Mapping the Binding Domains Involved in the Interaction between the *Plasmodium falciparum* Knob-associated Histidine-rich Protein (KAHRP) and the Cytoadherence Ligand *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1)*

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*The abbreviations used are: KAHRP, knob-associated histidine-rich protein; MESA, mature parasite-infected erythrocyte surface antigen; PfEMP, *P. falciparum* erythrocyte membrane protein; PCR, polymerase chain reaction; GST, glutathione S-transferase; PBS, phosphate-buffered saline; BSA, bovine serum albumin; *K*_D(kin) and *K*_D, dissociation constants determined by kinetic and Scatchard analysis, respectively; RESA, ring-infected erythrocyte surface antigen.

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*Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) clusters at electron-dense knob-like structures on the surface of malaria-infected red blood cells and mediates their adhesion to the vascular endothelium. In parasites lacking knobs, vascular adhesion is less efficient, and infected red cells are not able to immobilize successfully under hemodynamic flow conditions even though PfEMP1 is still present on the exterior of the infected red cell. We examined the interaction between the knob-associated histidine-rich protein (KAHRP), the parasite protein upon which knob formation is dependent, and PfEMP1, and we show evidence of a direct interaction between KAHRP and the cytoplasmic region of PFEMP1 (VARC). We have identified three fragments of KAHRP which bind VARC. Two of these KAHRP fragments (K1A and K2A) interact with VARC with binding affinities (KD(kin)) of 1 x 10^-7 and 3.3 x 10^-8 M, respectively, values comparable to those reported previously for protein-protein interactions in normal and infected red cells. Further experiments localized the high affinity binding regions of KAHRP to the 63-residue histidine-rich and 70-residue 5' repeats. Deletion of these two regions from the KAHRP fragments abolished their ability to bind to VARC. Identification of the critical domains involved in interaction between KAHRP and PfEMP1 may aid development of new therapies to prevent serious complications of *P. falciparum* malaria.

*Plasmodium falciparum* causes the most severe form of human malaria and is responsible for at least two million deaths worldwide each year. The excessive pathogenicity of *P. falciparum* appears to be related to an ability to cause infected red blood cells to adhere to vascular endothelium and sequester in the microvasculature of a variety of organs (1). Knob-like, electron-dense structures located at the membrane surface of infected red cells are the points of adhesion between infected red cells and the vascular endothelium (2). Knob formation is critically dependent upon the expression of the knob-associated histidine-rich protein (KAHRP) (3), an 85–105-kDa parasite protein that associates with the red cell cytoskeletal proteins spectrin and actin (4). In addition to KAHRP, several other parasite proteins are also present in knobs including the mature parasite-infected erythrocyte surface antigen (MESA), *P. falciparum* erythrocyte membrane protein 3 (PfEMP3) and *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) (5). PfEMP1 is a large (200–350 kDa) antigenically diverse parasite protein that is exported from the intracellular parasite and inserted into the red cell membrane (6, 7) where it clusters over the knobs (8). It is the ligand on the surface of infected red cells which mediates adhesion to a number of endothelial cell receptors including intercellular adhesion molecule 1, CD36, and thrombospondin (9), resulting in the accumulation of infected red cells in the microvasculature (2).

PfEMP1 proteins are encoded by var genes, a highly variable gene family with some 30–50 copies present per parasite genome (10). The first exon of a var gene encodes both the ectodomain and the transmembrane domain of PfEMP1 (11); the second exon encodes a relatively conserved cytoplasmic domain (VARC), also called the acidic terminal segment (11). Amino acid sequence alignments of VARC regions from various var genes have shown that there is general conservation with some diversity among var genes which allows assignment of the highly charged VARC sequences into two general families or into intermediate hybrid groups (12).

The mechanism by which PfEMP1 is anchored into the membrane of infected red cells has not yet been elucidated, but detergent extraction experiments of infected red cells have revealed that PfEMP1 extraction requires ionic detergents such as SDS (13), suggesting that PfEMP1 is associated with the cytoskeleton, perhaps by interaction of the VARC domain with the various components of the knobs. Previous studies have shown that parasites lacking a functional KAHRP gene, resulting either from loss of the structural gene by spontaneous deletion or by targeted gene disruption (14, 15), do not express knobs (termed knobless) and show defects in their ability to adhere to endothelial cells (15, 16). Knobless infected red cells still express PfEMP1 on their membrane surface and are capable of adhering at normal levels when tested in static assays.

