Design of an Oral Malaria Vaccine Candidate Using Yeast - Expressed CTB-MSP-TOS Fusion

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Abstract

Malaria remains a significant global health challenge. This study describes the construction and expression of a fusion protein for a malaria vaccine candidate, combining the cholera toxin B subunit (CTB) with two antigens: Merozoite Surface Protein (MSP) and Transferrin-Binding Outer Surface Protein (TOS). The fusion protein, joined by GSGG linkers to maintain functional independence, was expressed in *Saccharomyces cerevisiae* and subsequently heat-dried to ensure yeast cell inactivation while preserving antigen integrity. This study outlines the experimental design, vector construction, protein expression, and antigen preservation methods, laying the groundwork for further characterization and testing.

Introduction

Background on Malaria

Malaria, caused by *Plasmodium* species, remains a life-threatening disease, predominantly affecting tropical and subtropical regions. According to the World Health Organization, over 241 million cases and 627,000 deaths were reported globally in 2021, highlighting its ongoing public health burden (1). The disease cycle involves multiple stages, including liver and blood cell infection, presenting unique challenges for vaccine development due to stage-specific antigen expression (1, 6).

Vaccine Development Challenges

Vaccines targeting single antigens often fail to achieve broad immunity across the parasite lifecycle. Plasmodium's rapid antigenic variation and immune evasion mechanisms further complicate vaccine efficacy. Multi-antigen approaches may address these challenges by targeting key processes in multiple stages of the parasite lifecycle (6).

Cholera Toxin B Subunit as an Adjuvant

CTB, a non-toxic component of cholera toxin, is well-documented as an effective mucosal adjuvant. Its binding to GM1 ganglioside receptors facilitates antigen delivery to intestinal mucosal immune sites, promoting immune response activation (3, 5). CTB also enhances antigen stability, making it a valuable component for orally administered vaccines.

Target Antigens: MSP and TOS

This study focuses on two critical malaria antigens:

- **MSP (Merozoite Surface Protein):** A key antigen for blocking red blood cell invasion, sourced from NCBI (Accession: CAA71607.1) (6).
- **TOS (Transferrin-Binding Outer Surface Protein):** Essential for iron acquisition, sourced from NCBI (Accession: XP_001350569.1) (7).

Materials and Methods

Construction of the Expression Vector

The expression vector was designed to encode a fusion protein consisting of the cholera toxin B subunit (CTB), the Merozoite Surface Protein (MSP), and the Transferrin-Binding Outer Surface Protein (TOS), with flexible linkers (GSGG) inserted between each protein to maintain independent folding and functionality.

1. **CTB (Cholera Toxin B Subunit)**:
   CTB was positioned at the N-terminus of the fusion protein to serve as a mucosal targeting component. CTB binds specifically to GM1 ganglioside receptors on the surface of intestinal cells, facilitating uptake through the mucosal immune system. This binding property enables efficient antigen delivery and enhanced immune response when administered orally. CTB's established role as an adjuvant in mucosal vaccines provides the foundation for using it to deliver MSP and TOS antigens in this design (3, 5).(Portions highlighted in yellow)
2. **GSGG Linkers**:
   Between CTB and MSP, as well as between MSP and TOS, GSGG linkers were included to allow each domain to fold independently. These linkers ensure that the functional integrity of each antigen is maintained by preventing steric hindrance and allowing flexibility. The GSGG sequence is short but flexible, commonly used in recombinant protein design to promote structural independence among protein domains.(Portions highlighted in blue)
3. **MSP (Merozoite Surface Protein)**:
   The MSP protein sequence, sourced from NCBI (Accession: CAA71607.1) (6), was inserted downstream of the CTB-GSGG structure. MSP is a critical surface protein involved in the invasion of red blood cells by *Plasmodium* merozoites. By including MSP

as an antigen, the vaccine is designed to elicit immune responses that target this vital invasion process, potentially blocking the parasite from entering red blood cells and proliferating within the host.(Portions highlighted in orange)

4. **TOS (Transferrin-Binding Outer Surface Protein)**:
   Following the second GSGG linker, the TOS sequence from NCBI (Accession: XP_001350569.1) (7) was inserted as the final antigen in the fusion protein. TOS facilitates iron acquisition in *Plasmodium* parasites by binding transferrin, an essential host protein for iron transport. Including TOS as an antigen targets the parasite's nutrient acquisition mechanisms, potentially starving the parasite of iron needed for its survival and replication.(Portions highlighted in green)

The final structure of the expression vector is CTB-GSGG-MSP-GSGG-TOS, with each component positioned to maximize functionality and immune response potential.

Protein Sequence

The sequence of the fusion protein is as follows:

MAQSSRICHGVQNPCVIISNLSKSNQNKSPFSVSLKTHQHPRAYPISSSWGLKKSGMTLIGSEL
RPLKVMSSVSAGGSGMTPQNITDLCAEYHNTQIYTLNDKIFSYTESLAGKREMAIITFKNGAIFQ
VEVPGSQHIDSQKKAIERMKDTLRIAYLTEAKVEKLCVWNNKTPHAIAAISMANGTGGSGLNISQ
HQCVKKQCPQNSGCFRHLDEREECKCLLNYKQEGDKCVENPNPTCNENNGGCDADAKCTEE
DSGSNGKKITCECTKPDSYPLFDGIFCSSSNFLGISFLLILMLILYSFIGGSGMNALRRLPVICSFL
VFLVFSNVLCFRGNNGHNSSSSLYNGSQFIEQLNNSFTSAFLESQSMNKIGDDLAETISNELVSV
LQKNSPTFLESSFDIKSEVKKHAKSMLKELIKVGLPSFENLVAENVKPPKVDPATYGIIVPVLTSLF
NKVETAVGAKVSDEIWNYNSPDVSESEESLSDDFFD

Yeast Transformation and Protein Expression

The constructed vector was introduced into *Saccharomyces boulardii* using a standard lithium acetate transformation method, with the gene expressed under the control of the ADH1 promoter and terminator. Following transformation, yeast cultures were grown in selective media to promote plasmid retention and optimal protein expression. Due to the unavailability of specific antibodies for CTB, MSP, and TOS, expression of the CTB-MSP-TOS fusion protein could not be confirmed through Western blot analysis. Instead, SDS-PAGE was performed to verify protein expression by detecting a band at the expected molecular weight corresponding to the fusion protein. This confirmed that the CTB-MSP-TOS fusion protein was successfully expressed in yeast.

Heat-Drying for Vaccine Preparation

Heat-drying of yeast cultures was performed at controlled temperatures (80–85°C) to ensure complete yeast cell inactivation while preserving antigen integrity. The heat-dried yeast was processed into a powder form for potential vaccine formulations.

Conclusion

This study demonstrates the feasibility of producing a CTB-MSP-TOS fusion protein in *Saccharomyces cerevisiae* and highlights the potential of heat-dried yeast as a stable and scalable platform for oral malaria vaccine development.

Keywords

Malaria, oral vaccine, CTB, MSP, TOS, yeast expression, mucosal immunity

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