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Overexpression, Extraction, Purification and Characterisation of DppA from *Escherichia coli*

Zainol, M.K[1,3*], Mohd Zin, Z[1]., Linforth, R.S.T[2] and Scott, D.J.[3]

[1]School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia.
[2]Department of Food Science, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, UK
[3]The National Centre for Macromolecular Hydrodynamics, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, UK

*Corresponding Author: mkhairi@umt.edu.my
School of Food Science and Technology,
Universiti Malaysia Terengganu, 21030 Kuala Terengganu,
Terengganu, Malaysia.
Tel : +609 668 4971

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ABSTRACT

Components such as protein within the periplasmic space serve some vital purposes: such as buffering against the outside environment, processing of essential nutrients for transport into the cytoplasmic space, and cell wall biosynthesis. Dipeptide binding protein a (DppA) is a member of a family of ABC proteins and is involved in the transportation of potentially beneficial dipeptides as nutrient source through the periplasmic space and into cell. DppA was successfully cloned into expression vectors and over expressed in *Escherichia coli*, extracted, purified, and characterized. DppA was subjected to biophysical characterization using mass spectrometry. Mass spectrometry (ms) analysis and analytical ultra-centrifugation were used to evaluate the recombinant DppA's molecular weight. The findings presented in this study highlighted the ability to overexpress, extract and purify the recombinant DppA and reflect the differences techniques to asses its purity.

INTRODUCTION

Proteins are known to be involved in every function performed by a cell, including signal transduction, transportation and metabolism [1]. Proteins rely on interactions to other molecules in order for them to perform their biological functions which are crucial to a wide range of biological processes such as antigen recognition, enzyme-substrate interactions and hormone, neurotransmitter or drug binding. A protein is a combination of 20 naturally occurring amino acids arranged in a specific order along a linear polymer chain, which is folded up into a unique three-dimensional structure. The structure consists of a carboxyl and amide group connected to a central carbon atom (the α-carbon) and are distinguished by a particular side chain [2]. Some of these proteins are involved in transportation of compound through the periplasmic space of *E. coli*. These proteins are classified as transporter proteins that are crucial for peptide transportation through cells which occur in many species [3]. In *E. coli*, the permeases transporters such as dipeptide permeases that aid transportation of other proteins are categorized as dipeptide permeases. It is known that dipeptide permease protein transporter (DppA) has a preference for dipeptide substrates, but it can also transport tripeptides to a lesser extent [4].

A recombinant *E. coli* expression system was used to produce DppA in this study as the process has many advantages when compared to the other available systems including rapid growth, cheap cultivation, a well-studied genetic background and high expression level in large scale, and thus represents a widely used system for heterologous protein expression [5]. Recombinant protein expression was carried out in *E. coli* as it is known to be advantageous and by far the most well characterized of any system to date. It also has the ability to grow rapidly and high density in inexpensive media [6]. Because of the amount of knowledge about its genetics, biochemistry and molecular biology, *E. coli* is the system of choice for expression in many heterologous protein. They are easy, and cheap to grow, and many foreign proteins are well tolerated and may be expressed at high levels. Expression into periplasmic space also increase the yield of end product as it is easier to extract from periplasmic space as compared to that of the inner cells. The disadvantages of recombinant protein

expression are that the protein can be sequestered into inclusion bodies that will make purification of these recombinant proteins becomes difficult, and the functional proteins could be produced in a very small quantity. The aim of this study is to describe the expression, extraction, purification and characterization of periplasmic dipeptide binding protein (DppA) from *E. coli*.

MATERIALS AND METHODS

Chemicals

All chemical reagents used in the study were purchased from Sigma Aldrich, Poole, Dorset, UK and Fisher Scientific, Loughborough, UK unless otherwise stated.

Bacterial Strains

In order to produce DppA, *E. coli* BL21 (DE3) pLysS (Invitrogen, Paisley, UK) was used for transformation by electroporation and subsequent propagation of plasmid DNA. For transformation of mutagenesis products XL-10 gold ultracompetent *E. coli* cells were used (Agilent technologies, West Lothian, UK).

