at high flow rates
- Separation times five-fold faster than conventional beaded columns

Polyphosphoinositides and their metabolites are important intracellular signals involved in the responses to a number of hormones and growth factors. The discovery of a phosphatidylinositol kinase that phosphorylates PI at the 3 position of the inositol ring uncovered a new pathway of PI metabolism and potential intracellular signals.

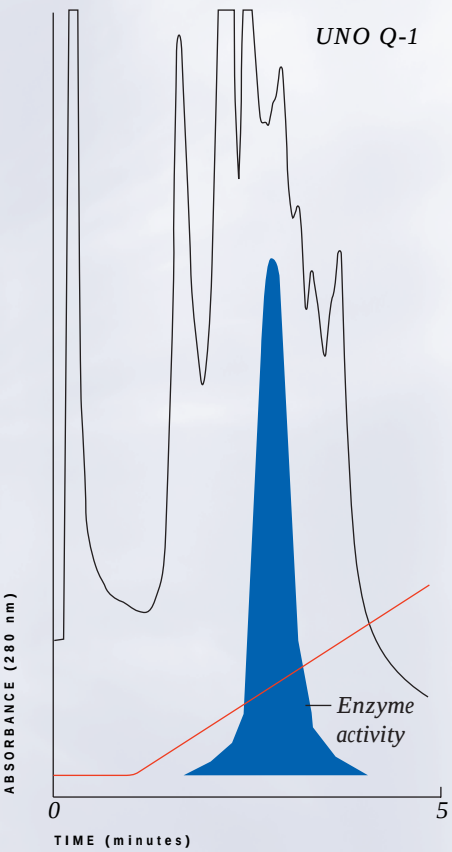
Phosphoinositide 3-kinase has been purified to homogeneity from rat liver and bovine thymus. The enzyme from rat liver is a heterodimer of 110 and 85 kDa proteins. Two forms of the 110 kDa protein exist, and have similar but not identical amino acid sequences. Recent studies have suggested that the 110 kDa protein is the catalytic protein and that the 85 kDa protein plays a regulatory role.

Figure 1. Purification of Phosphoinositide-3-Kinase at 0.5 ml/min.



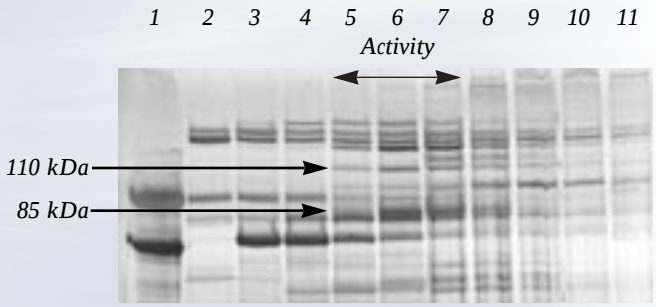
Buffer A: 40 mM TEA, pH 7.6
Buffer B: A + 1 M KCl, pH 7.6
Gradient: 0–35% B in 20 ml
Flow rate: 0.5 ml/min
System: FPLC®

Figure 2. Purification of Phosphoinositide-3-Kinase at 2.5 ml/min.



Buffer A: 40 mM TEA, pH 7.6
Buffer B: A + 1 M KCl, pH 7.6
Gradient: 0–35% B in 20 ml
Flow rate: 2.5 ml/min
System: FPLC®

Figure 3. SDS-PAGE of PI-3K Active Fractions.



Phosphoinositide 3-kinase has proven difficult to purify in an active form when overexpressed in various expression systems. Consequently, for continued biochemical studies of this phosphoinositide pathway, a routine purification protocol for phosphoinositide 3-kinase from rat liver has been established. The protocol involves multiple steps including size exclusion, hydroxyapatite, and ion-exchange chromatography.

The chromatograms (Figures 1 and 2) illustrate the use of an UNO Q1 column to partially purify a cytosolic phosphoinositide 3-kinase from a rat liver homogenate at a flow rate 5-fold higher than previously used with a conventional monodispersed beaded media.

Both the overall resolution and protein activity profile are maintained at the higher flow.

Acknowledgement

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