Rapid purification of GST-tagged proteins from large sample volumes

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Rapid purification of GST-tagged proteins from large sample volumes

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Introduction

The expression of recombinant proteins or domains fused to Glutathione S-transferase (GST) using the pGEX vectors allows for easy purification by affinity chromatography on Glutathione Sepharose™ under mild conditions. The GST-tag can be removed by a site-specific protease either on the column before elution, or after elution. We have developed a chromatography medium, Glutathione Sepharose 4 Fast Flow, for purification of GST-tagged proteins. It has high binding capacity for GST (10 mg/ml medium), and high rigidity makes the medium suitable for large-scale chromatography.

A GST-tagged protein was purified and cleaved by protease on a GSTrap™ FF 5-ml prepacked column to obtain a pure protein without the GST-tag. A second GST-tagged protein, expressed at a low level, was rapidly purified on a 34-ml column by application of a large sample volume at a high flow rate. The high volumetric flow rate allowed preparation to be done four times faster than before.

Summary and conclusions

- One-step purification on GSTrap FF was used to obtain a highly pure SH2 domain. SH2 was expressed as a GST-tagged protein and purified with on-column proteolytic removal of the GST-tag.
- Glutathione Sepharose 4 Fast Flow was used for rapid purification of a GST-tagged glycoprotein (expressed at a low level) by the application of a large sample volume to the column. This procedure offers a realistic alternative to the need to obtain higher expression levels by the optimization of the fermentation process or the preparation of a new gene construct.

Materials and methods

Samples

1. Clarified homogenate of *E. coli* containing the SH2 domain of a phosphatase fused with GST (SH2-GST) (M_r 37 000).
2. Clarified medium from a culture of Human Embryo Kidney cells (HEK293) expressing a M_r 120 000 glycosylated and secreted protein. Preliminary experiments revealed expression levels of 0.5 to 1.5 µg GST-tagged protein per ml of culture medium.

Chromatography

Chromatography was done at room temperature on Glutathione Sepharose 4 Fast Flow using ÄKTAexplorer™ 10 and ÄKTAexplorer 100. GSTrap FF 1-ml and GSTrap FF 5-ml prepacked columns were used for purification and on-column cleavage of SH2-GST.

A 34-ml Glutathione Sepharose 4 Fast Flow medium bed (1.6 x 17 cm) was packed in a XK 16/20 column and used for purification of the low-expressed eukaryotic protein.

On-column cleavage with protease

After sample application and wash, the GSTrap FF 5-ml column was disconnected from the ÄKTAexplorer and filled using a syringe with 7 ml of 20 U/ml thrombin protease from GE Healthcare in binding buffer. The column was reconnected and left overnight (14 hours) at room temperature before elution.

Mass spectrometry analysis

A desalted sample of purified SH2 domain was adjusted to 50% acetonitrile/0.5% trifluoroacetic acid, before mixing with saturated sinapinic acid in 50% acetonitrile/0.5% trifluoroacetic acid, and air drying. Mass spectrometry analyses were performed in linear m/z window on a MALDI-TOF-MS instrument.

Results

Purification and on-column cleavage of an SH2-GST fusion protein

Chromatography of 2 ml of clarified *Escherichia coli* (*E. coli*) homogenate on GSTrap FF 1-ml, for estimation of expression level, yielded about 2 mg of SH2-GST fusion protein (Fig 1A). The eluted material mostly contained SH2-GST, although a small amount of GST was detected (Fig 1B). No detectable contaminants were recovered.

Column: GSTrap FF, 1 ml
Sample: 2 ml clarified *E. coli* homogenate expressing a M_r 37 000 SH2-GST fusion protein
Binding buffer: 150 mM NaCl, 20 mM phospate buffer, pH 7.3
Elution buffer: 20 mM reduced glutathione, 50 mM Tris-HCl, pH 8.0
Flow rate: 2 ml/min (sample application and washing) and 0.5 ml/min (elution)
System: ÄKTAexplorer 10

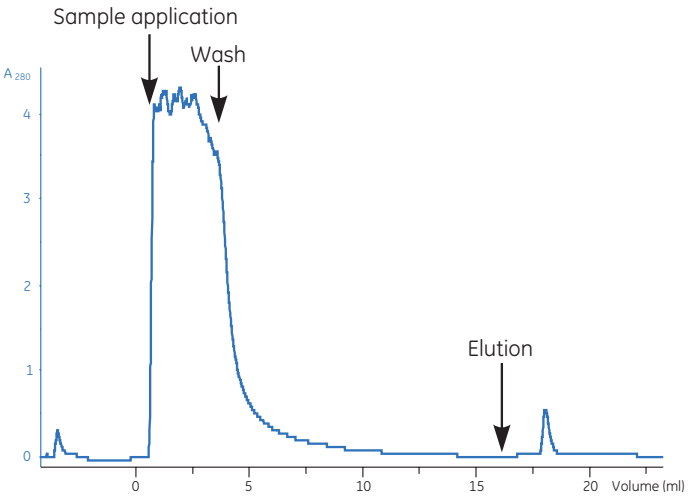
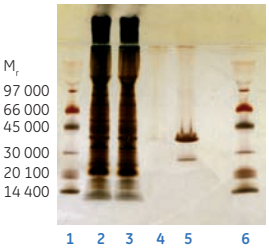


Fig 1A. Purification of SH2 phosphatase domain-GST fusion protein.