Plasmid

A plasmid construct for wild type DppA expression (*p*JT01) were cloned in pET-21 plasmid by Jeremy Tame (Yokohama City University, Japan). It contains T7 promoter to express the recombinant protein following induction by IPTG.

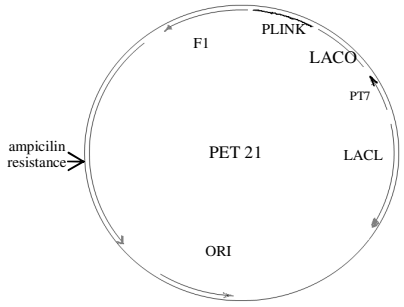

Fig. 1 The pET21 plasmid (Qiagen). The gene encoding *dpp* is inserted within the plasmid. The plasmid carries ampicillin resistance to allow selection of the plasmid.

DNA sequencing

The DNA encoding the DppA protein was sequenced using the ABI 310 genetic analyser for DNA sequencing (GMI, USA). This uses the didoxy chain termination method of sequencing. This method (Sanger, 1977) involved a single stranded DNA template incubated with oligonucleotides to copy the DNA strand under the influence of the enzyme DNA polymerase. The set of DNA primers used in the study were the universal T7 forward and reverse primers for the purified plasmid.

The reaction mixture also included nucleotide analogues that caused the new strand to be terminated at a specific DNA base. This leads to a series of DNA fragments that differ by one nucleotide in length which can be reassembled to decipher the sequence of the original section of DNA. DNA sequencing was carried out at Geneservice, Nottingham. All data interpretation and sequence analysis was carried out by the author.

Preparation of Electrocompetent Cells

Procedure for making electrocompetent cells includes preparation of 250 ml of sterile RO-water, 125 ml sterile water containing 10 % glycerol (in 4 different lab bottles), 10 ml of sterile water containing 10 % glycerol (in 2 lab bottles), 100 ml GYT broth (glucose, yeast and tryptone) an 500 ml of LB broth. All the materials were autoclaved at 121 °C for at least 15 min and stored at 4 °C.

One colony of *E. coli* BL21 (DE3) pLysS was added to a 25 mL LB and left to grow overnight at 37 °C, agitated at 200 rpm. The overnight culture was then added to 250 mL LB and allowed to grow until it reached log phase, OD_{600} = 0.6-0.8. The culture was then split across four centrifuge flasks and centrifuged at 6500 rpm for 20 min at 4 °C. The resulting supernatant was discarded and the pellets were resuspended twice in 125 mL 10% glycerol. Cells were maintained in 4 °C from here on. The cells were then centrifuged as before, and the supernatants were discarded and resuspend as before. The pelleted cells were then resuspend in 1 mL of 10% glycerol. All the fractions were combined and aliquoted into 40 µL in 1.5 ml centrifuge tubes. The cells were frozen in liquid nitrogen immediately and stored at -80 °C for further use.

Transformation of *E. coli* by electroporation

LB agar Plates containing Ampicillin and LB agar media were prepared 1 day before use and kept at 4 °C. The plates were then transferred to a 37 °C incubator, 30 minutes prior using them in order to remove the excess water. Plasmid DNA (pJT01) was prepared by dialysing 10 ml of the pJT01 plasmid on a 0.25 µm filter paper (Milipore Corporation, MA, USA) against pure water (miliQ) for 15 min. Two µl the dialysed pJT01 DNA plasmid was added to 40 µl of ice-cold electrocompetent cells (thawed for 5 minutes on ice). The mixture was then incubated on ice for 1 minute before transferred into an ice cold 0.2 cm electroporation cuvette (Biorad, Hercules, CA, USA). Electric current (25 µF, 2.5 v and 200 ohms) was administrated to the cuvette. The cells were immediately transferred into 1 ml of LB broth and incubated at 37 °C for 1 hour with 200 rpm agitation. After the 1 hour incubation, the electroporated cells were plated onto LB containing ampicillin, and incubated at 37 °C overnight. Colonies were counted and transformation efficiency was calculated. A number of colonies from the transformed cells were selected and screen for the presence of the desired plasmid by mini prepping and digestion analysis. The dish containing the transformed colony was then kept at 4 °C for further usage.

