CO-EXPRESSION OF HUMAN WDR1 GENE WITH A CHAPERONE INCREASES ITS PROTEIN SOLUBILITY

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Human WDR1 (tryptophan-aspartate repeat containing protein 1) is a cytoplasmic protein involved in actin cytoskeleton regulation. It is the vertebrate homolog of yeast Aip1 and was first identified in chickens (1). WDR1 contains nine WD repeats of 30 to 40 amino acids, and these WD domains are recognized as domains which interact most in eukaryotes (2, 3). Here, we report the co-expression of human *wdr1* gene with tig chaperone, which is known to assist protein folding (4). Protein solubility was significantly increased in the presence of chaperone. The identity of the purified protein has been confirmed by mass spectrometry and western blot. WDR1 obtained in this way can be further used for different *in vitro* studies.

Key words: actin-interacting protein 1, prokaryotic expression, *trigger* factor chaperone, mass spectrometry.

INTRODUCTION

Aip1 protein, also known as WDR1 (WD-repeat containing protein 1) in mammals, was discovered in yeast as an actin interacting protein (5). Intensive studies revealed that this protein is involved in disassembly of actin filaments in conjunction with ADF/cofilin family of proteins (6-9). Aip1 orthologs have been identified in a large number of eukaryotic species, having 20 to 95% sequence identity (10). Human WDR1 protein contains 10 tryptophan-aspartate repeats, WD domains, which are found in a large number of proteins (2, 3, 11) and function as protein-protein or protein-DNA interaction platform (2, 11). So far, no WD domain was found to have enzymatic activity (2).

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The main function of WDR1 reported so far is to bind cofilin/actin complex, and thus to enhance cofilin's capacity to sever actin filaments (7-9, 12). In chickens, WDR1 was found over-expressed in auditory epithelium of the inner ear upon noise damage (1, 10). This epithelium has the ability to regenerate the damaged tissues restoring hearing capability, suggesting that WDR1 is involved in cytoskeletal reorganization during regeneration (1, 10). The mRNA of WDR1 was found to be downregulated in oligodendrocytes differentiation and in central nervous system myelination, suggesting a potential neuronal function (13). Since 2003 there are over 600 records registered on PhosphoSitePlus web site, regarding the tyrosine phosphorylation of human WDR1 protein (http://www.phosphosite.org (14). Phosphorylated WDR1 was identified in many diseases by mass spectrometry, like leukemia, lung, liver and gastric cancer; however, none of the potential phosphorylation sites are documented by other experimental methods (*i.e.*, *in vitro* phosphorylation, immunoprecipitation, western blot). Toward *in vitro* analyses of this protein, we first need to obtain the protein. Despite the important role played by WDR1, in both physiological and phathological conditions, very few studies have been performed *in vitro* (7-10). Lack of reports for prokaryotic expression of WDR1 could be an important reason in this respect.

The most common technique used in order to obtain proteins for *in vitro* studies is represented by the gene expression in a prokaryotic system. Frequently, recombinant proteins obtained are found in the soluble fractions, thus facilitating the purification steps. In some situations, the cellular stress encountered by the host cells makes impossible to obtain soluble recombinant proteins, leading to their accumulation in inclusion bodies (15). To overcome this problem, different expression systems can be used, like yeast systems, insect cell system or eukaryotic cell system, each of them being more expensive than the prokaryotic one. To obtain more soluble proteins and also taking advantage of the cheap prokaryotic expression system, different strategies have been developed. One of them is the co-expression of the target proteins with molecular chaperones (16). Molecular chaperones assist the folding of newly synthesized proteins, preventing aggregation and degradation of proteins (17, 18).

Trigger (tig, peptidyl-prolyl *cis-trans* isomerase) factor was discovered in 1995 (19), and since then, a large set of experimental data were reported on the function of this chaperone. It is known to assist folding by protecting nascent chains with long hydrophobic stretches during synthesis and by acceleration of *cis/trans* isomerization (4, 19, 20).

Here, we report the co-expression of human *wdr1 gene* with trigger chaperone in *E. coli* BL21 strain. Taking the advantage of this system we could obtain soluble WDR1 protein. The identity of affinity purified protein was proven by western blot and mass fingerprintig analysis. Purified protein, thus obtained, can be further used for *in vitro* functional studies.

