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Identification of novel proteins on the surface of *Plasmodium falciparum* infected erythrocytes

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Malaria is one of the oldest diseases of human beings and is still widespread and dangerous in the world today, with 300 – 500 million clinical cases and 1 – 3 million malaria related deaths every year. The biggest current problem in malaria control is the worldwide spread of drug-resistance in Plasmodium ssp. The discovery of new anti-malarial drugs and vaccines is urgent. Most of the vaccines and many drugs focus on cell surface proteins. Furthermore, the characteristics of *Plasmodium falciparum*-infected erythrocytes, such as increased nutrient import, cytoadhesion and antigenic variation, can still not be fully explained by the proteins known to be located on the infected erythrocyte surface. A comprehensive investigation of surface parasite proteins may help to further understanding of the pathogenesis of malaria, as well as to provide new opportunities for therapeutic and prophylactic interventions. The complementation of *P. falciparum* DNA sequencing and the rapid improvement of mass spectrometry present the possibility to study *P. falciparum* membrane proteins using a proteomic approach.

In this study, we used a proteomic approach to search for novel *P. falciparum* membrane proteins on the host erythrocyte membrane. The host erythrocyte lacks a nucleus and so cannot generate new proteins itself. Thus, all the novel proteins generated during *P. falciparum* infection originate from the parasite. This offers us an easy and reliable tool to distinguish between the proteins of parasite or host origin by ^{35}S-methionine metabolic labeling during *in vitro* culture.

There are several methods to separate membrane proteins, such as sucrose gradient centrifugation and hypotonic lyses methods. But with these methods, it is very difficult to avoid contamination with cytosolic proteins. We used a non-cell permeable biotin product (Sulfo-NHS-LC-biotin) to label only the surface proteins followed by proteomic methods to separate and analyses novel surface proteins. Hemoglobin was used as a biomarker for cytoplasmic proteins because of its high concentration inside of erythrocytes. We have evidence from an Immunofluorescent assay and two dimensional gel electrophoresis that hemoglobin is not labeled by Sulfo-NHS-LC-biotin, which suggests that this biotin cross linker does only label cell surface proteins but not cytosolic proteins.

By overlaying two dimensional gel profiles of biotinylation and metabolic label, we found some candidate proteins, which were confirmed both by biotinylation and metabolic labeling. These proteins were analyzed by mass spectrometry. Those proteins found in two dimensional gel electrophoresis are: exported protein 1, exported protein 2, early transcribed membrane proteins and two uncharacterized proteins.

Since 2DGE has difficulties in resolving proteins with high molecular size, high hydrophobic character or extreme isoelectric point, we also used an avidin column to purify the biotinylated proteins and further separated them in one dimensional SDS-PAGE. From the soluble fraction of freeze-thaw lyses, we found some proteins involved in glycolysis, like enolase, glyceraldehydes-3-phosphate dehydrogenase and L-lactate dehydrogenase. Other soluble proteins include serine-repeat antigen protein (p126), heat shock protein/70, elongation factor 1α and hemoglobin chain B mutant. From Triton X-100 extracts of membrane proteins that were purified by the avidin column, we found major surface protein-1 (p195), erythrocyte membrane band 3, primase, merozoite surface protein-1 and heat shock protein. In other experiments using magnetic streptavidin beads to purify biotinylated proteins, there were some unspecific binding of proteins to the beads. Thus, some of the proteins found using the avidin column may also be attributed to unspecific binding. There are also interesting proteins in the SDS extracts, but unfortunately, both two dimensional gel electrophoresis and SDS-PAGE did not separate the proteins well. Chaperones and enzymes involved in glycolysis (for energy) account for the largest fraction of protein purified by the avidin column. This phenomenon may be explained by these proteins helping membrane proteins unfold and translocation to the cell surface.

We have no evidence to show whether the biotin cross linker has labeled proteins beneath the erythrocyte membrane or in the Maurer's clefts close to the membrane, because of the low resolution of IFA. Most of the proteins we found in 2DGE have been previously associated with the parasitophorous vacuolar membrane. It cannot be excluded that proteins in the PVM can also localize to the surface of infected erythrocyte, either transiently or stably; and it also cannot be excluded that the biotin cross linker can penetrate into the parasitophorous vacuole by some unknown mechanisms. If this biotin does penetrate inside the *P. falciparum*-infected erythrocyte without passing through the host cytoplasm, these proteins may help us elucidate the import pathway of biotin or other similar molecular nutrients or drugs into the parasite-infected erythrocyte.