Production of DppA

The protein (wild type DppA) was expressed in BL21 (DE3) pLysS cells containing the *p*JT01 plasmid construct in a *p*ET21 vector system. Expression, extraction and purification of the protein were carried out using an adapted method to that described previously by [7].

Overexpression of DppA

Protein expression was carried out by increasing the incubation temperature or by the addition of an inducing chemical such as isopropyl-β-D-thiogalactoside (IPTG) to the culture medium [8]. The plasmids with promoters based on the *lac* operon can be induced when IPTG added as it behaves like lactose removes the repression on their expression without getting inactivated itself [11]. IPTG was used to induce the DppA expression. The cells were incubation at 29 °C and grow for 20 hours. The culture (cell) was centrifuged at 6500 rpm for 20 minutes, and the pellet was kept at -20 °C for further usage. The presence of protein was confirmed using SDS-PAGE [9] and ESI-LCMS analysis.

Extraction
Recombinant DppA was extracted using osmotic shock procedure based on the modified method by Chen and Co-workers [10]. Pellets of cells obtained in overexpression procedure were then resuspended in 75 ml of 30 mM Tris-HCL, 20% sucrose, pH 8, and then 60 µl (or 1 drop) of 0.5 M EDTA added. The cells were then collected by centrifugation (Beckman, Buckinghamshire, UK) at 8000 xg, 20 min at 4 ^{o}C. The remaining pellet was then resuspended in 75 ml of ice–cold 5 mM Mg_2SO_4. The suspension was gently stirred in icy condition for 10 min. The suspension was then centrifuged at 8000 xg for 20 min at 4 ^{o}C in order to pellet the shocked cells.

At this point, the cells were in osmotic shock and the expressed DppA (in the periplasmic space) was released into the buffer. The suspension was again centrifuged at 8000 g for 20 min at 4 ^{o}C. The supernatant (the lysed sample) was then kept at 4 ^{o}C or straight to dialysis against chromatography buffer (10 mM Tris, 10 mM NaCl, 1 mM EDTA, pH 8). The OD_{280} of the supernatant was determined and protein concentration determined using the Beer Lambert's Law, (A =C(Σ), where A= absorbance at 280 nm, c = concentration of the protein, Σ= extinction coefficient was also performed on the supernatant to confirm the presence of DppA.

Purification
Two steps of purification were used in the study namely ion exchange chromatography (IEX) on an AKTA^{TM} Purifier (GE Healthcare Life Sciences Buckinghamshire, UK) and gel filtration chromatography (GF) on an AKTA^{TM} Prime (GE Healthcare Life Sciences Buckinghamshire, UK).

Ion Exchange Chromatography
In ion exchange chromatography, 20 ml of Q-Sepharose was used as the stationary phase and packed in the XK 16 column (GE Healthcare Life Sciences Buckinghamshire, UK). The column was conditioned with HPLC grade-water and reconditioned with 250 ml of 10 mM NaCl, 10mM TRIS, 1mM EDTA, pH 8 (Buffer A). The sample (20 mg in 200 ml of extracted, dialysed DppA) was loaded onto the column using a peristaltic pump prior to purification.

The chromatography system was started with 100 % of Buffer A and 0 % Buffer B (500 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 8), ending with 0 % of Buffer A and 100 % Buffer B. Fractions collected were based on peak visualised in the chromatogram at 280 nm indicating the presence of protein. The collected fractions were then run on SDS-PAGE to confirm the presence of DppA.

Gel filtration
Gel filtration chromatography (GF) separates proteins according to size. Protein samples were gel filtrated using an AKTA Prime Fast Performance Liquid Chromatography (FPLC) system. A Superdex 75 gel filtration column (GE Healthcare Life Sciences Buckinghamshire, UK) was equilibrated with Phosphate Saline Buffer (PBS) as the gel filtration buffer (300 ml) and the spin concentrated protein was injected onto the column. Fractions of protein (monitored by absorbance at 280 nm) were collected and run on an SDS-PAGE gel to confirm the presence of the protein.