MATERIALS AND METHODS

Cloning of WDR1 sequence

The full human WDR1 sequence (pEYFP-Aip1/WDR1) was a kind gift from Prof. K. Mizuno (6). The sequence of WDR1 was amplified by PCR and subcloned into the pGEX-6P1 (GE Healthcare) a prokaryotic expression vector at the *Bam*H1 and *Xho*I sites. This vector allows both to obtain the fusion protein with Gluthation S-transferase tag, and also the untagged protein. A PreScission recognition sequence is inserted between the tag and the protein of interest. Primer sequences used for PCR are as follows: Forward primer 5' CGGGATCCATGCCGTACGAGATCAAGAAGGTG and Reverse primer 5' CCGCTCGAGTCAGTAGGTGATTGTCCACTCCTTGACAG**.**

Expression of *wdr1* gene

Bacterial strain – The expression of *wdr1* gene was carried out either in *E. coli* BL21 competent cells alone or in *E. coli* BL21 cells made competent together with pTf16 plasmid which encodes for *tig* factor ((21) and TAKARA BIO INC, cat. no. 3340), which acts as chaperone (46kDa). pTf16 plasmid was a kind gift from Dr. M. Ciubotaru (Yale University).

Media and culture conditions – Luria Broth media was used for expression. BL21 cells harboring for the expression vector of *wdr1* gene alone were grown in medium containing 100 mg/mL of ampicillin and the BL21 cells harboring for the two expression plasmids (one for the WDR1 recombinant protein and the other for *tig* chaperone) were grown in medium containing 50 mg/mL of ampicillin and 20 mg/mL of chloramphenicol. To induce chaperone expression, 0.5 mg/mL L-arabinose was used at an optical density (OD_{600}) of 0.5 to 0.6. The expression of the recombinant protein was induced using 0.3mM isopropylthio-β-galactoside (IPTG) when OD_{600} of cells reached 0.9 to 1.

Purification of WDR1 protein

The obtained *E. coli* cells were harvested by centrifugation (5000 x g, 10 min, and 4°C) and pellets were resuspended in Phosphate Buffer Saline (PBS) supplemented with 0.1% Triton x-100, 10% glycerol, 1mM PMSF and 1x cocktail of protease inhibitors. Cell disruption was realized by sonication (10 pulses of 10 sec, each at 70% power) with a Bandelin Sonicator. Following sonication the cell debris were separated from the soluble fraction by centrifugation (20.000 x g, 45 min and 4°C). The obtained cell extract containing the GST-tagged WDR1 protein was incubated for 1h at 4°C with Glutathione-Sepharose 4B beads (GE Healthcare), and further on, the GST-fusion proteins were either eluted with 10 mM reduced glutathione or were subjected to PreScission protease cleavage over night at 4°C . The obtained proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 7 or 10% polyacrylamide gels.

Western blot

Small aliquots of Glutathione-Sepharose 4B beads with GST-WDR1 before and after PreScission cleavage, and the supernatant after protease cleavage were first separated by SDS-PAGE and then transferred to Immobilon P membrane (Millipore) using a semidry transfer system. The western blot was performed using the SNAP I.D. system (Millipore) following the manufacture instruction. Goat anti-human WDR1 antibody (G13, Santa Cruz Biotechnology) was used to evidence the obtained protein.

Mass spectrometry

Protein trypsinization and peptide extraction. In gel digestion was performed according to previous published protocols with minor differences (22, 23). Briefly, the gel bands containing the proteins of interest were excised, washed for 5 minutes with a solution containing 50 mM ammonium bicarbonate with 50% acetonitrile and then with 100% acetonitrile, at room temperature. The gel pieces were subject to reduction with a solution containing 10 mM DTT in 100 mM ammonium bicarbonate by incubation at 56 ^{o}C for 45 minutes. The washing procedures were repeated once again using the same solutions, described earlier and then the proteins were alkylated using a solution of 100 mM iodacetamide in 100 mM ammonium bicarbonate in the dark at room temperature. After repeating the initial washing steps, the gel pieces were dried in a speed-vac for 15 minutes. Trypsin digestion was performed overnight at 37 ^{o}C using a solution of 10 ng/µl trypsin in 40 mM ammonium bicarbonate. The peptides were extracted in a two step procedure, first using a solution of 50 mM ammonium bicarbonate in 50% acetonitrile with 5% formic acid (twice) and then using a 5% formic acid solution in acetonitrile. The resulting fractions were combined and then dried in a speed-vac concentrator. The peptides were kept at -20 ^{o}C until further use.

