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Immunology

# Identification of two integral membrane proteins of *Plasmodium falciparum*

(malaria/merozoite surface/Triton X-114/glycosylphosphatidylinositol anchor/rhoptry antigen)

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**ABSTRACT** **We describe the isolation and cloning of two integral membrane protein antigens of *Plasmodium falciparum*. The antigens were isolated by Triton X-114 temperature-dependent phase separation, electrophoretically transferred to nitrocellulose, and used to affinity-purify monospecific human antibodies. These antibodies were used to isolate the corresponding cDNA clones from a phage λgt11-Amp3 cDNA expression library. Clone Ag512 corresponds to a $M_r$ 55,000 merozoite rhoptry antigen, and clone Ag513 corresponds to a $M_r$ 45,000 merozoite surface antigen. Both proteins can be biosynthetically labeled with [$^3$H]glucosamine and [$^3$H]myristic acid, suggesting that they may be anchored in membranes via a glycosylphosphatidylinositol moiety. Similarities in the C-terminal sequences of the $M_r$ 45,000 merozoite surface antigen and the *Trypanosoma brucei* variant surface glycoproteins provides further evidence that this antigen has a glycosylphosphatidylinositol anchor.**

Integral membrane proteins of *Plasmodium falciparum* sporozoites and merozoites are potential components of a malaria vaccine. One such protein is the precursor to the major merozoite surface antigens (PMMSA), which is proteolytically processed to generate three antigens on the surface of the merozoite (1, 2). Others antigens have been reported to be present on the merozoite surface. Two of these are apparently adsorbed as they lack structural features required for anchoring in the membrane (3–5). Other may be integral membrane proteins, although structural studies have yet to be reported (6–8). We describe here a novel approach to the selection of recombinant clones expressing *P. falciparum* integral membrane protein antigens. Integral membrane proteins were isolated by temperature-dependent phase separation using the nonionic detergent Triton X-114 and blotted onto nitrocellulose. Human antibodies affinity-purified on these immobilized antigens were used to identify cDNA clones encoding the corresponding polypeptide. With this strategy we have isolated clones encoding two *P. falciparum* merozoite antigens. One is an integral membrane protein of $M_r$ 45,000 associated with the merozoite surface. The other is a polypeptide of $M_r$ 55,000 located in the rhoptries, a pair of flask-shaped organelles at the apical end of the merozoite. Both antigens were biosynthetically labeled with [$^3$H]myristic acid and [$^3$H]glucosamine, consistent with posttranslational modification by a glycosylphosphatidylinositol (OsePtdIns) anchor. The cDNA for the $M_r$ 45,000 merozoite surface antigen (MSA) has been completely sequenced.‡ The deduced primary structure provides further evidence that this antigen is anchored in the merozoite surface membrane by a OsePtdIns moiety.

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## MATERIALS AND METHODS

**Parasites.** The origin of *P. falciparum* isolate FCQ27/PNG (FC27) has been described elsewhere (9). Parasites synchronized by sorbitol treatment were cultured at 0.25% hematocrit with parasitemias ranging from 2% to 7%, washed, and stored as 1-ml packed-cell aliquots at −70°C.

**Triton X-114 Solubilization and Phase Separation.** Triton X-114 solubilization and temperature-dependent phase separation of parasite antigens were performed essentially as described by Bordier (10) and adapted in this instance as follows. Triton X-114 was precondensed in human tonicity phosphate-buffered saline (HTPBS; 137 mM NaCl/2.7 mM KCl/8.1 mM sodium phosphate, pH 7.2). A 1-ml aliquot of pelleted parasitized erythrocytes was solubilized in 15 ml of 0.5% Triton X-114 for 90 min on ice, with mild mixing at 10-min intervals. A 1-ml sample of the total material was removed and snap-frozen. The remaining 15 ml was then centrifuged at 10,000 × *g* for 15 min at 4°C to remove insoluble material. The supernatant was collected, and the centrifugation step was repeated. The insoluble pellet material was washed three times in 0.5% Triton X-114 and then frozen. The remaining 15 ml of detergent-soluble material was carefully layered over a cold 10-ml sucrose cushion (6% sucrose/0.06% Triton X-114) in a 50-ml tube with minimal disruption to the interface and placed in a 37°C warm room for 5 min. The tube was then transferred to a centrifuge in the warm room and spun at 500 × *g* for 5 min. After centrifugation, the 15-ml detergent-depleted upper layer was collected and chilled on ice. The 10-ml sucrose cushion was discarded, and the detergent-enriched pellet (1–2 ml) was resuspended on ice with 10 ml of cold HTPBS. The resuspended detergent-enriched phase was again layered over a sucrose cushion, brought to 37°C for 5 min, and repelleted by centrifugation. After this second precipitation, the detergent-enriched pellet, containing the putative integral membrane proteins, was resuspended to 5 ml in HTPBS and snap-frozen. The upper layer from the sucrose cushion separation was further depleted of hydrophobic proteins by the addition of 1 ml of 11.4% (wt/vol) Triton X-114. It was then chilled on ice, mixed, and warmed to 37°C for 5 min, and the detergent was sedimented by centrifugation. The pellet was discarded. This cycle of depletion was repeated three times, and the resulting detergent-depleted aqueous solution was then snap-frozen. All samples were stored at −70°C until analysis.

