Abstract

Malaria remains a serious human health problem and the disease is particularly prevalent in developing countries. Malaria is caused by a parasite of the genus *Plasmodium*. Diagnosis of malaria is required before any treatment or intervention. The gold standard for malaria diagnosis is microscopy. Rapid diagnostic tests (RDT) have been used at point-of-care because of their relative ease of use. Correct and accurate diagnosis of malaria is a prerequisite as a point-of-care intervention aimed at eradication, detection of an asymptomatic reservoir, quantification of parasite load and tracking of drug resistance to malaria. Therefore, RDTs that have high sensitivity and specificity are required.

Plasmodium LDH (*P*LDH) is one of the three proteins in current use as antibody targets in RDTs to detect human malaria. The other two proteins are *Plasmodium falciparum* histidine rich protein II (*Pf*HRPII) and aldolase. Of the three proteins, HRPII is the most widely used protein in RDTs in sub-Saharan Africa. One of the requirements in improving the current RDTs is to improve specificity and sensitivity*. Plasmodium falciparum* heat shock protein 70-1 (*Pf*HSP70-1) has been found to be immunogenic in infected humans, abundantly expressed in the asexual stages and thought to be a potential diagnostic target for malaria. Conditions were optimized for the recombinant expression of *Pf*LDH and *Pf*HSP70-1 in different growth media, temperatures, concentrations of IPTG, times of induction, stages at which IPTG is introduced, use of a single colony as starting material or a dilution of an overnight culture to inoculate fresh media. Terrific Broth was found to be a better growth medium than Lysogeny Broth and does not require induction with IPTG. Use of a single starting colony was found to be better than dilution of an overnight culture as it saves time. Inducing at the stationary phase of bacterial growth yields more soluble protein than at mid-log phase. Expressing at lower temperatures lower than 37°C produces more soluble protein than growth at 37°C.

Methods of lysing the host bacterial cells expressing *Pf*LDH and *Pf*HSP70-1 by freezing and thawing, sonication, lysozyme digestion and a combination of these methods were compared and optimized. The combination of freeze and thaw followed by repeated sonication was found to be optimal for lysing the *E. coli* host cells.

Both proteins were affinity purified using an affinity Talon® resin and proteins were eluted with 150 mM imidazole. The purification protocol was monitored by separating the proteins on a 12.5% sodium dodecyl sulphate polyacrylamide electrophoresis gel. Purifying recombinant protein at 4°C produced higher yields of recombinant protein. The identity of the recombinant protein was confirmed by probing a western blot with anti-His-tag antibodies against each protein. The anti-His-tag antibodies detected both *Pf*LDH and *Pf*HSP70-1.

Preliminary experiments on *Pf*LDH enzyme found that the recombinant enzyme was active. *In silico* studies were done on *Pf*LDH and *Pf*HSP70-1 to identify potential immunogenic

peptides to be used in the production of antibodies in chickens using a prediction program Predict7™. Potential post-translational modifications that may affect the activity of the native protein were evaluated. There is evidence that some post-translational modifications may affect the activity of proteins such as *Pf*LDH and *Pf*HSP70-1. Potential lysine acetylation, phosphorylation, glycosylation and proteolytic sites were identified on both *Pf*LDH and *Pf*HSP70-1.

This research generated soluble *Pf*LDH which can be used to produce antibodies in chickens and can be evaluated to detect malaria in blood.