LC-MS/MS analysis. Before injection, the peptides were solubilized in ~20µL 0.1% formic acid solution and placed in nano-liquid chromatograph (nLC) vials. The peptides were injected into a nLC-MS system coupled online with a hybrid LTQ-Orbitrap mass spectrometer. The chromatographic system was composed of a desalting C18 (2 cm, ID 100 µm, 5 µ) precolumn coupled with an analytical C18 column (10 cm, ID 75 µm, 3 µ) for peptide separation. A 90 minute gradient of 2-45% organic solvent (0.1% formic acid in acetonitrile) with a 300 nL/min flow was applied for the elution of the peptides. The mobile phase was 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The eluted peptides were injected into the mass spectrometer using a nanoFLEX II ESI source provided with a metal coated emitter coupled online with the chromatographic system. The acquisition method involved an initial survey MS scan between 300 and 1800 m/z at a resolution of 30000 at m/z 400, followed by a data-dependent analysis of the 20 most intense peaks from the survey scan with +2, +3, and +4 charge using a CID

fragmentation method. Before injections external calibration of the mass spectrometer was performed using the LTQ ESI Positive Calibration Solution from Thermo Fisher Scientific. To increase the mass accuracy in the initial MS1 survey scan, also the lock mass option was enabled. Dynamic exclusion was enabled with a repeat count of 1, exclusion duration of 60 s and exclusion relative to reference mass of 10 ppm.

Protein identification. Spectrum files acquired over the entire run were searched using the SEQUEST algorithm in Proteome Discoverer v1.3 with the following parameters: database UniprotKB/Swiss-Prot 2012, precursor mass tolerance of 20 ppm, fragment mass tolerance of 0.6 Da, enzyme used: trypsin with maximum of two missed cleavage (full tryptic), carbamidomethylation on cysteine residues as a static side chain modification, methionine oxidation, as a dynamic modification. To eliminate the false positive results also a decoy data-base search (reversed sequences) was performed in parallel using a target FDR of 1%. To assess a positive identification of the proteins at least 2 unique peptides were required to be present with a 99% confidence of identification. At least two consecutive runs were searched from the same sample.

RESULTS

Prokaryotic expression of human wdr1 gene and protein purification

wdr1 gene was cloned in pGEX-6P1 a prokaryotic expression vector, which enables the expression of WDR1 recombinant protein fused with Glutathione S-Transferase tag. The *wdr1* gene expression was tested in *E. coli* BL21 strain at different temperatures and different IPTG concentrations. The cellular extracts containing the recombinant protein GST-WDR1 (~93kDa) were separated on standard SDS-PAGE gels and visualized by Coomassie brilliant-blue staining. Fig. 1A presents the over-expression level of *wdr1* gene achieved at either 30°C or 37°C. The expression of *wdr1* gene was considerably improved by decreasing the expression temperature at 18°C (Fig. 1A). The effect of the inducer concentration was also tested, the optimal concentration being 0.3 mM IPTG independent of temperature.

In the following step, we purified the recombinant GST-WDR1 protein by affinity chromatography on Glutathione-Sepharose 4B resin. In principle, the soluble recombinant proteins should be attached through their GST-tag to the Glutathione Sepharose resin. When examining the total recombinant protein bound to resin, we observed that only a small fraction from our protein of interest was bound, suggesting that the main part of GST-WDR1 was insoluble (Fig. 1B). This result suggests that the major part of WDR1 protein was in inclusion bodies, even if the expression was conducted at a lower temperature (which is known to improve protein solubility).