**Electrophoresis and Immunoblotting.** Samples for $NaDodSO_4$/PAGE were processed under reducing conditions and electrophoresed on 10% slab gels (11). Samples to

Abbreviations: VSG, variant surface glycoprotein; MSA, merozoite surface antigen; OsePtdIns, glycosylphosphatidylinositol; PMMSA, precursor to the major merozoite surface antigen; PNG, Papua New Guinean.

‡The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03828).

be analyzed by immunoblotting were then electrophoretically transferred to nitrocellulose, blocked, and probed with antibody and with $^{125}$I-labeled protein A (11).

**Affinity Purification of Polyclonal Monospecific Human Antibodies.** The source of human plasma from which antibodies were extracted has been described (11). Elution of antibodies from nitrocellulose strips after electrophoresis and immunoblotting of antigenic material (12) was modified in this instance as follows. Briefly, reduced samples of Triton X-114-extracted membrane antigens were electrophoretically separated and transferred to nitrocellulose. Radioactive $^{14}$C-labeled high molecular weight markers (Amersham) were used to locate the transferred proteins of interest. This region was then cut from the nitrocellulose sheet as a strip. Multiple strips were incubated with human serum from Papua New Guineans (PNG) chronically exposed to malaria. After incubation the serum was removed, and the strips were washed with several changes of 5% skim milk powder in HTPBS (blotto) and HTPBS, followed by a 15-min incubation in borate buffer (0.1 M boric acid/0.5 M sodium chloride, pH 8.0) and a final wash in HTPBS. The antibodies were then eluted at low pH with glycine buffer (0.1 M glycine/0.15 M sodium chloride, pH 2.8).

Eluted antibodies were immediately neutralized with 2 M Tris·HCl (pH 8.0) and stored at 4°C with 0.05% sodium azide. The affinity-purified antibodies were diluted in blotto to probe antigens electroblotted to nitrocellulose and to screen cDNA libraries (13). Monospecific antibodies were also eluted directly from immunopositive clones of *Escherichia coli* lysogens of phage λAmp-3 grown as a lawn on nitrocellulose. Lawns of clones were plated, induced, and lysed *in situ* to allow binding of the cellular protein to the nitrocellulose filter (11). After incubation with PNG sera, the monospecific antibodies were eluted as above. These antibodies were used to confirm the identity of the clones and, as probes, to localize the antigen within the parasite by immunofluorescence assays.

**Antibody Depletion.** One-milliliter cultures of immunopositive clones were induced and pelleted by centrifugation, and the supernatents were removed. Pellets were frozen and thawed three times, and the lysed cells were resuspended in 1 ml of HTPBS. A 20-μl aliquot of PNG pooled sera was added and incubated with the lysate for 3 hr at 4°C. The cellular debris was then spun down at 10,000 × *g*, and the supernatant was used to probe immunoblots of Triton X-114-extracted parasite membrane preparations.

**Indirect Immunofluorescence, Metabolic Labeling, and Immunoprecipitation.** Indirect immunofluorescence on thin blood films of cultured *P. falciparum* was as described (11). Preparations of stage-specific *P. falciparum* proteins biosynthetically labeled with either [$^3$H]glucosamine or [$^3$H]-myristic acid were provided by S. Lustigman (Walter and Eliza Hall Institute). Immunoprecipitation of parasite antigens from these preparations was performed by using affinity-purified human antibodies and *Staphylococcus aureus* as described (14).