SDS-PAGE
Determination for homogeneity and apparent molecular mass of the purified DppA was carried out on Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS–PAGE) utilizing 12% acrylamide gels according to Laemmli [9]. The molecular mass of the protein was calculated by a comparison with reference standards. The gels were casted using BioRad Mini Protean III apparatus (Bio-Rad, Hercules, CA, USA). SDS-PAGE gel consisted of 2 layers of acrylamide gels namely resolving gel and stacking gel. Resolving gel layer consisted of 950 µl of 40% bisacrylamide: acrylamide (19:1), 1250 µl of 3M Tris 5% SDS, 1120 µl RO-water, 400 ml Glycerol, 2 µl TEMED and 19 µl of ammonium persulphate.

The stacking gel layer consisted of 250 µl of 40% bisacrylamide: acrylamide (19:1), 620 µl of 3 M Tris 5% SDS, 1610 µl RO-water, 400 ml Glycerol, 2 µl TEMED and 20 µl of ammonium persulfate. The stacking gel was added above the resolving gel. All SDS–PAGE was performed using a BioRad Protean III system with 15% Tris-Tricine buffered gels (0.75 mm). 15µl of DppA preparation was mixed to 30 µl of loading buffer and 17 µl of the mixture was loaded into the wells of the SDS-PAGE gel. Loading buffer was made by mixing 3.55 ml deionised water with 1.25 ml of 0.5 M Tris-HCl pH 6.8, 2.5 ml glycerol, 2 ml 10% SDS and 0.2 ml bromophenol Blue. Samples for SDS-PAGE were prepared by mixing protein solution 1:2 with loading buffer (Biorad Tricine Sample Buffer). The mixture was then heated for 5 min at 95 ^{o}C, briefly centrifuged and then loaded onto the gel. A standard protein marker (#7720, Broad Range pre-stained marker, New England Biolabs) was used to enable assessment of the protein size. Electrophoresis was carried in 2 buffers namely anode and cathode, with anode consisted of 0.4 M Tris-HCl, pH 8.8. Cathode consists of 0.25 M Tris, 0.25 M Tricine, 0.25 % SDS at pH 8.25.

Quantification of protein using UV spectrophotometry
The amount of protein obtained in the purification was quantified using UV spectrometry at 280 nm. This is based on the presence of aromatic residues tryptophan, tyrosine and cysteine bonds that are known to be responsible for the UV absorption at 280 nm [11]. A linear relationship exists between protein concentration and absorbance at a particular wavelength for a dilute protein solutions as described bt the Beer-Lambert's Law. $A_\lambda = \varepsilon_\lambda bc$

Where, A_λ is the absorbance reading at λ wavelength, ε_λ is the Molar Extinction Coefficient chromophore at λ wavelength [ε_λ = 5500 x numbers of tryptophan + 1490 x numbers of tyrosine ($M^{-1}.cm^{-1}$),] [10]. C is the protein concentration in Molar (M), b is the path length of light in cm.

Assessment of DppA purity

Mass Spectrometry
Liquid Chromatography Mass Spectrometry (LCMS) using electrospray ionisation was used to confirm the molecular weight of the protein.

Analytical Ultra Centrifuge
AUC is a classic method to determine molecular mass and size of protein. Even nowadays, most protein can be determined using computer software, the use of AUC are still considered as a powerful technique for mass determination as it can still be used in a complex macromolecules assembly in solution. The sedimenting behaviour of DppA was observed using a Beckman XLA Analytical Ultracentrifuge Optima XLA-1 (Beckman, Palo Alto, CA, USA). A four hole rotor (An-Ti) housed cells containing quartz windows with conventional double-sector charcoal-filled centrepieces. Cell assembly, loading and experimental set up were as manufacturer's instruction. All the

cells containing DppA sample were balanced (based on weight) and the experiment was carried out at 40 000 rpm at 20 ^{o}C and data were recorded at 280 nm. Data obtained were analysed using SEDFIT programme to generate the molecular mass of the DppA [12].