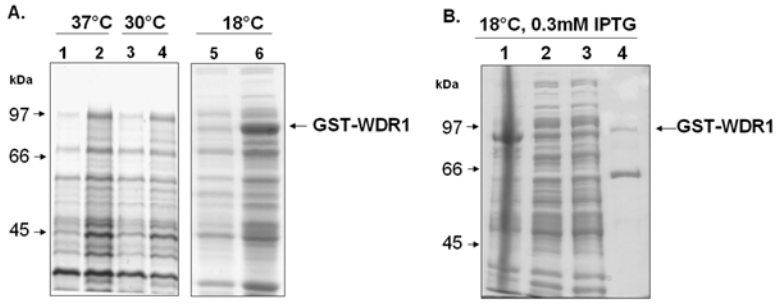


Fig. 1. – SDS-PAGE analysis of *wdr1* gene expression in *E. coli* BL21 cells and WDR1 protein purification. **A.** The expression was tested at three different temperatures (37, 30 and 18 °C) with 0.3 mM IPTG. Lanes 1, 3, 5: Samples were taken before adding the inducer; Lanes 2, 4, 6: Samples were taken at the end of induction time (4 h for 37 °C, 6 h for 30 °C and 16h for 18 °C). **B.** Purification of recombinant WDR1 fusion protein. Lanes: 1 – total cell lysate containing GST-WDR1 protein; 2 – supernatant of cell lysate of recombinant WDR1 protein; 3 – supernatant of cell lysate after incubation with Glutathione-Sepharose 4B resin; 4 – recombinant GST-protein bound on Glutathione-Sepharose 4B resin. Protein bands were visualized by Coomassie brilliant-blue staining.

Co-expression of wdr1 gene with tig chaperone increases protein solubility

Further on, we tried to optimize the expression of *wdr1* gene in order to increase protein solubility. To this purpose, we set up a different expression method based on co-expression with chaperones. It is well known that molecular chaperones assist proteins in folding and are also involved in preventing aggregation of folding intermediates (24). First, we co-expressed *wdr1* gene with pG-Tf2 plasmid (24) (Takara Bio Inc) which contains the genes of three chaperons - *groES* (10kDa), *groEL* (60kDa) and *trigger* factor (47kDa). Unexpectedly, the solubility of GST-WDR1 protein was not improved significantly (data not shown). Then, we tested the co-expression with pTF16 plasmid (Takara Bio Inc) (21) which caries only the gene expressing the *trigger* factor. During protein over-expression, first the *trigger* factor was induced using arabinose as inducer, and then later the *wdr1* gene was also induced, using IPTG as inducer. Overnight expression of *wdr1* gene did not considerably improve the production of WDR1 protein as revealed by SDS-PAGE of cell free extracts (Fig. 2A). However, analysing the affinity purified protein we showed that the amount of GST-WDR1 protein bound on resin was at least twice higher (Fig. 2B), as compared to the amount of protein bound in the absence of the chaperone, suggesting that the co-expression with *tig* chaperone increases the solubility of the GST-WDR1 fusion protein.

Human WDR1 protein is very unstable and co-purifies with chaperones

Next, we wanted to remove the GST tag in order to obtain untagged WDR1 protein. To this goal, we incubated the recombinant GST-WDR1 fusion protein bound on media with PreScission protease (the recognition sequence for this protease is present between the GST tag and the protein of interest) and we

obtained the WDR1 protein, as it can be seen both by Commassie brilliant staining (Fig. 2C) and western blot (Fig. 2D).

Carefully analysing the results obtained, we observed that after tag removal, the WDR1 protein was found in a small part in solution, and the most of it precipitated on the Glutathione-Sepharose beads (Figs. 2C and D).

