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Production and characterization of clinical grade *Escherichia coli* derived *Plasmodium falciparum* 42 kDa merozoite surface protein 1 ($MSP1_{42}$) in the absence of an affinity tag

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Abstract

The 42 kDa cleavage product from the carboxyl end of the *Plasmodium falciparum* merozoite surface protein 1 ($MSP1_{42}$) is an important blood-stage malaria vaccine target. Several recombinant protein expression systems have been used for production of $MSP1_{42}$ including yeast (*Saccharomyces cerevisiae* and *Pichia pastoris*), *Escherichia coli*, baculovirus and transgenic animals. To date, all of the reported recombinant proteins include a 6× His affinity tag to facilitate purification, including three $MSP1_{42}$ clinical grade proteins currently in human trials. Under some circumstances, the presence of the 6× His tag may not be desirable. Therefore, we were interested to produce clinical grade $MSP1_{42}$ without a 6× His affinity tag from *E. coli* inclusion bodies. We produced a recombinant $MSP1_{42}$ with a *P. falciparum* FUP (Uganda-Palo Alto) phenotype which accounts for a substantial proportion of the $MSP1_{42}$ protein observed in African isolates. Ec$MSP1_{42}$-FUP was produced in *E. coli* inclusion bodies by high cell mass induction with IPTG using 5 L and 60 L bioreactors. Isolated inclusion bodies were solubilized in 8 M guanidine–HCl and the Ec$MSP1_{42}$-FUP protein refolded by rapid dilution. Refolded Ec$MSP1_{42}$-FUP was purified using hydrophobic interaction chromatography, anion exchange chromatography, and size exclusion chromatography, and subject to biochemical characterization for integrity, identity, and purity. Endotoxin and host cell protein levels were within acceptable limits for human use. The process was successfully transferred to pilot-scale production in a cGMP environment. A final recovery of 87.8 mg of clinical-grade material per liter of fermentation broth was achieved. The Ec$MSP1_{42}$-FUP clinical antigen is available for preclinical evaluation and human studies.
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Keywords: *Plasmodium falciparum*; MSP1; *Escherichia coli*; Refold; Vaccine

Malaria continues to cause significant levels of morbidity and mortality in tropical regions. Current control efforts are hindered by the emergence of parasite drug resistance and insecticide resistance of the mosquito host. The development of a malaria vaccine which protects against clinical disease caused by *Plasmodium falciparum* would significantly enhance existing control measures. One of the major targets of a blood-stage malaria vaccine, the life-cycle stage responsible for clinical disease, is the merozoite surface protein 1 (MSP1)[1]. MSP1 as a full-length protein has an approximate molecular mass of 195 kDa. This molecule is naturally processed into several fragments on the surface of the asexual stage parasite [1]. Of particular interest is a primary cleavage product of ~42 kDa ($MSP1_{42}$) derived from the carboxyl end of the molecule that undergoes further processing at the time of merozoite invasion of the red

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[1] *Abbreviations used:* MSP1, merozoite surface protein 1; IPTG, isopropysl-1-thio-β-galactopyranoside; DTT, dithiothreitol; IB, inclusion bodies; SIB, solubilized and denatured IB; CV, column volume; SV, sedimentation velocity; SE, sedimentation equilibrium.

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blood cell to generate ~33 and ~19 kDa proteins [2]. The 19 kDa fragment consists of two EGF-like domains containing a total of six disulfide bonds [2]. *Plasmodium falciparum* $MSP1_{42}$ can be classified into two dimorphic alleles that contain the greatest diversity within the 33 kDa portion (~50%) and more limited diversity within the 19 kDa fragment (~5%) [3,4]. Antibodies raised against the 42 kDa fragment are protective in various primate [5–7] and rodent [8,9] models and may correlate with protection in humans from endemic regions [6,10]. Individuals showing high levels of protection tend to have elevated anti-MSP1 antibody titers [11,12].

Escherichia coli derived recombinant $MSP1_{42}$-3D7, $MSP1_{42}$-FVO, and the combination of the two proteins ($MSP1_{42}$-C1) are currently being tested in Phase I human studies by the Malaria Vaccine Development Branch (MVDB/NIAID/NIH). The $MSP1_{42}$-FUP (Uganda-Palo Alto strain) protein phenotype is commonly observed in infected patients in Africa [13,14]. The recombinant protein, $EcMSP1_{42}$-FUP, represents the 33 kDa fragment from the 3D7 *P. falciparum* variant and the E-K-NG point mutations identified in the 19 kDa fragment within the $MSP1_{42}$ native molecule (Fig. 1).

