UNO

Purification of elongation factors, EF-1βγδ and EF-1βγ, from Xenopus Laevis *Oocytes Using UNO Q1 Column*

Incorporating UNO into this purification scheme resulted in:

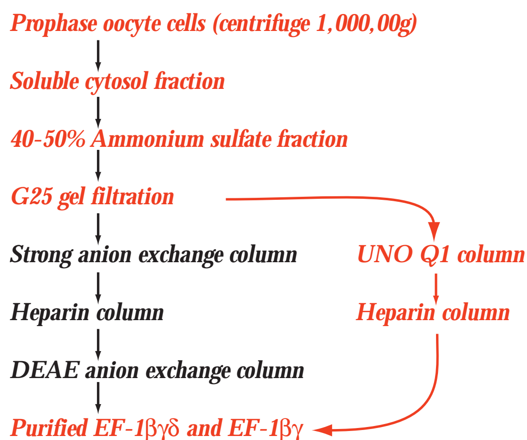
- **Reduction** of the number of purification steps
- **High degree of resolution** achieved at a flow-rate **3x faster** than a conventional beaded column

Introduction

Elongation factor 1(EF-1) mediates the elongation step of mRNA translation. The transfer of aminoacyl-tRNA to ribosomes mediated by hydrolysis of GTP is catalyzed by a GTP binding protein, EF-1. A guanine-nucleotide exchange complex, EF-1βγδ, replaces GDP by GTP on EF-1α. A complex, purified from *Xenopus laevis* oocytes as a substrate for the meiotic and mitotic $p34^{cdc2}$ protein kinase, was shown to contain the guanine-nucleotide exchange activity. This complex is composed of three main proteins, p30, p36, and p47. This application describes the isolation of the EF-1βγδ and EF-1βγ complexes using UNO Q1 column.

Purification

The traditional protocol to purify the EF-1βγδ and EF-1βγ complexes required six steps as outlined below.



The traditional separation protocol involved separation of the gel filtration desalted *Xenopus laevis* prophase oocyte sample on a strong anion exchange column (see Figure 1). The collected fraction contained both the EF-1βγδ (p47, p36 and p30) and EF-1βγδ (p47, p30) complexes, a p40 contaminate and some high molecular weight contaminates (see Figure 2, Mono Q lane). The high molecular weight and p40 contaminates were removed using a Heparin-Sepharose column (see Figure 2, Heparin lane). Fractionation of the heparin fraction on a DEAE column resulted in the separation of the EF-1βγ and EF-1βγδ complexes. (see Figure 2, DEAE lanes 1&3).

Purification of the EF-1βγδ and EF-1βγ complexes using the UNO Q1 column required only five steps. The gel filtration fraction was separated on the UNO Q1 column (see Figure 3) at 4.5 ml/min. This was accomplished at **3x** faster flow rate than that of the traditional strong anion exchange column separation, (1.5 ml/min). Note the high degree of resolution achieved on the UNO Q1 column.

The collected fractions from the UNO Q1 separation were subjected to electrophoretic analysis. The EF-1βγδ complex was resolved into p47, p36 and p30 bands in fraction 16 and the EF-1βγ complex was resolved into p30 and p47 bands in fraction 14 (see Figure 4). The densitometry scan of fraction 16, showed that the p40 contaminate that was present in the Mono Q fraction was not present in the UNO fraction (data not shown). The high molecular weight contaminants in fraction 14 and 16 were removed using a heparin column (data not shown).

Incorporating the UNO Q1 column into the purification scheme of elongation factors from *Xenopus laevis* prophase oocytes resulted in improved purification and a reduction of the number of purification steps. This was achieved using an UNO Q1 high resolution separation at 4.5 ml/min.

References:

Mulner-Lorillon O. et al., (1989), *FEBS Letters*, **251**, 219-224.

Janssen, G. et al, (1991), *J. Biol. Chem*, **266**, 23, 14885-14888.

Mulner-Lorillon O. et al., (1994), *J. Biol. Chem*, **269**, 31, 20201-20207.