RESULTS

Gene sequencing

All plasmid DNA sequencing was carried out by Genesequence, Nottingham. The concentration was checked using a Nanodrop™ ND1000 spectrometer (Thermo Fisher Scientific, Wilmington, USA) by measuring the absorbance at a wavelength of 260 nm. The gene sequencing data provided by NCBI BLAST programme indicates that DppA is a protein containing 535 amino acids (**Fig.** 2). In order to find a template to model DppA on a BLAST search was conducted on the primary amino acid sequence of DppA against all proteins in the PDB.

The closest sequence identity found was to α DppA (E234869, PDB code: 1034), which is unsurprising as DppA was initially identified as having a very similar nucleotide sequence. Alignment of the amino acid sequences shows them to have 97 % sequence identity, when the pre-sequences are excluded (**Fig.** 2).

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1   MRISLKKSGM LKLGLSLVAM TVAASVQAKT LVYCSEGSPE GFNPQLFTSG TTYDASSVPL
61  YNRLVEFKIG TTEVIPGLAE KWEVSEDGKT YTFHLRKGVK WHDNKEFKPT RELNADDVVF
121 SFDRQKNAQN PYHKVSGGSY EYFEGMGLPE LISEVKKVDD NTVQFVLTRP EAPFLADLAM
181 DFASILSKEY ADAMMKAGTP EKLDLNPIGT GPFQLQQYQK DSRIRYKAFD GYWGTKPQID
241 TLVFSITPDA SVRYAKLQKN ECQVMPYPNP ADIARMKQDK SINLMEMPGL NVGYLSYNVQ
301 KKPLDDVKVR QALTYAVNKD AIIKAVYQGA GVSAKNLIPP TMWGYNDDVQ DYTYDPEKAK
361 ALLKEAGLEK GFSIDLWAMP VQRPYNPNAR RMAEMIQADW AKVGVQAKIV TYEWGEYLKR
421 AKDGEHQTVM MGWTGDNGDP DNFFATLFSC AASEQGSNYS KWCYKPFEDL IQPARATDDH
481 NKRVELYKQA QVVMHDQAPA LIIAHSTVFE PVRKEVKGYV VDPLGKHHFE NVSIE

gi|215488827|ref|YP_002331258.1| dipeptide transporter
[Escherichia coli O127:H6 str. E2348/69]
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Fig. 2. Amino acid sequence of DppA prior to export into the extracellular space form the cytoplasm, where the export signal, shown in orange, is cleaved.

Expression DppA in *E. coli* strain BL21 (DE3) pLysS

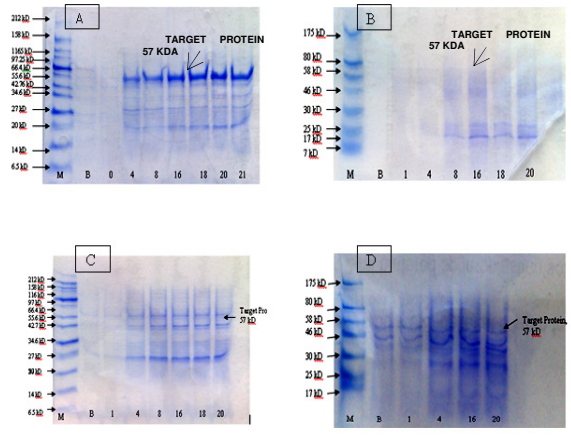

Fig. 3 (A, B, C and D). 4-20 % SDS- PAGE of samples taken from the soluble fractions before IPTG induction and then at regular intervals during a 20 hour over-expression period. A= *E. coli* BL21 (DE3) pLysS + pJT01 + IPTG, B = *E. coli* BL21 (DE3) pLysS – pJT01 – IPTG, C = *E. coli* BL21 (DE3) pLysS + pJT01 – IPTG and D (negative control) = *E. coli* BL21 (DE3) pLysS - pJT01 + IPTG. Lane M is the marker, Lane B is sample taken before the induction of IPTG; lane 1 is sample taken after 1 h, lane 2 is sample taken after 2 h; lane 3 is after 3 h, lane 4 is sample taken after 4 h and lane 20 is at 20 h.