**Sequence Determination.** Subcloning and sequence determination were performed as previously described (15).

## RESULTS

**Identification of Antigenic Integral Membrane Proteins of *P. falciparum*.** Proteins of asynchronous or stage-specific cultures of *P. falciparum* were fractionated by phase separation with Triton X-114 by the procedure of Bordier (10), modified as in *Materials and Methods* to obtain a Triton X-114 phase free of nonintegral membrane proteins. The volumes of 0.5% Triton X-114 used, the incubation times, and the mixing for complete solubilization were found to be critical factors.

When immunoblots of the extracted material were probed with sera from individuals chronically exposed to malaria, we identified a subset of antigens presumed to be integral membrane proteins (Fig. 1). Particularly prominent in the Triton X-114 phase was a set of antigens with $M_r$ values of 55,000, 45,000, 42,000, 30,000, and 21,000. When these extracts were probed with antibodies to various *P. falciparum* antigens cloned in *Escherichia coli*, we found that the $M_r$ 21,000 antigen was the circumsporozoite protein-related antigen. This antigen is known to have a 26-amino acid hydrophobic sequence typical of integral membrane proteins (15, 16) and has been localized to membranous structures by immunoelectron microscopy (17).

As the other prominent Triton X-114 soluble antigens did not correspond to known *P. falciparum* antigens, we isolated the corresponding clones from a cDNA expression library. The strategy we adopted was to isolate antibodies from human sera by affinity purification on the $M_r$ 30,000–65,000 antigens immobilized on nitrocellulose and then to use these antibodies to identify recombinant clones expressing the corresponding polypeptides. The advantage in this methodology was that, although none of the antigens described (Fig. 1) were abundant enough to be detected by Coomassie staining of NaDodSO$_4$/PAGE gels, it was possible to affinity-purify enough antibody to screen a cDNA expression library of >15,000 clones by using <1 ml of parasitized erythrocytes. Ten immunopositive clones were isolated after screening the 15,000 colonies. Sibling analysis revealed clones of two classes, and the most immunoreactive clone of each group, Ag512 and Ag513, was selected for characterization. Antibodies purified on the two selected recombinant clones were used to probe immunoblots of proteins derived from asynchronously cultured *P. falciparum*. Antibodies to Ag512 recognized a $M_r$ 55,000 parasite protein, whereas antibodies to Ag513 recognized a $M_r$ 45,000 protein. These antigens were identified as the corresponding Triton X-114-soluble proteins. Sonicates of both clones Ag512 and Ag513 were also found to be capable of depleting the antibodies present in adult PNG immune sera that react with these parasite antigens (see Fig. 5*C*).

**Ultrastructural Location and Stage Specificity of Ag512 and Ag513.** Indirect immunofluorescence microscopy with human antibodies purified on Ag513 stained mature asexual blood forms. A grape-like pattern was observed on seg-

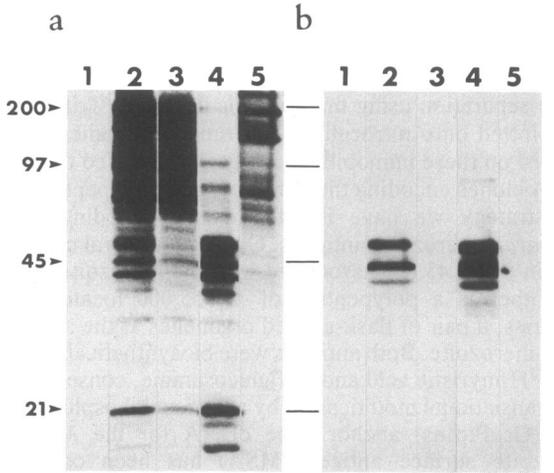


FIG. 1. Identification of putative integral membrane protein antigens of *P. falciparum*. Immunoblots of fractions generated by Triton X-114 extraction probed with pooled PNG sera (*a*) or affinity-purified antibody to antigens included in the $M_r$ 30,000–65,000 region (*b*). Lanes: 1, uninfected erythrocytes; 2, total parasitized erythrocytes; 3, aqueous phase; 4, Triton X-114 phase; 5, detergent-insoluble material. Molecular weights are shown × $10^{-3}$.

