Production and Purification of PIAS1

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Objective:

Huntington's Disease (HD) is a hereditary neurodegenerative disease. The cause of this disease is a CAG repeat extension in the HTT Gene. This extension is translated into an elongation of exon 1 in Huntingtin protein. Although we know the cause, the mechanism of this disease still eludes us. A part of the difficulty is our lack of understanding of the role of normal HTT in our cells. We at the SGC have set out to explore the HTT interactome to further our understanding of the role of HTT. This will give us a better basis for further study of mutant Huntingtin. Our first step on this journey was to conduct a literature review alongside a BioID experiment to come up with a list of putative huntingtin interaction partners. One of the hits on the list was PIAS1. This means that we must obtain a pure sample of PIAS1 for use in future experiments to further validate the claim that these proteins interact. Previously, I purified a shorter construct of PIAS1 in *E. coli*: 10.5281/zenodo.3367282. At the SGC we have access to many different constructs of the same protein. The previously purified product was only a portion of the full protein. For physiological relevance, it is important to test full length proteins so we decided to order another purification of PIAS1, and this time try to purify the whole protein.

Methods & Results:

Construct:

Construct ID	Tag	Sequence	Mol. Weight kDa	PI
TOC016:E03	MHHHHHH EFMDEKTT GWRGGHV VEGLAGEL EQLRARLE HHPQGQRE PSSGRENL YFQG	MADSAELKQMVMSLRVSELQVLLGYAGRNKHGRKHE LLTKALHLLKAGCSPAVQMKIKELYRRRFPQKIMTPAD LSIPNVHSSPMPATLSPSTIPQLTYDGHPASSPLLPVSLL GPKHELELPHLTSALHPVHPDIKLQKLPFYDLLDELIKP TSLASDNSQRFRETCFAFAL TPQQVQQISS SMDISGTK CDFTVQVQLRFCLSETSCPQEDHFPPNLCVKVNTKPCSL PGYLPPTKNGVEPKRPSRPINITSLVRLSTTVPNTIVVSW TAEIGRNYSMAVYLVKQLSSTVLLQRLRAKGIRNPDHS RALIKEKLTADPDSEIATTSLRVSLLCPLGKMRLTIPCR ALTCSHLQCFDATLYIQMNEKKPTWVC PVCDKKAP YEHLIIDGLFMEILKYCTDCDEIQFKEDGTWAPMRSKK EVQEVSASYNGVDGCLSSTLEHQVASHHQSSNKNKKV EVIDLTIDSSSDEEEEEPSAKRTCPSLSPTSPLNNKGILSL PHQASPVSRTPSLPAVDTSYINTSLIQDYRHPFHMTPMP YDLQGLDFFPFLSGDNQHYNTSLLAAAAAAVSDDQDL LHSSRFFPYTSSQMFLDQLSAGGSTSLPTTNGSSS GSNS SLVSSNSLRESHSHTVTNRSSTDTASIFGIIPDIISLD	71.8 (without tag) 78.5 (with tag)	6.9 (without tag) 6.7 (with tag)

Expression/ Growth:

The construct described above was grown if Sf9 cells. 4 L of culture was provided to me by Ashley.

Harvest:

The cell culture was spun down at 4500 rpm for 15 minutes in the Beckman JLA 8.1000. Cells were collected and resuspended in ~ 160 mL of lysis buffer [20 mM Tris pH 8(at 4°C), 300 mM NaCl, 1 mM TCEP, 5% Glycerol], 1:100 dilution of protease inhibitors were added to the resuspended cell pellet. These cells were then flash frozen and stored at -80°C. This process of freezing is sufficient to lyse the cells, so clarification of lysate can begin as soon as cells are thawed.

Lysate Clarification:

The cell pellet was thawed and diluted with lysis buffer to a final volume of 400 mL. This was followed by the clarification of the lysate via centrifugation (Beckman JLA 16.250) at 15000 rpm for 1 hour.

Nickel Purification:

Clarified lysate was Rocked with 5 mL of NiNTA resin for 25 minutes; the slurry was then spun down on a benchtop centrifuge at 1000 rpm for 5 min. The supernatant was decanted off the resin and collected (Ni Flow Though). The resin was then resuspended and washed in an open column (BioRad) with 250 mL wash buffer (lysis buffer + 15 mM Imidazole) (Wash). Beads eluted with elution buffer (lysis buffer + 300 mM Imidazole) (Nickel Elution). The elusion was then placed in 1.5L of dialysis buffer with 5 mg of TEV for 16 hours at 4°C.

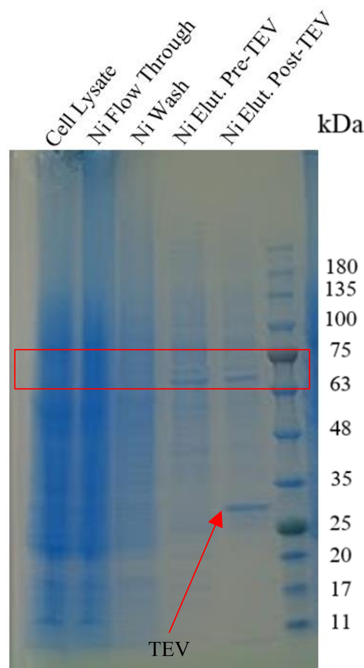

Figure 1. SDS Page, Tris-Glycine 4-20% Gel. Analysis of Steps of Nickel Purification.

Discussion:

The SDS Page does not show any protein at the appropriate molecular weight; on top of this, the NiNTA resin seems to be binding indiscriminately. Both of these facts point toward to idea that PIAS1 was not adequately expressed in Sf9 cells. The problem could have arisen for many reasons, but it seems likely that either there was some issue with Sf9 production resulting in no expression of PIAS1 or PIAS1 may be incompatible with this expression system, limiting its expression. *E. coli* production of the same construct seemed to work to a much larger degree so I would think that going forward, E. coli expression would we the preferred method.