The use of a 6× His affinity tag to capture recombinant proteins by immobilized metal affinity chromatography is a common method for initial protein purification. Expression and purification of recombinant *P. falciparum* $MSP1_{42}$ proteins for evaluation in clinical human trials to date have all used a 6× His affinity tag [MVDB and 5,10,15,16] due in part to the capacity of immobilized metal affinity chromatography to significantly remove host derived impurities including endotoxin. However, inclusion of this 6× His affinity tag, adds at least six non-native histidine residues which may have an unknown immunological effect on larger human populations. Also of concern is the possibility of heavy metals (Ni, Co, and Zn) leaching from the affinity column during purification that may elicit an inappropriate immune response in humans if not completely removed. Although the concentration of heavy metals in bulk substance may be tested their presence is still a cause of concern. The objective of this study was to produce an *E. coli* derived $MSP1_{42}$-FUP protein without a 6× His affinity tag at pilot-scale in a cGMP environment of suitable quality and quantity for preclinical studies and human trials. Here, we report on the expression, purification and biochemical characterization of clinical-grade recombinant $EcMSP1_{42}$-FUP protein.

Materials and methods

Plasmid construction for expression in E. coli

The recombinant $MSP1_{42}$-FUP protein is based on the C-terminal $MSP1_{42}$ amino acid sequence of the Uganda-Palo Alto *P. falciparum* isolate (GenBank Accession No. M37213). A *E. coli*-codon optimized DNA sequence encoding the amino acid sequence of $MSP1_{42}$-FUP was generated by amplifying the 5′ region of synthetic $MSP1_{42}$-3D7 DNA (GenBank Accession No. DQ414722) and the 3′ region of synthetic $MSP1_{42}$-FVO DNA (GenBank Accession No. AY343089). These two PCR products were annealed and used as a template in a second round of amplification which produced the synthetic $MSP1_{42}$-FUP DNA (GenBank Accession No. DQ437518). This product was cloned into pET-24a(+), the sequence verified and used to transform BL21(DE3) *E. coli*. A stop codon was engineered upstream of the pET-24a(+) encoded 6× His tag thus, the antigen was expressed without a 6× His tag. The $EcMSP1_{42}$-FUP antigen corresponds to the coding region from Ala-1333 to Asn-1708 (GenBank Accession No. AAA29611) with the exception of three amino acid differences at H 1564, N 1565, and D 1568.

Fermentation

The $EcMSP1_{42}$-FUP recombinant protein was expressed in small-scale shake flask and at bench-scale in 5 L fermentations (6.6 L vessel, BioFlo 3000 Bioreactor, New Brunswick Scientific, Edison, NJ) for research as previously described [6]. Sixty liter fermentations (80 L vessel, BioFlo 5000 Bioreactor, New Brunswick Scientific) were performed for production using a scaled-up version of the research protocol. In brief, fermentations were performed using defined media with NH_4OH used to maintain the pH at 7.0 and also as a nitrogen source for protein production. Glucose was the primary carbon source. Oxygen supplementation was used to maintain dissolved oxygen at 30% in

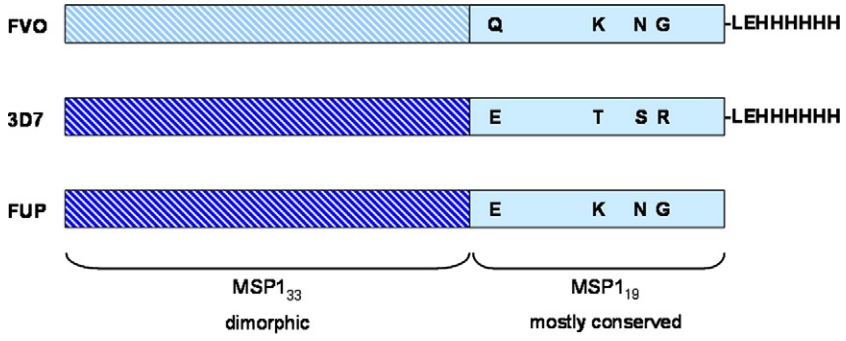

Fig. 1. Schematic of the two different allelic variants (light or dark blue) of *P. falciparum* $MSP1_{42}$ produced by MVDB as *E. coli* derived proteins for human studies with and without 6× His affinity tags.