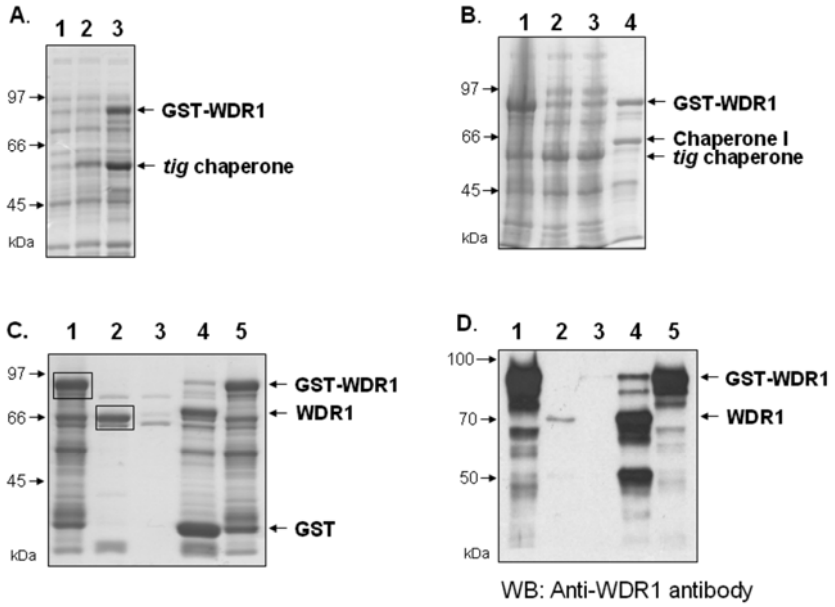


Fig. 2. – SDS-PAGE analysis of *wdr1* gene expression in *E.coli* BL21 cells and WDR1 protein purification. **A.** The expression was tested at three different temperatures (37, 30 and 18 °C) with 0.3 mM IPTG. Lanes 1, 3, 5: Samples were taken before adding the inducer; Lanes 2, 4, 6: Samples were taken at the end of induction time (4 h for 37 °C, 6 h for 30 °C and 16 h for 18 °C). **B.** Purification of recombinant WDR1 fusion protein. Lanes: 1 – total cell lysate containing GST-WDR1 protein; 2 – supernatant of cell lysate of recombinant WDR1 protein; 3 – supernatant of cell lysate after incubation with Glutathione-Sepharose 4B resin; 4 – recombinant GST-protein bound on Glutathione-Sepharose 4B resin. Protein bands were visualized by Coomassie brilliant-blue staining.

Then, in order to prove the identity of the recombinant WDR1 protein, thus obtained (Fig. 2C), we used mass spectrometry analysis. WDR1 protein as such or fused with GST were analyzed to assess the identity of the proteins, bands from the SDS–PAGE gel were excised, digested with trypsin and analyzed using reverse-phased liquid chromatography coupled with tandem mass spectrometry. To validate the results, two biological replicates were analyzed and two injections from each sample were performed (technical replicates). The results consisted of both biological and technical replicates. Table 1 lists the proteins identified with 99% confidence with the SEQUEST algorithm after removing contaminants like keratins or bovine serum albumin (BSA).

Table 1

Proteins identified by mass spectrometry

A. Identified proteins when GST-WDR1 fusion protein was analyzed

Accession	Description	ΣCoverage	Σ# Unique Peptides	Pfam IDs	# AAs	MW [kDa]	Calc. pI
A1AJ51	60 kDa chaperonin 1 OS=*Escherichia coli* O1:K1 / APEC GN=groL1 PE=3 SV=1 – [CH601_ECOK1]	36.31	10	Pf00118	548	57.3	4.94
P08515	Glutathione S-transferase class-mu 26 kDa isozyme OS=*Schistosoma japonicum* PE=1 SV=3 - [GST26_SCHJA]	32.57	9	Pf00043 ; Pf02798	218	25.5	6.54
O75083	WD repeat-containing protein 1 OS=*Homo sapiens* GN=WDR1 PE=1 SV=4 - [WDR1_HUMAN]	47.69	9	Pf00400 ; Pf04762 ; Pf08662	606	66.2	6.65

B. Identified proteins when WDR1 protein was analyzed

Accession	Description	ΣCoverage	Σ# Unique Peptides	Pfam IDs	# AAs	MW [kDa]	Calc. pI
A1AJ51	60 kDa chaperonin 1 OS=*Escherichia coli* O1:K1 / APEC GN=groL1 PE=3 SV=1 - [CH601_ECOK1]	82.12	6	Pf00118	548	57.3	4.94
B7MD95	Trigger factor OS=*Escherichia coli* O45:K1 (strain S88 / ExPEC) GN=tig PE=3 SV=1 - [TIG_ECO45]	41.26	13	Pf00254; Pf05697; Pf05698	429	47.8	4.88
O75083	WD repeat-containing protein 1 OS=*Homo sapiens* GN=WDR1 PE=1 SV=4 - [WDR1_HUMAN]	33.50	10	Pf00400; Pf04762; Pf08662	606	66.2	6.65

Human WDR1 was successfully identified in all samples, before and after the digestion with PreScission protease, with 9 unique peptides with an average mass accuracy under 3 ppm for peptide the precursors. The identified peptides provide good sequence coverage, their localization covering the full sequence of the protein. (Fig. 3A). In the samples analyzed before the incubation with protease, the GST from *S. japonicum* was also identified providing a good sequence coverage. Figure 3B displays the extracted ion chromatograms for some of the peptides identified from WDR1 protein. MS/MS spectra corresponding to peptides from WDR1 and GST are shown in Figure 3C. Besides the expected proteins, LC-MS/MS analysis revealed the presence of trigger chaperone. The band corresponding to the end cleavage product of PreScission protease also contained, in high amounts (indicated by the large number of peptide spectrum matches – 618 and the high score) the 60 kDa chaperonin 1, that could be involved in the folding of the protein after its release from the GST tag.