DppA from *E. coli* was overexpresed using *E. coli* BL21 (DE3) pLysS. Increase in the thickness of the protein was visible after 4 hours IPTG induction. Yet the yield of the DppA was not enough for the biophysical analysis. To overcome this, incubation time was increased to 20 hours while the temperature was reduced to 29^{o}C after 4 hour.

Fig. 3 (A, B, C, and D) exhibited the results of SDS-PAGE of DppA expressed in *E. coli* BL21 (DE3) pLysS containing pJT01 plasmid induced with IPTG, and incubated for 20 hours. Clear and thick bands of DppA were visualised at 57 kDa (the size of DppA in expressed in periplasmic space of *E. coli*) on the SDS gel indicating a successful DppA expression (Gel A).

DppA expressions were carried out at 29 ^{o}C for 20 hours, this gave a significantly high yield of protein as thick bands were visualised in the SDS-PAGE. The results also showed that there were several other proteins expressed along with the desired DppA, but with lower intensity to that of DppA. These proteins are removed after extraction and purification, leaving only the purified DppA.

The results also showed that the intensity of the bands increased over time. The expression started at the second hour and fully matured at the sixteenth hour. From this point forward, the intensity of the bands never increased showing that they had reached the maximum level of expression.

Extraction of DppA using osmotic shock procedure

Fig. 4 shows the results for DppA extraction utilising the osmotic shock procedure. It clearly depicted that a band was present on the SDS PAGE indicating that DppA was extracted from the experiment and also located in the periplasmic space of *E. coli* as periplasmic expression procedure is normally used to extract and confirm that protein originates from the periplasmic space of the bacteria [13].

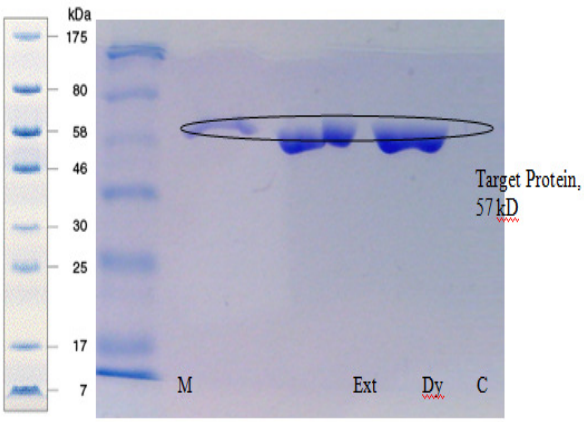

Fig. 4 SDS PAGE analysis of extracted DppA by osmotic shock, M= Marker, Ext = Extracted DppA , Dy = DppA dialysed against 10 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 8.

Purification of DppA

Fig. 5 (A) depicts the chromatographic profile of the purification of DppA utilizing ion exchange chromatography (IEX) procedure. IEX separates proteins on the basis of differences in their net surface charge (GE Healthcare Life Sciences, Buckinghamshire, UK). Q-sepharose beads were used as the stationary phase and 10 mM Tris, 10 mM, 1 mM EDTA and 500 mM Tris, 10 mM, 1 mM EDTA as the mobile phases. One large peak was clearly visible in fractions 23–32 at 280 nm indicating the presence of protein in the system. The purified protein was collected in tubes and analysed using SDS-PAGE in order to re-confirm the presence of DppA. Results showed that DppA was eluted in fractions 23 to 32. SDS-PAGE confirmed that DppA was present in fraction 23-32 as thick and clear bands were visualised at 57 kDa on the gel (**Fig.** 5 B). The same sample was then put through a gel filtration chromatography procedure to further purified it and also change the buffer to 50 mM ammonium acetate in preparation for further biophysical analysis.