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1 The abbreviations used are: KAHRP, knob-associated histidine-rich protein; MESA, mature parasite-infected erythrocyte surface antigen; PfEMP, *P. falciparum* erythrocyte membrane protein; PCR, polymerase chain reaction; GST, glutathione S-transferase; PBS, phosphate-buffered saline; BSA, bovine serum albumin; *K*_D(kin) and *K*_D, dissociation constants determined by kinetic and Scatchard analysis, respectively; RESA, ring-infected erythrocyte surface antigen.
Remarkably, however, when tested under flow conditions that mimic those in the vasculature in vivo (15) they show a grossly reduced ability to adhere. This decreased adhesiveness under flow can be best interpreted as being the result of defective anchoring of PfEMP1 into the membrane of knobless infected red cells, perhaps due to the absence of an anchoring interaction.

In this study we have examined whether VARC may bind to KAHRP using recombinantly expressed fusion fragments of KAHRP and VARC. The protein binding data obtained demonstrate that VARC binds to at least two distinct regions of the KAHRP protein with dissociation constants ($K_D$) comparable to those of other important protein-protein interactions among red cell cytoskeletal proteins and between red cell cytoskeletal proteins and parasite proteins in infected red cells. Experiments using smaller recombinant fusion proteins and deletion mutant proteins have mapped the two main VARC binding regions of KAHRP to regions of 63 and 70 amino acid residues, respectively.

### EXPERIMENTAL PROCEDURES

**Construction of Protein Expression Clones in pGEX**—The VARC domain of PfEMP1 was amplified from genomic *P. falciparum* 1G2 DNA by PCR using oligonucleotide primers p575 and p576 (Table I), designed with reference to the nucleotide sequence of *var* 2 (11). Regions of the second exon of KAHRP were amplified by PCR using the oligonucleotide primers listed in Table I. PCR products were cloned into the *Escherichia coli* pGEX protein expression plasmids (Amersham Pharmacia Biotech), and the nucleotide sequence and reading frame of cloned inserts were confirmed by automated dye terminator sequencing.

**Expression and Purification of Recombinant GST Fusion Proteins**—VARC and regions of KAHRP were expressed as glutathione S-transferase (GST) fusion proteins in *E. coli* BL21(DE3) cells (Novagen Inc., Milwaukee, WI) and purified using standard techniques (17). The purified GST fusion proteins were dialyzed overnight against phosphate-buffered saline (PBS; 10 mM Na$_2$HPO$_4$/NaH$_2$PO$_4$, pH 7.4, containing 0.15 M NaCl) at 4 °C, with one buffer change before proteins were concentrated by centrifugal filtration. Total protein concentrations were determined by Bio-Rad protein assays.

**In Vitro Binding Assays**—Binding assays were performed using an assay based on that described previously by Bennett et al. (17). Briefly, KAHRP GST fusion proteins were coated into the wells of 96-well microtiter plates (Dynatech Laboratories, Inc., Chantilly, VA). For each protein, wells were coated in triplicate using ~3 μg of protein/well and incubated at 4 °C overnight. After blocking with 5% (w/v) BSA in PBS overnight at 4 °C and washing twice with PBS, 5 μg of GST-VARC, diluted in PBS containing 0.05% (v/v) Tween 20, was added to each well and incubated at 4 °C overnight. Wells were washed five times with PBS, and then proteins were stripped from the wells using heated (70 °C) reducing SDS sample buffer and resolved by SDS-polyacrylamide gel electrophoresis in 10% polyacrylamide gels. Proteins were transferred to Polyscreen™ polyvinylidene difluoride transfer membrane (NEN Life Science Products) and interacting GST-VARC1 proteins immunoblotted using a polyclonal rabbit anti-GST-VARC1 antisera (preabsorbed for anti-GST reactivity) followed by a sheep anti-rabbit IgG-horseradish peroxidase conjugate. Detection was performed using NEN Renaissance Western blot chemiluminescence reagent according to the manufacturer’s instructions (NEN Life Science Products) and visualized on x-ray film.