A.

O75083 WD repeat-containing protein 1 OS=Homo sapiens

MPYEIKKVFA SLPQVERGVS KIIGGDPKGN NFLYTNGKCV ILRNIDNPAL ADIYTEHAHQ VVVAKYAPSG FYIASGDVSG KLRIWDTTQK
EHLLKYEYQP FAGKIKDIAW TEDSKRIAVV GEGREKFGAV FLWDSGSSVG EITGHNKVIN SVDIKQSRPY RLATGSDDNC AAFFEGPPFK
FKFTIGDHSR FVNCVRFSPD GNRFATASAD GQIYIYDGKT GEKVCALGGS KAHDGGIYAI SWSPDSTHLL SASGDKTSKI WDVSVNSVVS
TFPMGSTVLD QQLGCLWQKD HLLSVSLSGY INYLDRNNPS KPLHVIKGHS KSIQCLTVHK NGGKSYIYSG SHDGHINYWD SETGENDSFA
GKGHTNQVSR MTVDESGQLI SCSMDDTVRY TSLMLRDYSG QGVVKLDVQP KCVAVGPGGY AVVVCIGQIV LLKDQRKCFS IDNPGYEPEV
VAVHPGGDTV AIGGVDGNVR LYSILGTTLK DEGKLLEAKG PVTDVAYSHD GAFLAVCDAS KVVTVFSVAD GYSENNVFYG HHAKIVCLAW
SPDNEHFASG GMDMMVYVWT LSDPETRVKI QDAHRLHHVS SLAWLDEHTL VTTSHDASVK
EWTITY

P08515 Glutathione S-transferase class mu 26 kDa isozyme OS=Schistosoma japonicum,

MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA
EISMLEGAVL DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPK

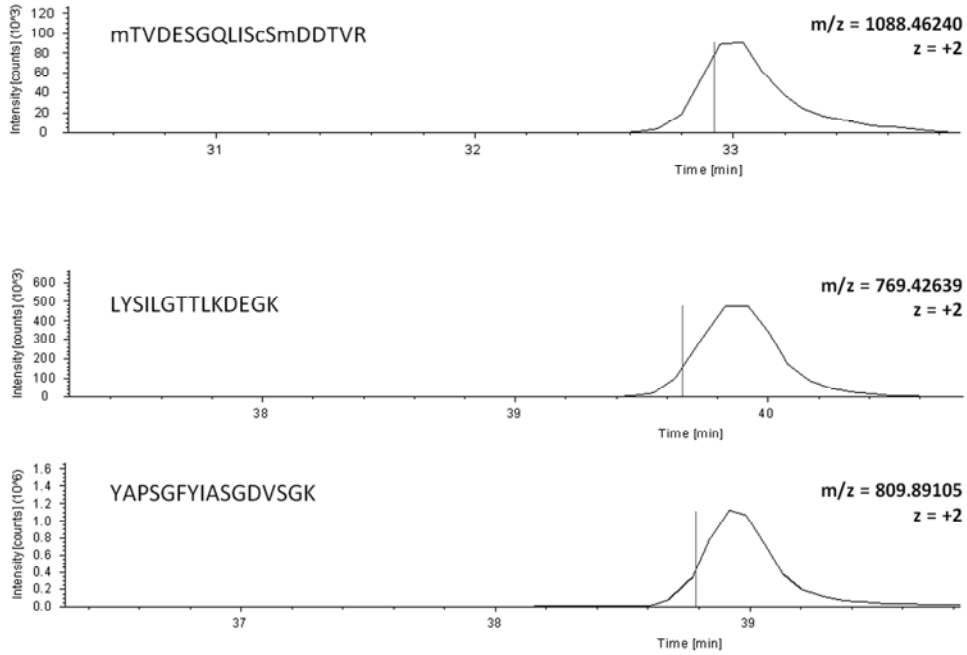
B7MD95 Trigger factor OS=Escherichia coli O45:K1