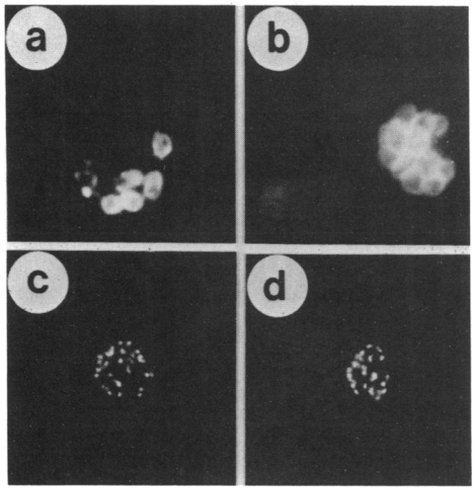


FIG. 2. Immunofluorescence microscopy of late-stage parasites probed with affinity-purified antibodies to Ag513 (*a* and *b*) or Ag512 (*c* and *d*).

mented schizonts (Fig. 2, *a* and *b*), a characteristic pattern noted previously with a MSA (1). We refer to this molecule as the $M_r$ 45,000 MSA. In contrast, antibodies purified on Ag512 from the same pool of human sera gave a punctate pattern of immunofluorescence within merozoites (Fig. 2, *c* and *d*), characteristic of antigens located in the rhoptries (18). A number of other rhoptry proteins are components of either a high or low molecular weight complex (18–21). Immunoprecipitation studies (data not shown) suggest that Ag512 is not related to either complex.

Immunoblotting studies showed that the antigens corresponding to both Ag512 and Ag513 are present in various amounts in all stages of the asexual life cycle. Consistent with the indirect immunofluorescence microscopic patterns, the $M_r$ 45,000 MSA is least abundant in the ring stage and increases in abundance as the parasite matures. The $M_r$ 55,000 rhoptry antigen also increases in abundance as the parasite matures but, in contrast to the $M_r$ 45,000 MSA, which in all stages partitioned totally into the Triton X-114 phase, the solubility of the rhoptry antigen varied in different life-cycle stages: the antigen in the late schizont and merozoite stages was only found in the Triton X-114 phase, but in the ring and trophozoite stage, equal amounts of the antigen could be found in the insoluble pellet. This may indicate that this rhoptry antigen associates with components of the erythrocyte membrane skeleton during the process of invasion, as appears to be the case with the ring-infected erythrocyte surface antigen, another antigen located to secretory organelles within merozoites (22).

**Ag513 Has the Primary Structure of an Integral Membrane Protein.** The primary structure of the $M_r$ 45,000 MSA determined from the nucleotide sequence of the full-length 1138-base-pair (bp) cDNA of Ag513 (Fig. 3) was consistent with that of an integral membrane protein. The sequence contains only one long open reading frame, commencing at base 94 and continuing to base 886. The A+T content of the 5′ sequence preceding base 94 is >94%, whereas the A+T content of the predicted coding region is ≈65%, as found for all *P. falciparum* genes studied, suggesting that base 94 is the actual initiation codon (23). At the N-terminal end of the predicted polypeptide, there is a sequence of 15 hydrophobic amino acids flanked by charged residues—typical of a signal sequence. The sequence encodes two identical copies of a 32-amino acid sequence, arranged in tandem, beginning at residue 60 and continuing to residue 124. Preceding the C terminus is a sequence of 17 hydrophobic amino acids. As there is no charged residue between the hydrophobic sequence and the C terminus, this is not a typical transmembrane anchor. The predicted polypeptide is rich in serine and threonine, which compose ≈28% of the sequence.