**Binding Assays Using Resonant Mirror Detection**—Protein-protein interactions were studied using the resonant mirror detection method (18–20) of the IAsys™ (Affinity Sensors, Cambridge, U. K.). Two dissociation constants ($K_D$$_{(Scat)}$ and $K_D$$_{(hemi)}$) were determined by kinetic and Scatchard analysis using the results from the binding assay as outlined below (21). GST, BSA, and GST-VARC1 were immobilized on an aminosilane cuvette, which had been activated with bis-(sulfosuccinimidyl) suberate (Pierce Chemical Co.) according to the manufacturer’s instructions, with slight modifications. After immobilization the cuvette was blocked with 1% (w/v) BSA in PBS. All experimental procedures were carried out in PBS containing 0.05% (v/v) Tween 20 at 25 °C under constant stirring. Then both the association rate constant ($k_a$) and the dissociation rate constant ($k_d$) were measured by using the software package FASTfit™ (Affinity Sensors, Cambridge, U.K.). $K_D$$_{(Scat)}$ was calculated from Equation 1 (18–20). $K_D$$_{(Scat)}$ = $k_d$/ $k_a$ (Eq. 1)

Dissociation constants by Scatchard analysis ($K_K$ = $k_d$/ $k_a$) were also derived from the binding data. The maximal extent of binding ($R_{max}$) at various concentrations of [B] were derived from the binding curves.

$$K_D$$$_{(Scat)}$ = $k_d$/ $k_a$ (Eq. 1)

The slope of the plot of $R_{eq}$ versus $R_{max}[B]$ provides the value of $K_D$$_{(Scat)}$ is then calculated.

$$K_D$$$_{(hemi)}$ = $R_{max}/[B]$ (Eq. 2)

In the present study, the $K_D$$_{(hemi)}$ derived under a variety of experimental conditions closely matched the corresponding $K_D$$_{(Scat)}$ values calculated.

At least two cuvettes were used to validate the simulation process.

### Table I

| Oligonucleotide primer | Use | Nucleotide sequence (5’ → 3’) |
|------------------------|-----|------------------------------|
| p296                   | K1 (F), K1A (F) | TGC AAT AAT GGA AAC GGA TCC |
| p297                   | K2 (F)          | GGT GAT GAA AAA CAC CAT TCC |
| p298                   | K1 (R)          | GGA ATG GTC TTT TTC ATC ACC |
| p299                   | K3 (F)          | CAA TGT GCT GGA GAA GCA |
| p300                   | K2 (R)          | TGC TCC TTC AGC AGC TTG |
| p301                   | K3 (R)          | TTA ACC ACA GCA TCC TCT TTT |
| p375                   | VARC (F)        | cgc gga tcc GAA AAA AAC CA AAT ATC TG |
| p376                   | VARC (R)        | cgc gaa ttc TTA GAT ATT CCA TAT ATC TGA |
| p542                   | K2A (F)         | cgc gga tcc GST GAT GAA AAA CAC CAT TCC |
| p543                   | K2A (R)         | cgc gaa ttc TTA GAT ATT CCA TAT ATC TGA |
| p544                   | K2B (F)         | cgc gga tcc AGG GAG AAA AGC ATA ATG G |
| p545                   | K2B (R), K2Δ5’ (R) | cgc gaa ttc TCG TCC TTC AGC AGC ATA TTG |
| p573                   | K1A (R)         | cgc gaa ttc TGT TCC CTG GGG TTG TTA GAG |
| p574                   | K1B (F)         | cgc gaa ttc CTT CAC GAA GAT GGA GCA |
| p575                   | K1B (R)         | cgc gaa ttc ATT TGG ACC ATT TTC TGT GCT |
| p576                   | K1C (F)         | cgc gaa ttc GAT TAT AGC GAA GAT GGT |
| p577                   | K1C (R)         | cgc gaa ttc TCT TCT TAA ATT ACC GTT AGA |
| p578                   | K1D (F)         | cgc gaa ttc GAC ATG GGA GCA |
| p579                   | K1D (R), K1Δhis (R) | cgc gaa ttc GAC ATG GGA GCA |
| p713                   | K1Δhis (F)      | cgc gaa ttc GST GAT GAA AAA CAC CAT TCC |
| p714                   | K2Δ5’ (F)       | TCA AAA AAA AGT GCT AAA GAA |
RESULTS