MQVSVETTQG LGRRVTITIA ADSIETAVKS ELVNVAKKVR IDGFRKGKVP MNIVAQRYGA SVRQDVLGDL MSRNFIDAII KEKINPAGAP
TYVPGEYKLG EDFTYSVEFE VYPEVELQGL EAIEVEKPIV EVTDADVDGM LDTLRKQQAT WKEKDGAVEA EDRVTIDFTG SVDGEEFEGG
KASDFVLAMG QGRMIPGFED GIKGHKAGEE FTIDVTFPEE YHAENLKGKA AKFAINLKKV EERELPELTA EFIKRFGVED GSVEGLRAEV
RKNMERELKS AIRNRVKSQA IEGLVKANDI DVPAALIDSE IDVLRRQAAQ RFGGNEKQAL ELPRELFEEQ AKRRVVVGLL LGEVIRTNEL
KADEERVKGL IEEMASAYED PKEVIEFYSK NKELMDNMRN VALEEQAVEA VLAKAKVTEK ETTFNELMN

A1AJ51 60 kDa chaperonin 1 OS=Escherichia coli O1:K1/APEC

MAAKDVKFGN DARVKMLRGV NVLADAVKVT LGPKGRNVVL DKSFGAPTIT KDGVSVAREI ELEDKFENMG AQMVKEVASK ANDAAGDGTT
TATVLAQAII TEGLKAVAAG MNPMDLKRGI DKAVTAAVEE LKALSVPCSD SKAIAQVGTI SANSDETVGK LIAEAMDKVG KEGVITVEDG
TGLQDELDVV EGMQFDRGYL SPYFINKPET GAVELESPFI LLADKKISNI REMLPVLEAV AKAGKPLLII AEDVEGEALA TLVVNTMRGI
VKVAAVKAPG FGDRRKAMLQ DIATLTGGTV ISEEIGMELE KATLEDLGQA KRVVINKDTT TIIDGVGEEA AIQGRVAQIR QQIEEATSDY
DREKLQERVA KLAGGVAVIK VGAATEVEMK EKKARVEDAL HATRAAVEEG VVAGGGVALI RVASKLADLR GQNEDQNVGI KVALRAMEAP
LRQIVLNCGE EPSVVANTVK GGDGNYGYNA ATEEYGNMID MGILDPTKVT RSALQYAASV AGLMITTECM VTDLPKNDAA DLGAAGGMGG
MGGMGGMM

B.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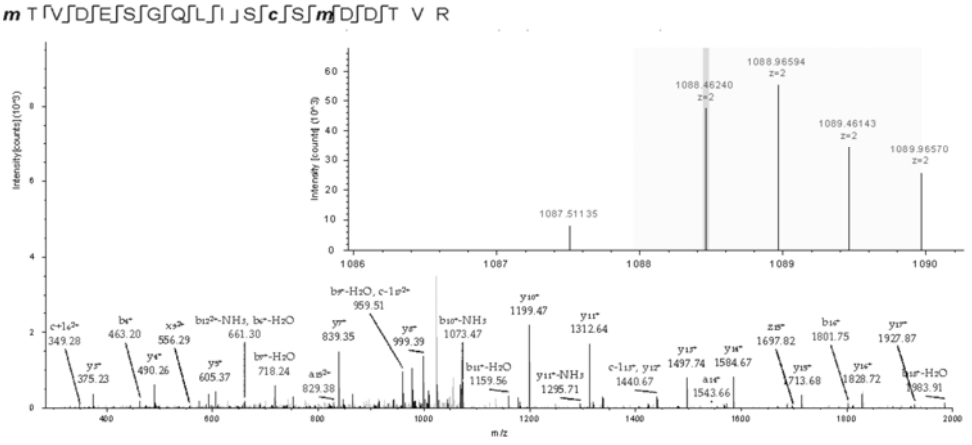
C.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D.