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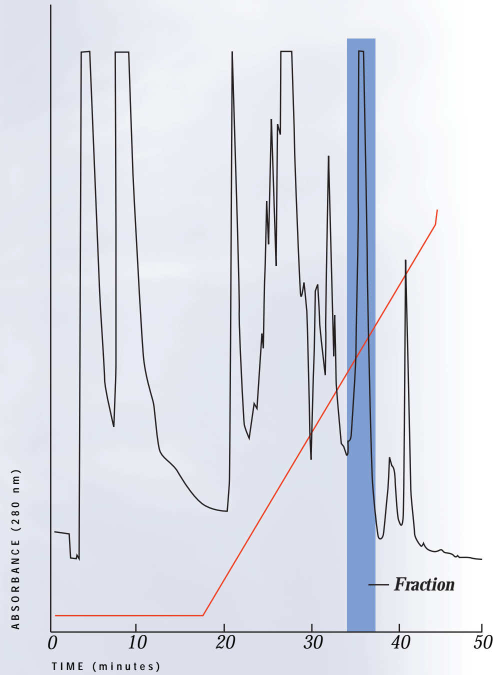
Figure 1. $(NH_4)_2SO_4$ Precipitate Fractionation of desalted *Xenopus laevis* oocytes on Strong Anion Exchanger.



Protein load: 12 mg; Buffer A: 50 mM Tris, 1 mM DTT, 2 mM EDTA, pH 7.4; Buffer B: A + 1 M NaCl, Gradient: 0-70%B in 40 ml; Flow Rate: 1.5 ml/min System: FPLC®

Figure 2. SDS-PAGE of Mono Q, Heparin Sepharose, and DEAE column fractions containing EF-1βγ and EF-1βγδ.

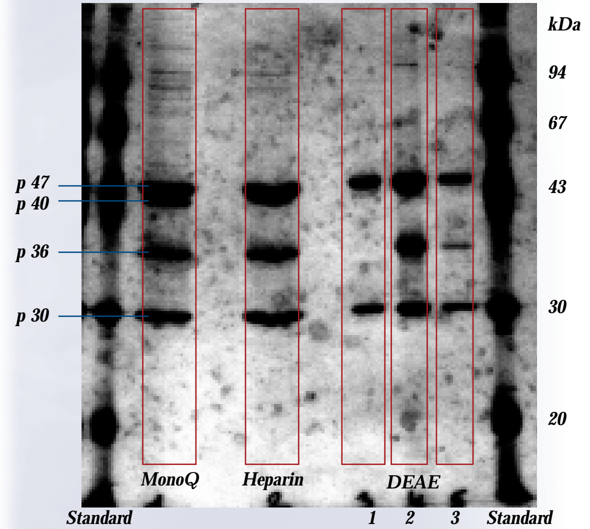
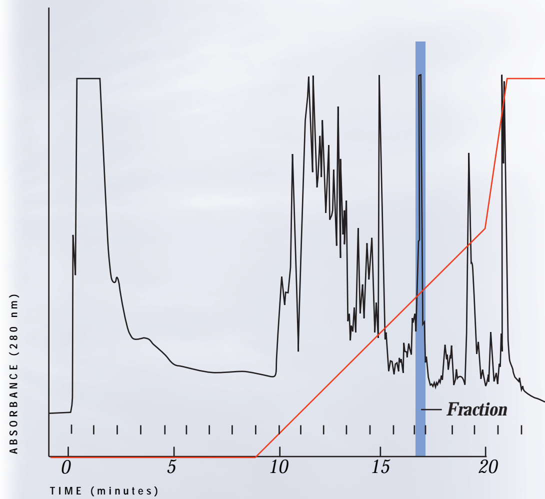
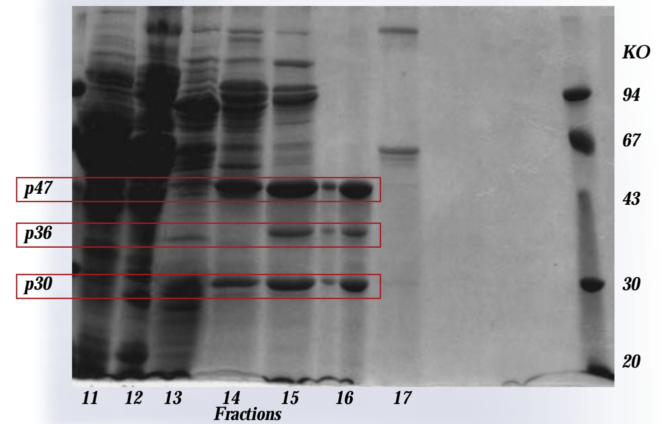


Figure 3. Fractionation of desalted *Xenopus laevis* oocytes extract on UNO Q1.



Protein load: 6 mg; Buffer A: 50 mM Tris, 1mM DTT, 2 mM EDTA, pH 7.4; Buffer B: A + 1 M NaCl, Gradient: 0-60%B in 50 ml; Flow Rate: 4.5 ml/min; System: FPLC®

Figure 4. SDS-PAGE of UNO Q1 Fractions.



Data courtesy of R. Poulhe, Laboratoire de la reproduction, CNRS, Université Pierre et Marie Curie, Paris, France.

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