A search of the available data bases failed to detect any overall homology of Ag513 with known proteins, including the $M_r$ 43,000 fragment of the PMMSA. However, one of several proteins having a short region of homology with the $M_r$ 45,000 MSA was a variant surface glycoprotein (VSG) of *Trypanosoma brucei*. The homologous sequences in both

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GTATATTAAATTTCATTTTGTAATTTAATATTTTTTAATAATTAATTTAAAAGAATTGTATTTATTAATTCTTAACATATTATATTAGTC    90

   MetLysValIleLysThrLeuSerIleIleAsnPhePheIlePheValThrPheAsnIleLysAsnGluSerLysTyrSerAsnThr
AAAATGAAGGTAATTAAAACATTGTCTATTATAAATTTCTTTATTTTTGTTACCTTTAATATTPPAAATGAAAGTAAATATAGCAACACA  180

PheIleAsnAsnAlaTyrAsnMetSerIleArgArgSerMetAlaAsnGluGlySerAsnThrAsnSerValGlyAlaAsnAlaProAsn
TTCATAAACAATGCTTATAATATGAGTATAAGGAGAAGTATGGCAAATGAAGGTTCTAATACTAATAGTGTAGGTGCAAATGCTCCAAAT  270

AlaAspThrIleAlaSerGlySerGlnArgSerThrAsnSerAlaSerThrSerThrThrAsnAsnGlyGluSerGlnThrThrThrPro
GCTGATACTATTGCTAGTGGAAGTCAAAGGAGTACAAATAGTGCAAGTACTAGTACTACTAATAATGGAGAATCACAAACTACTACTCCT  360

ThrAlaAlaAspThrIleAlaSerGlySerGlnArgSerThrAsnSerAlaSerThrSerThrThrAsnAsnGlyGluSerGlnThrThr
ACCGCTGCTGATACTATTGCTAGTGGAAGTCAAAGGAGTACAAATAGTGCAAGTACTAGTACTACTAATAATGGAGAATCACAAACTACT  450

ThrProThrAlaAlaAspThrProThrAlaThrGluSerIleSerProSerProProIleThrThrThrGluSerSerLysPheTrpGln
ACTCCTACCGCTGCTGATACCCCTACTGCTACAGAAAGTATTTCACCTTCACCACCCATCACTACTACAGAAAGTTCAAAGTTCTGGCAA  540

CysThrAsnLysThrAspGlyLysGlyGluGluSerGluLysGlnAsnGluLeuAsnGluSerThrGluGluGlyProLysAlaProGln
TGCACAAATAAAACAGACGGTAAAGGAGAAGAGAGTGAAAAACAAAATGAATTAAATGAATCAACTGAAGAAGGACCCAAAGCTCCACAA  630

GluProGlnThrAlaGluAsnGluAsnProAlaAlaProGluAsnLysGlyThrGlyGlnHisGlyHisMetHisGlySerArgAsnAsn
GAACCTCAAACGGCAGAAAATGAAAATCCTGCTGCACCAGAGAATAAAGGTACAGGACAACATGGACATATGCATGGTTCTAGAAATAAT  720

HisProGlnAsnThrSerAspSerGlnLysGluCysThrAspGlyAsnLysGluAsnCysGlyAlaAlaThrSerLeuLeuSerAsnSer
CATCCACAAAATACTTCTGATAGTCAAAAAGAATGTACCGATGGTAACAAAGAAAACTGTGGAGCAGCAACATCCCTCTTAAGTAACTCT  810

SerAsnIleAlaSerIleAsnLysPheValValLeuIleSerAlaThrLeuValLeuSerPheAlaIlePheIle***
AGTAATATTGCTTCAATAAATAAATTTGTTGTTTTAATTTCAGCAACACTTGTTTTATCTTTTGCCATATTCATATAATTCTCTTCATTT  900
TAAAACATTGACTTATATAATATATATATATATATATATATATATATATATATATATATATGTATATATATATATATATGTACATATAGATGTGTT  990
TATTTTTTTATATAATAAAATGCCATATATACATTCGAATTTTTTATGTCACCACATGATTATGTATACGTTTGAATATTTATTATAATG 1080
CATGGTTTTTACATATGAAATAAATAATGCACGCATTGAAAAAACTAAAAAATAACAT                                 1138
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FIG. 3. Nucleotide and predicted amino acid sequence of Ag513. The predicted signal peptide and the region of the Ag513 sequence that shows homology with the signal for acylation in the trypanosome VSG are underlined. The two copies of the 32-amino acid repeat are in bold type.