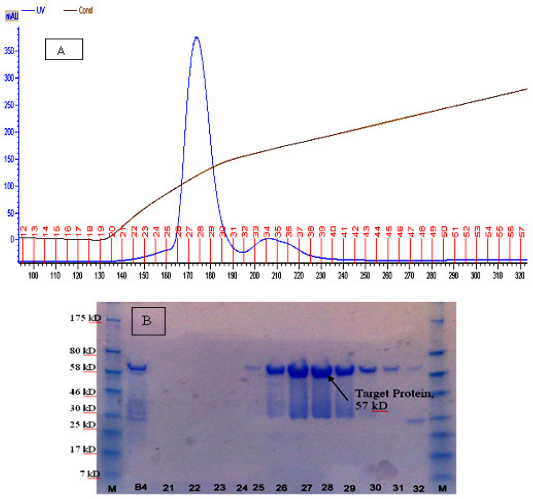

Fig. 5 (A and B) A: Chromatogram showing protein (DppA) purified using ion exchange chromatography (IEX). Peak of DppA was visualised at fractions number 23 – 31. B: 4-20 % SDS-PAGE confirming DppA presence in IEX analysis. M= Protein marker, B4= DppA before purification, 21 – 32= Number of fractions collected aligned to peak generated in IEX.

Gel filtration chromatography (GF) was used as second step in protein (DppA) purification, which was designed to polish the separated DppA from the previous technique. Superdex 75 was utilised in the study as the stationary phase and ammonium sulfate as the mobile phase. Protein that was collected from IEX was pooled together and concentrated to 5 ml using 3 kDa MWCO Spin concentrator (Sortorius Stedim, Goettingen, Germany) prior to the GF procedure. **Fig.** 6 (A) illustrates that DppA was eluted from the system at fraction 12-18. The method was used to achieve a high level of purity from possible impurities that might still be in the DppA fractions after the IEX procedure. GF also allows buffer exchange in order to prepare DppA for the next experiment (Binding experiment using ITC) as the buffer for ITC is different from the buffer used in purification as well as extraction and expression. As confirmation, SDS-PAGE was used to analyse the gel filtrate. **Fig.** 6 B shows the DppA after gel filtration chromatography with bands at 57 kDa clearly visible indicating that the procedure was a success.

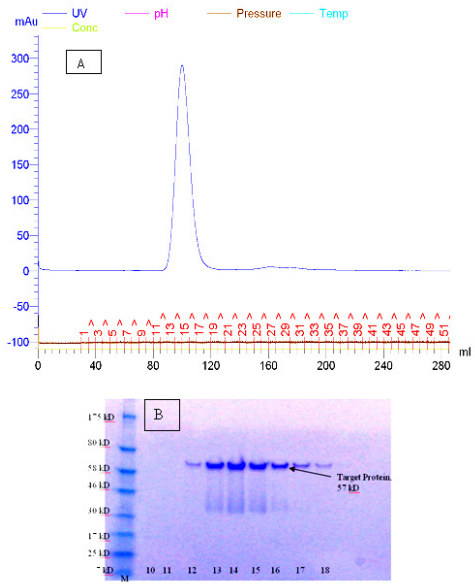

Fig. 6 (A and B) A: Chromatogram showing protein (DppA) purified using gel filtration chromatography (GF). Peak of DppA was visualised at fractions number 23 – 31. B: 4-20 % SDS-PAGE confirming DppA presence in GF analysis. M= Protein marker, B4= DppA before purification, 12–18= Number of fractions collected align to peak generated in GF.

Assessment of Purified DppA purity

Electrospray Liquid Chromatography Mass Spectrometry (ESI-TOF LCMS)

Electrospray Ionisation Time of Flight (ESI-TOF) mass spectrometry was used to determine the exact mass of the successfully purified DppA. In addition, it confirmed that the purified DppA sample was of high enough purity. Electrospray Ionisation Mass Spectrometry was used to determine the purity of the purified protein. Freeze dried DppA (water-base) was diluted 1:25 into 50% Acetonitrile, 1 % formic acid. **Fig.** 7 illustrates the profile of the purified protein at the mass of 57409.8 ± 0.2 Da with very few additional masses present. This result indicates that DppA purified here was highly pure and ready for further in the biophysical analysis.