PCR Amplification of VARC and Nucleotide Sequence Analysis—Oligonucleotide primers, designed using the var2 nucleotide sequence reported by Su et al. (11), were used to amplify the VARC region of var genes from *P. falciparum* genomic DNA (line 1G2). Resultant DNA fragments were cloned into the E. coli cloning vector pUC18 (22), and the nucleotide sequence of two of these amplification products, VARC1 and VARC2, was determined. Both VARC1 and VARC2 nucleotide sequences showed significant similarity to each other and other VARC regions reported in sequence data bases (data not shown).

Recombinant Protein Expression and Purification—VARC1 and PCR-amplified regions of KAHRP were cloned into the E. coli pGEX protein expression plasmids. Fragments from the second exon of KAHRP were used in these experiments because we have been unable to express full-length recombinant KAHRP in usable amounts (data not shown). The first exon of KAHRP was not examined for VARC binding regions because it encodes a predominantly hydrophobic region believed to be a signal sequence that is cleaved from the mature polypeptide (23). The various GST-KAHRP fusion proteins used in the protein-protein binding assays are shown in Fig. 1.

Binding of VARC to K1, K2, and K3—Initial binding assays were performed using the GST-KAHRP fusion proteins GST-K1, GST-K2, and GST-K3. Approximately 3 μg of total protein was added per well followed by blocking of nonspecific protein-plastic interactions with BSA in PBS. 5 μg of purified GST-VARC1 was then added to each well. After removal of nonspecifically bound GST-VARC1, proteins remaining in the wells were removed by stripping the wells with SDS sample buffer, subjected to SDS-polyacrylamide gel electrophoresis before transfer to membrane. Immunoblot detection of GST-VARC1 was performed using a polyclonal rabbit anti-GST-VARC1 antiserum (preabsorbed to remove anti-GST reactivity). Fig. 2 shows the immunoblot results of such a binding assay. GST-VARC1 (full length ~97 kDa) was detected binding to each of the GST-K1, GST-K2, and GST-K3 (lanes 1, 2, and 3) fusion proteins. Smaller molecular mass immunoreactive GST-VARC1 bands were detected in each lane using the anti-GST-VARC1 antiserum (preabsorbed for anti-GST reactivity). Because these bands are immunoreactive with both anti-GST-VARC1 (preabsorbed for anti-GST reactivity) and anti-GST antiserum (data not shown) they most likely represent proteolytic cleavage or premature termination products of full-length GST-VARC1. GST-VARC1 was not detected binding to the control proteins, GST or BSA (lanes 4 and 5). Thus each of the K1, K2, and K3 regions of KAHRP appeared to encode a separate domain capable of binding the cytoplasmic tail of PfEMP1.

Fine Mapping of the VARC Binding Regions in KAHRP—With the data obtained in initial binding assays indicating the existence of multiple regions in KAHRP able to bind VARC, we set out to map the location of these binding regions more precisely by constructing a series of protein expression clones encoding smaller fragments of the KAHRP gene. For this series of experiments the KAHRP subclones K1A, K1B, K1C, K1D, K2A, and K2B (Fig. 3) were cloned into pGEX, and their corresponding GST fusion proteins were expressed and purified (Fig. 1). Immunoblots of these binding assays (Fig. 3) show binding of GST-VARC1 to several regions. GST-VARC1 binds to GST-K1, and its subfragments GST-K1A (with minimal binding to GST-K1B, and GST-K1D; lanes 1, 2, 3, and 5, respectively), to GST-K2 and its subfragment GST-K2A (lanes 6 and 7), and to GST-K3 (lane 9) as evidenced by the detection of a ~97-kDa band by the anti-GST-VARC1 antiserum (preabsorbed for anti-GST reactivity). No binding of GST-VARC1 was detected to GST-K1C, GST-K2B or the control protein GST and BSA (lanes 4, 8, 10, and 11, respectively). These data provided direct evidence for the existence of at least three VARC binding sites in KAHRP. These VARC binding regions are encoded within K1A, K2A, and K3.