| *T.brucei* VSG's | | | ref |
|---|---|---|---|
| MITat 1.4a | ENNACKD | SSILVTKKFALTVVSAAFVALLF | 24 |
| ILTat 1.3 | EGETCKD | SSFILNKQFALSVVSAAFAALLF | 26 |
| ILTat 1.4 | TTNTTGS | HSFVINKTPLLLAFLLF | 26 |
| TxTat 1 | TSNTTAS | NSFVINKAPLLLGFLLT | 24 |
| ***P.falciparum* Ags** | | | |
| Ag 513 | SLLSNSS | NIASINKFVVLISATLVLSFAIFI | |
| Pf 195 FC/27 | DGIFCSS | SNFLGISFLLILMLILYSFI | 27 |
| Pf 195 WELL | MVIFCSS | SNFLGISFLLILMLILYSFI | 28 |
| Pf CSP | KMEKCSS | VFNVVNSSIGLIMVLSFLFLN | 29 |

FIG. 4. C-terminal sequences of *T. brucei* VSGs and several *P. falciparum* antigens assumed to be anchored by OsePtdIns moieties. The space in the sequences represents the cleavage site for processing of the polypeptide to a OsePtdIns-anchored membrane protein. In the case of the VSG sequences, the mature C-terminal residue is known. Alignment of the *P. falciparum* sequences is based on the predictions of Ferguson and Williams (24).

proteins precede and include part of the hydrophobic sequence at the C terminus of the polypeptide chain (Fig. 4). In the VSGs this sequence is thought to be a signal for attachment of the OsePtdIns anchor moiety (ref. 24; P. Englund, personal communication) that, after cleavage of the C-terminal hydrophobic sequence, anchors the VSG to the trypanosome membrane. This homology suggested that the $M_r$ 45,000 MSA may also be modified by PtdIns-dependent acylation. This was confirmed by metabolic labeling of cultured parasites with [$^3$H]myristate and [$^3$H]glucosamine (Fig. 5*b*). Interestingly, the $M_r$ 55,000 rhoptry antigen was also labeled with both these radioactive metabolites, suggesting that both these merozoite integral membrane antigens are modified with OsePtdIns moiety.

## DISCUSSION

Temperature-dependent phase separation with the nonionic detergent Triton X-114 coupled with affinity purification of human antibodies has allowed us to identify a number of putative integral membrane protein antigens of the asexual blood stages of *P. falciparum*. cDNA clones in phage λgt11-Amp3 for two of these antigens were selected by using human antibodies affinity-purified on the antigens electrophoretically transferred to nitrocellulose. Recombinant clones corresponding to the $M_r$ 45,000 MSA and the $M_r$ 55,000 rhoptry antigen have not been identified previously. However, monoclonal antibodies specific for the $M_r$ 45,000 MSA exist and are capable of blocking merozoite invasion of erythrocytes *in vitro* (7, 8). Using these monoclonals, we have been able to establish that the $M_r$ 45,000 MSA described here corresponds to the $M_r$ 55,000 antigen described by Epping *et al.* (7) and the $M_r$ 45,000 antigen referred to as GYMMSA by Ramasamy (8). Given the range of apparent molecular weights in different studies, it is possible that the gp56 MSA (6) is also the same antigen. The sequence of Ag513 shows that the $M_r$ 45,000 MSA is not the C-terminal fragment of the PMMSA, which is approximately of the same size. The sequence also suggests that the $M_r$ 45,000 MSA identified here, like the PMMSA, is an integral membrane protein.

