Expression and purification of full-length recombinant Plasmodium falciparum PfMC-2TM Maurer’s cleft protein

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Abstract

Malaria caused by Plasmodium falciparum remains the most virulent form of malaria, resulting in 216 million cases and 445,000 deaths worldwide each year. Invasion of red blood cells by P. falciparum leads to the formation of membranous structures known as Maurer’s clefts (MC). Virulence markers of P. falciparum such as PfEMP1 are transported across the MC to the surface of the infected red blood cell. Insight into the formation and function of the MC will be important for the discovery of new vaccine and drug candidates. The PfMC-2TM is encoded by a multi-gene family of 13 members. PfMC-2TM is a protein localized to the MC. We induced expression of PfMC-2TM encoded by 1 family member (PF3D07_0114000 [PFA04680c]) in BL21 DE3 strain of Escherichia coli following transformation with recombinant pET-28a plasmid containing a chemically synthesized gene. The purpose of this study was to determine immunogenic properties of the resulting recombinant protein using western blot analysis. The recombinant plasmid was isolated and analyzed in 1% agarose gel and an approximately 5kb band was identified. Pilot expression of transformants showed expression of recombinant PfMC-2TM by western blot. Recombinant PfMC-2TM protein will be expressed and purified for antibody production to allow subsequent domain analysis and characterization.

Introduction

Malaria

- 216 million cases worldwide and 445,000 deaths worldwide (World Health Organization, www.who.int)
- Infection transmitted by female Anopheles mosquito
- Infection caused by Plasmodium parasites (protozoans)
  - P. falciparum - most virulent form
  - P. ovale
  - P. malariae
  - P. knowlesi
  - P. vivax

Plasmodium falciparum

- Cause of Complicated/Severe Malaria
- Only species that develops Maurer’s clefts (MC)
- MC responsible for protein transport from red cell cytoplasm to erythrocyte membrane (RCBM)
- Virulence protein PfEMP1 is associated with knobs on the surface of RBC
- PfMC-2TM is localized to the MC

Rationale

- The rationale behind this project is to determine if the full-length chemically synthesized gene, following bacterial transcription and subsequent translation, can yield a protein with immunogenic properties.

Objectives and Hypothesis

Objectives

- Transform DH5a strain E.coli bacteria with full-length pET-28a(+) PfMC-2TM recombinant plasmid
- Induce expression of the PfMC-2TM protein in BL21 DE3 strain of E. coli
- Analyze bacterially synthesized protein by western blot analysis and determine potential immunogenicity

Question

- Do E. coli transformed with chemically synthesized PfMC-2TM recombinant pET-28a(+) plasmid yield an immunogenic PfMC-2TM protein?

Hypothesis

- E. coli bacteria transformed with chemically synthesized PfMC-2TM recombinant plasmid pET-28a(+) yield immunogenic PfMC-2TM protein

Methods

- Transformed recombinant PfMC-2TM into E. coli/strains DH5s and BL21 DE3
- Selected 4 DH5s and 6 BL21 DE3 transformants
- Plated transformants on LB-Kanamycin plates and LB broth

Results and Conclusions

- pET28a plasmid was isolated from DH5a E. coli cultures
- PfMC-2TM was successfully expressed in BL21-DE3 E. coli cultures
- Full-length recombinant PfMC-2TM reacted strongly with rabbit-anti-PfMC-2TM antibodies

Future Directions

- Repeat pilot expression to obtain more protein
- Express protein for purification
- Purified protein will be used for antibody production
- Antibodies will be used for domain and epitope characterization

References


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