Lane 1: Low Molecular Markers
Lane 2: Sample
Lane 3: Flowthrough fraction
Lane 4: Last wash fraction
Lane 5: Eluate
Lane 6: Low Molecular Markers

Fig 1B. SDS-PAGE analysis on PhastGel™ Gradient 8–25, silver staining.

To obtain the pure SH2 domain without its GST-tag, chromatography was scaled-up on GSTrap FF 5 ml, and on-column cleavage was done overnight with thrombin before elution of released SH2 domain with binding buffer (Fig 2A). The eluted SH2 domain fraction contained 2 mg of protein and the GST fraction eluted by glutathione contained 4 mg. SDS-PAGE indicated that the prepared SH2 domain was pure and that the protease cleavage was complete (Fig 2B). Mass spectrometry revealed essentially two peaks corresponding to the single-charged (m/z 12 472) and double-charged protein (m/z 6 241), which agrees with the expected M_r of the SH2 domain (Fig 3). The spectra contained no other peaks in the m/z window used (insert in Fig 3).

Column: GSTrap FF, 5 ml
Sample: 100 ml clarified *E. coli* homogenate expressing a M_r 37 000 SH2-GST fusion protein
Binding buffer: 150 mM NaCl, 20 mM phospate buffer, pH 7.3
Elution buffer: 10 mM reduced glutathione, 50 mM Tris-HCl, pH 8.0
Protease treatment: 20 U/ml thrombin protease (GE Healthcare) for 14 hours at room temperature
Flow rate: 10 ml/min (sample application and washing) and 2.5 ml/min (elution)
System: ÄKTAexplorer 10

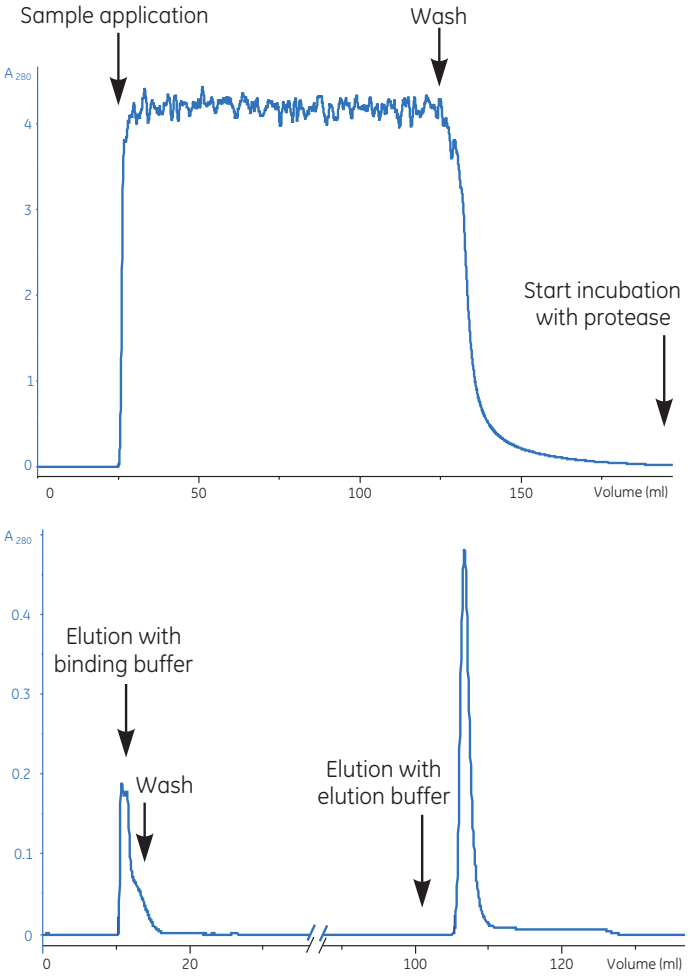
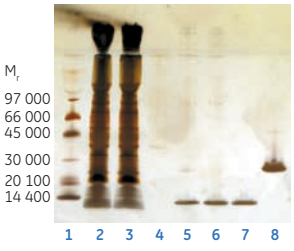


Fig 2A. Purification of SH2 domain with concomitant removal of the GST-tag.



Lane 1: Low Molecular Markers
Lane 2: Sample
Lane 3: Flowthrough fraction
Lane 4: Last wash fraction
Lane 5: Eluate with cleaved-off material eluted with binding buffer, first part of peak
Lane 6: as lane 5, middle part of peak
Lane 7: as lane 5, latter part of peak
Lane 8: Eluate desorbed by elution buffer

Fig 2B. SDS-PAGE analysis on PhastGel Gradient 8–25, silver staining.