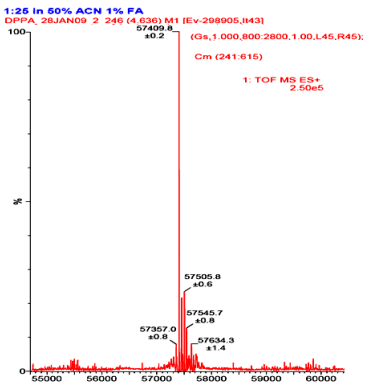

Fig. 7. ESI-TOF mass spectrum of DppA denatured in acetonitrile/formic acid. The values represent molecular weight of the protein following ionisation. ESI-TOF mass spectrum of DppA (mass 57409.8 Da).

Analytical Ultra Centrifuge (AUC) Analysis

AUC is useful to provide essential properties of the analysed protein such as, the molecular weight, size, and shape in its native state. Detection is achieved by macromolecule's natural properties. **Fig.** 8 shows a big peak appeared at 57 kDa along with another 3 small peaks in the sedimentation coefficient profile. A possible explanation might be protein degradation.

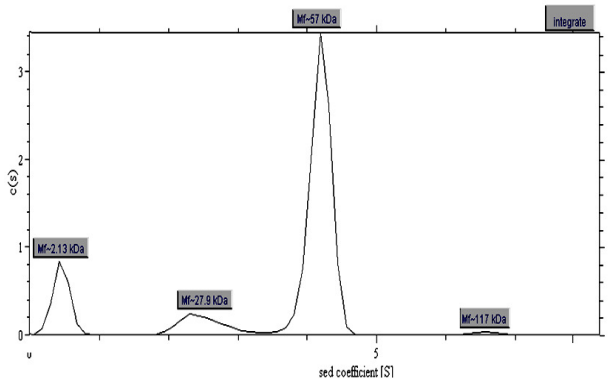

Fig. 8 AUC spectrum of DppA expressed in *E. coli* BL21. The values represents molecular weight of the protein following ionisation of 57 KDa).

DISCUSSION

Identification of the correct expressed protein proved interesting as leaking in the expression. The transformation of *Dpp* gene proved successful as 3 bands (supercoiled) visualised in agarose gel analysed after the transformation procedure. Furthermore, the gene sequencing results indicated that the expressed protein would contain 535 amino acids. DppA expression is categorised as periplasmic expression indicating that DppA was expressed in the periplasm. This was being confirmed when the osmotic shock extraction showed protein being extracted as it is known that osmotic shock was normally being used to confirm the protein expressed in the periplasm space of the cell. This offers several advantages for protein targeting, unlike the cytosolic compartment, the periplasm contains only 4% of the total cell protein [12].

Recombinant DppA was produced for purification in this study. Expression of the DppA was induced with IPTG at OD_{600}0.7 and cells harvested 20 hour after induction. The cells were extracted by the osmotic shock procedure. Recombinant DppA was purified using a procedure consisting of two steps, ion exchange (IEX) chromatography and gel filtration (GF) chromatography [14]. The results showed in protein purification indicated that two steps of purification yielded a highly pure protein. Furthermore, in the end of the GF procedure, one peak and one band visualised on the chromatogram and SDS-PAGE gave a high confidence that the purified protein was pure.

In order to further analyse the purity of the purified protein, LCMS and AUC analysis were carried out. In LCMS analysis, the data showed a slightly different value as compared to that of the value in AUC. This might be due to the presence of salts such as potassium and sodium in the buffers. A reduction or increase in molecular weight value of analysed protein in MS might also be due to protein slicing or truncation of some protein [15]. Analysis by electrospray or even nanospray ionisation, is limit to a mass accuracy of within 0.01% of the molecular weight is routinely cited as achievable, which in this case represents +/- 5.7 kDa. Losses of water [16] ammonia [17] and carbon monoxide [18] from the sequence fragment ions are often present.

CONCLUSION

In conclusion, DppA was over-expressed from *E. coli* BL21 (DE3) pLysS containing pJT01 plasmid. The size of DppA expressed was the expected 57 kDa, and the mass was confirmed by mass spectrometry. In order to produce better DppA several parameters were altered such as the use IPTG as an inducer to the expression and incubation at low temperature showed better or more DppA was expressed. This was visualized by the heavier bands that appeared on SDS gel, and the resultant higher yields after purification.

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