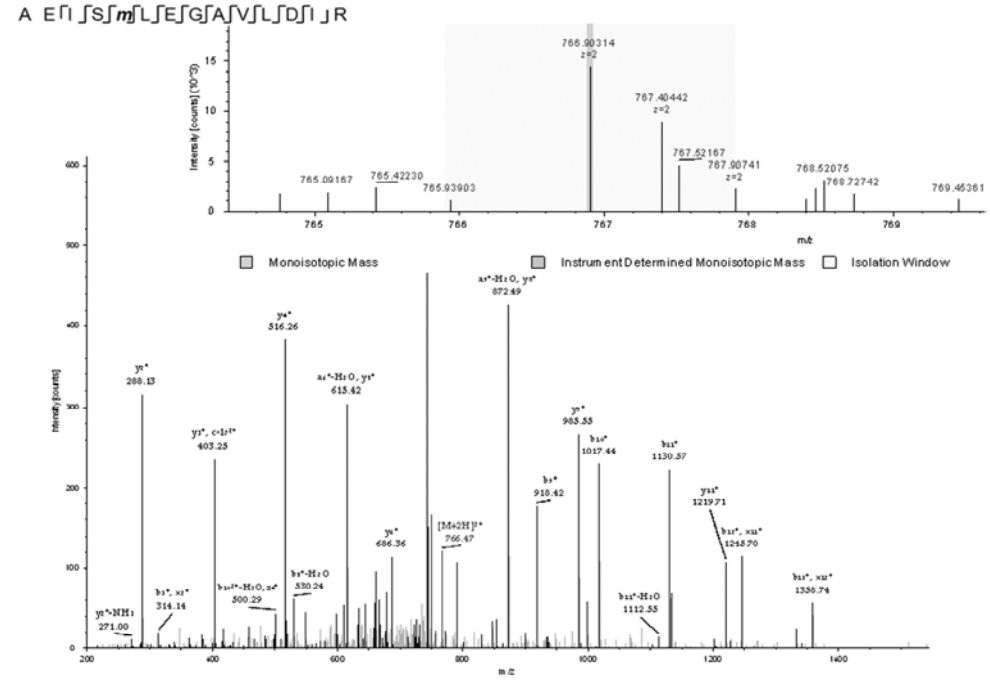

Fig. 3. – Mass spectrometry analysis of WDR1 protein **A.** Sequence coverage for WDR1 protein, GST, Trigger factor protein and 60 kDa chaperonin. Accession number from Uniprot database and name of the protein are provided in black bold. The amino acid sequence is displayed for each protein. Underlined are the peptides identified using LC-MS/MS. **B.** Extracted ion chromatograms (XICs) for some of the peptides identified from WD repeat-containing protein 1 from human. Vertical line represents the moment when the precursor was fragmented. Peptides were fragmented using CID with normalized collision energy of 35.0. **C.** Precursor isolation and MS/MS spectra of ions corresponding to peptides from WDR1 protein and to GST. A double charged peak at m/z 1088.4624 (precursor isolation in the inset) was fragmented using collision induced dissociation (CID). The analysis of the MS/MS spectrum led to the identification of b and y ions (alongside with water and/or ammonia loss ions) for a peptide with the sequence MTVDESGQLISCSMDDTTVR corresponding to the WDR1 protein. **D.** A double charged peak at m/z 766.9031 (precursor isolation in the inset) was fragmented using CID. The analysis of the MS/MS spectrum led to the identification of b and y ions (alongside with water and/or ammonia loss ions) for a peptide with the sequence MTVDESGQLISCSMDDTTVR corresponding to the GST sequence.

We also observed the WDR1 protein association with chaperones, when we tried to purify the GST-WDR1 fusion protein by gel filtration (data not shown).

Altogether, our results indicate that WDR1 protein is unstable precipitating after tag removal, and also that WDR1 protein needs chaperones for proper folding.

DISCUSSION

We optimized the expression of human *wdr1* gene; a low-temperature expression improved the target protein production. Co-expression with *tig* chaperone, which is known to be involved both in ribosomes and nascent polypeptides binding (19, 25), improved the solubility of WDR1 fusion protein. GST-tag removal by protease cleavage makes WDR1 protein to precipitate, only a small part being found in the soluble fraction, suggesting that both the GST-tag and chaperones may contribute to the proper folding and solubility of human WDR1 protein in *E. coli* expression system. Our findings are also supported by the nLC-MS/MS analysis, which revealed the presence of WDR1 protein together with the *tig* and of the 60 kDa chaperonin 1. This is partly surprising given that SDS-PAGE should dissociate non-covalently bound proteins. However, this finding can be explained by the relatively close molecular weights of WDR1 protein and chaperones (Table 1B). As an additional support to the association of WDR1 and chaperones is the presence of both proteins in GST-fused WDR1 sample analysed by mass spectrometry, the fusion protein having a reasonable difference in molecular weight as compared to chaperones.

The co-expression of *tig* chaperones with different proteins has previously shown that increases protein solubility (16-18), and once more in our report it showed its utility in increasing WDR1 protein solubility.

In conclusion, the WDR1 protein obtained through this method can be further used for different *in vitro* studies.

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