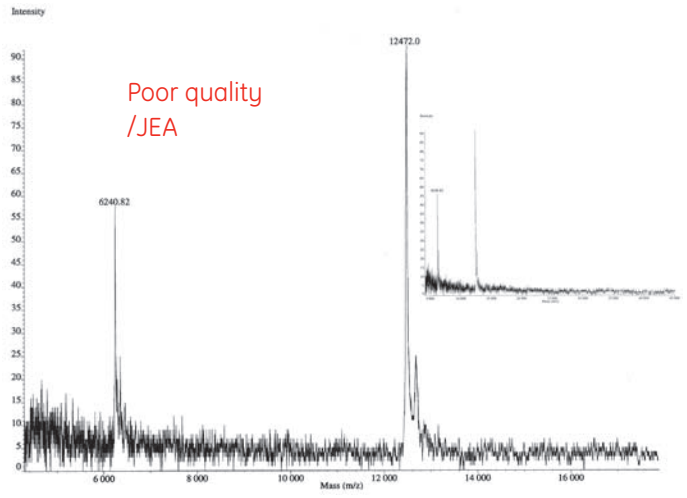


Fig 3. MALDI-TOF-MS analysis of the SH2 domain.

Purification of a eukaryotic GST-tagged protein for crystallography

A large volume (1.5 liters) of clarified cell culture medium from HEK293 cells expressing small amounts of a M_r 120 000 glycosylated and secreted protein was applied to a 34-ml Glutathione Sepharose 4 Fast Flow column. The column was washed with binding buffer and 1 mg of pure protein was eluted by a step-gradient of glutathione (Fig 4), all within five hours. The protein was concentrated by ultrafiltration (cut-off M_r 10 000) and used for successful crystallization trials (Fig 5).

Column: 1.6 x 17 cm Glutathione Sepharose 4 Fast Flow (34 ml) packed in XK 16/20 column
Sample: 1500 ml clarified cell culture medium HEK293 expressing a M_r 120 000 glycosylated and secreted protein
Binding buffer: 140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.3
Elution buffer: 20 mM reduced glutathione, 50 mM NaCl, 50 mM Tris-HCl, pH 8.0
Flow rate: 10 ml/min (sample application and washing) and 1 ml/min (elution)
System: ÄKTAexplorer 100

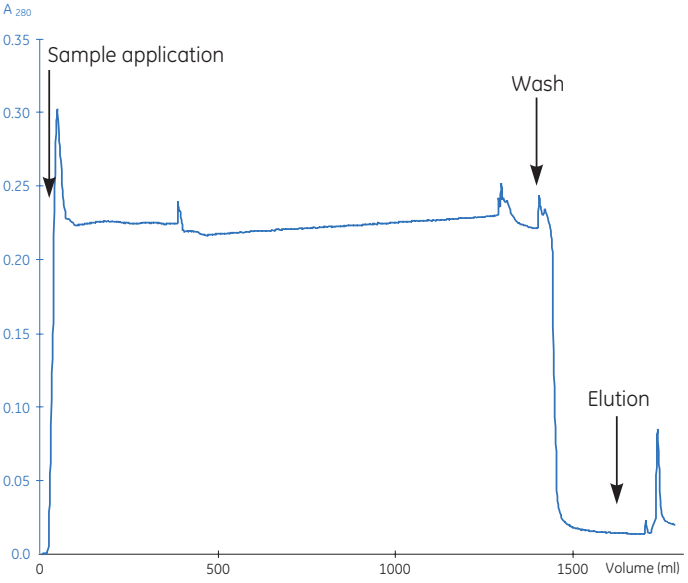
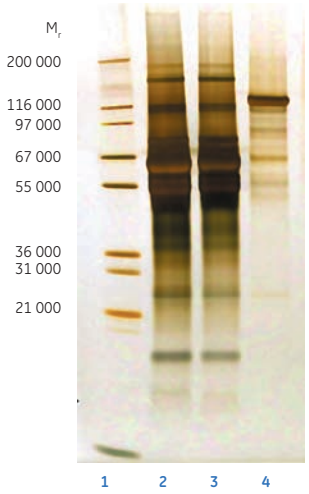


Fig 4A. Purification of low-expressed eukaryotic protein.



Lane 1: Low Molecular Markers
Lane 2: Sample
Lane 3: Flowthrough fraction
Lane 4: Eluate

Fig 4B. SDS-PAGE analysis on 4% to 12% gel followed by silver staining.

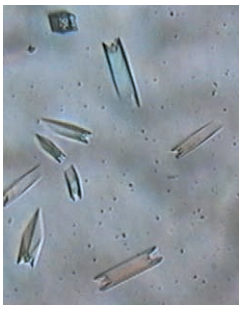


Fig 5. Crystals of the eukaryotic GST-tagged protein obtained in the initial crystallization trial.

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