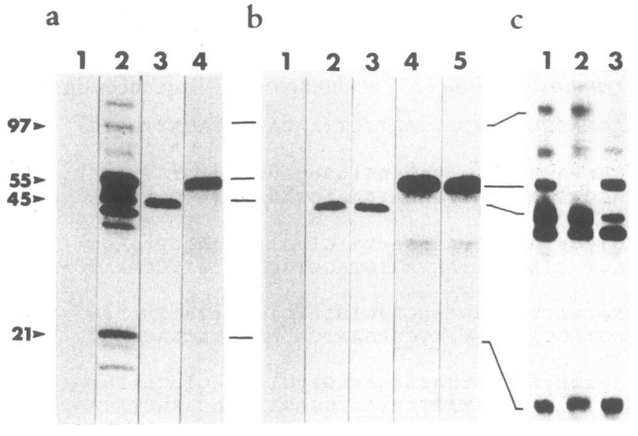


FIG. 5. (*a*) Clones Ag512 and Ag513 correspond to two different integral membrane proteins. Immunoblots with antibodies affinity-purified on Ag513 (lane 3) and Ag512 (lane 4) reacted with $M_r$ 45,000 and $M_r$ 55,000 polypeptides, respectively, two of the dominant antigens recognized by PNG pooled sera in the Triton X-114 extract (lane 2). This pooled sera did not react with any antigens in uninfected erythrocytes (lane 1). (*b*) The $M_r$ 45,000 MSA and the $M_r$ 55,000 rhoptry antigen are labeled with [$^3$H]glucosamine and [$^3$H]myristate. Lysates of parasites metabolically labeled with [$^3$H]glucosamine (lanes 2 and 4) or [$^3$H]myristate (lanes 3 and 5) were immunoprecipitated with affinity-purified anti-Ag513 antibodies (lanes 2 and 3) or with affinity-purified anti-Ag512 antibodies (lanes 4 and 5). No labeled polypeptides were precipitated with a normal human serum from a lysate of parasites labeled with both metabolites (lane 1). (*c*) Absorption of antibody reactivity to parasite antigens with clones Ag512 and Ag513. A PNG sera pool was unabsorbed (lane 1) or absorbed with sonicates of Ag512 (lane 2) or Ag513 (lane 3) and used to probe immunoblotted Triton X-114-extracted antigens. Molecular weights are shown $\times 10^{-3}$.

The PMMSA has been shown to be acylated via a diglyceride moiety, and it is assumed that this reflects posttranslational attachment of a OsePtdIns anchor (24, 25). The results presented here indicate that both the $M_r$ 45,000 MSA and the $M_r$ 55,000 rhoptry antigen also have been modified with a OsePtdIns anchor. By analogy with the processing of the VSG in *T. brucei*, the $M_r$ 45,000 MSA is assumed to be anchored into the merozoite surface membrane by a OsePtdIns moiety, which is attached to the polypeptide after cleavage of the C-terminal hydrophobic sequence. The C-terminal sequences of several *T. brucei* VSGs are aligned to Fig. 4 with the C-terminal sequences of the *P. falciparum* antigens that are assumed to be modified with a OsePtdIns anchor. The C-terminal residues of the anchored VSGs are known, whereas those of the *P. falciparum* antigens are suggested (24, 26–29). This alignment provides two serine residues on the N-terminal side of the cleavage site in all three *P. falciparum* antigens. This structural feature is also found in many VSGs. There are a number of VSGs that have a serine doublet on the C-terminal side of the cleavage site, and there are others in which the serine residues span the cleavage site and are separated by an intervening residue (Fig. 4).

When merozoites invade erythrocytes, they shed material from the merozoite surface (30). However, as the acylated C-terminal fragment of the PMMSA is carried into the ring-stage parasite, cleavage of the putative OsePtdIns anchor of this molecule is probably not involved in the release of the merozoite coat (31). The $M_r$ 45,000 MSA is in low abundance in the ring stage and, therefore, is presumably released from the merozoite at the time of invasion. The

release of this protein during invasion may indicate a role of this membrane molecule in the merozoite attachment to the erythrocyte. After attachment the merozoite is known to reorient. It has been shown that OsePtdIns anchors may confer a higher rate of lateral diffusion in the phospholipid bilayer than would more conventional protein anchors (24, 32). Such an increased mobility of the $M_r$ 45,000 MSA because of the lipid nature of the anchor moiety might facilitate the reorientation event preceding invasion.

The finding that the $M_r$ 55,000 antigen is located in the rhoptries was unexpected. It has not yet been possible to determine whether this antigen is part of the rhoptry secretion or whether it is a component of the membrane of this organelle. Six other distinct polypeptides have been described in the rhoptries of *P. falciparum* merozoites (18–21), but none of them appear to correspond to the $M_r$ 55,000 antigens described here. Although the antigen has the characteristics of an integral membrane protein, such proteins may be contained in the rhoptry secretions. The studies of Bannister *et al.* (33) and Stewart *et al.* (34) indicate that the rhoptry discharge includes lipid capable of forming membranous structures. The release of material possibly containing integral membrane proteins may play a critical role in the formation of the parasitophorous vacuole in which the intracellular parasite resides.

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