Although binding of GST-VARC1 to GST-K1 was detected, it is of lesser intensity than the binding of GST-VARC1 to GST-K1A. This is likely to be the result of the relatively lower amount of full-length GST-K1 used compared with full-length GST-K1A (Fig. 1, lanes 1 and 2) resulting in the lower signal intensity detected than, for example, full-length GST-K2 and GST-K2A, which are both expressed as predominantly full-length products (Fig. 1, lanes 6 and 7) and produce bands of similar intensity.

To determine the significance of the observed interactions, biophysical data was obtained using an IAsys™ system in which the aminosilane cuvettes were coated with GST, BSA, or GST-VARC1 proteins and the GST-KAHRP fusions applied in aqueous solution. The *K_d(kin)* values determined for the interaction between GST-VARC1 and GST-K1A, and GST-VARC1 and GST-K2A were 1 × 10^−7 M and 3.3 × 10^−6 M, respectively, whereas the interaction of GST-VARC1 and GST-K3 gave a *K_d(kin) = 1.3 × 10^−5 M* (Table II). Other subfragments of K1 and K2 and the GST and BSA control proteins gave no detectable binding in this system (data not shown). It is noteworthy that the binding constants for K2 and K2A (Table II) are very similar, suggesting that essentially all binding sequences of K2 are found in K2A. Similar values were obtained when the binding of these fragments was examined using a procedure that provides *K_d(Scat)*. The concordance of the estimates is good, giving confidence in the derived values.

Deletion Mutagenesis and Confirmation of VARC Binding to Peptide Repeat Regions in KAHRP—Comparison of the locations of the K1A and K2A regions to the KAHRP gene structure (Fig. 3) revealed that the K1A and K2A regions contain peptide repeat regions (23). We wondered whether the binding regions were located within these regions particularly as they would be predominantly positively charged, and VARC has regions of high net negative charge. To examine this, two KAHRP mutants lacking the peptide repeats were generated by PCR, these being the K1Δhistidine-rich repeats and K2Δ5’ repeats (designated K1Δhis and K2Δ5’, respectively) and cloned into the pGEX protein expression plasmids. Recombinant GST fusion proteins were expressed and purified (Fig. 1) and then used in
binding assays to demonstrate the effect of removal of these repeat regions on the ability of K1 and K2 to bind VARC. In these assays, the amount of GST-K1 added to the wells was corrected for the low amount of full-length GST-K1 present in the purified sample (Fig. 1). We increased the amount of GST-K1 added 3-fold so that approximately equivalent amounts of full-length GST-K1 and GST-K1A proteins were being added to the wells.

The removal of both the histidine-rich repeat region (63 amino acid residues) in GST-K1Dhis and the 5'9 repeats (70 residues) in GST-K2D59 resulted in the complete absence of GST-VARC1 binding to these mutant proteins (Fig. 4, panel B, lane 3; panel C, lane 8), whereas binding to the KAHRP fragments retaining these peptide repeat regions was observed (Fig. 4, panel B, lanes 1 and 2; panel C, lanes 6 and 7). Binding experiments performed using the IAaoys™ confirmed that GST-
FIG. 4. Binding of GST-VARC1 to GST-KAHRP deletion mutants. The KAHRP mutants, K1-histidine repeats (K1his) and K2Δ5′ repeats (K2Δ5′) are shown with respect to their locations in the KAHRP gene (panel A). Amino acid residue numbers are shown adjacent to the protein schematic and KAHRP fragment names. Binding assay immunoblots showed GST-VARC1 (~97 kDa) bound to GST-K1 and GST-K1A (panel B, lanes 1 and 2) but not to the KAHRP deletion mutant GST-K1his (lane 3). Similarly, GST-VARC1 bound to GST-K2 and GST-K2A (panel C, lanes 6 and 7) but not to the GST-K2Δ5′ KAHRP mutant (lane 8). In both of these assays there was little or no GST-VARC1 bound to the control proteins GST and BSA (panel B, lanes 4 and 5, and panel C, lanes 9 and 10).

DISCUSSION

Modification of red cell properties during parasite infection has been attributed to both the presence of parasite-encoded proteins at the red blood cell cytoskeleton and parasite-induced changes to red cell cytoskeletal proteins (for review, see Ref. 5). Perhaps the most important acquired property is the capacity of red cells infected with mature parasites to adhere to the vascular endothelium and consequently, disappear from the peripheral circulation. This property is implicated strongly in the causation of severe forms of *P. falciparum* infection such as cerebral malaria. The work presented here suggests that the parasite adhesin PfEMP1 is anchored at the cell membrane by knobs, the electron-dense protuberances on the cytoplasmic face of the infected red cell membrane (24, 25). This interaction helps explain why PfEMP1 is found to be clustered at knobs (8). Previous work has identified an association between KAHRP and spectrin and actin (4). Thus the net effect of the interaction between parasite proteins and host proteins of the red cell membrane skeleton. For example, the interaction between MESA, which is localized on the cytoplasmic side of the red cell membrane (26), and the red cell cytoskeletal protein, protein 4.1 (27) has been localized to 19 residues located in the NH₂-terminal region of MESA (17). The ring-infected erythrocyte surface antigen (RESA), which associates with the red cell cytoskeletal protein spectrin (28), contains a single 48-residue spectrin binding domain (29), whereas a 30-residue domain confers binding of merozoite surface protein 1 to spectrin (30). Mapping studies of KAHRP binding to the red cell cytoskeletal proteins spectrin and actin are less advanced with the binding domain localized to a 271-residue binding region that has not been further characterized (4).

Determination of the affinities (KD) for both GST-K1A and GST-K2A binding to GST-VARC1 gave values indicative of moderate affinity interactions: KD(K1A) = 1 × 10⁻⁷ M and KD(K2A) = 3.3 × 10⁻⁸ M, respectively. A value of KD(K3A) = 1.3 × 10⁻⁵ M was obtained for the binding of GST-K3 to GST-VARC1, indicating an interaction of less significance than the aforementioned regions. Comparison of these KD values with those obtained for other protein-protein interactions at the membrane of normal and infected red cells reveals that they are within the range of previously determined KD values. The binding of normal red cell cytoskeletal proteins spectrin and band 2.1 was measured at KD ~ 10⁻⁷ M, whereas binding between spectrin and band 4.1 returned a similar affinity (KD ~ 10⁻⁷ M) (31). The KD(K2A) obtained for the binding between the parasite protein MESA and its partner protein 4.1 was found to be (6.3 ± 1.2) × 10⁻⁷ M (17). The K1A and K2A regions contribute individually to the interaction between KAHRP and VARC at affinities comparable to the single binding domains identified in spectrin-protein 4.1, spectrin-band 2.1, and MESA-protein 4.1. When considering the data obtained for each of K1A, K2A and K3 in conjunction with one another, it seems reasonable that the three regions may act cooperatively to result in an interaction of very high affinity. One caveat is that
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in the absence of a three-dimensional structure for KAHRP, it is not known whether it is possible for all three regions of KAHRP to interact with a single VARC. However, it is also possible that the three regions could react with separate VARC molecules providing a cross-linking effect that would serve to anchor a number of PfEMP1 molecules in a compact space. This would provide a high density of PfEMP1 ectodomains at the knob and improve binding affinity for endothelial cells. Perhaps it is the loss of clustering of PfEMP1 in the absence of knobs which explains the loss of adherence of knobless infected red cells under flow conditions while their binding ability appears to be maintained in the absence of flow-induced hemodynamic stress. Alternatively, the weakened adhesive properties may be caused by PfEMP1 being “pulled” out of the membrane of infected red cells, due to inadequate anchoring, when subjected to the physiological shear stresses that occur in the circulation in vivo.

A prominent feature of many malaria proteins is the presence of extensive regions of sequences repeated in tandem (32). It has been quite difficult to assign functional roles to these repeat regions. They are often the target of antibody-induced immunity in individuals living in endemic areas (33), and it has been suggested that they act as a form of immunological smoke screen diverting the immune system to low-affinity nonprotective antibody responses (34, 35). Occasionally, additional roles have been suggested. For example, the repeats of the circumsporozoite protein have been proposed to play some role in the interaction of the sporozoite with the hepatocyte (36). However, more recently the binding site has been mapped to nonrepetitive sequence elsewhere in the circumsporozoite protein (37). Similarly, the binding site of a second sporozoite protein, thrombospondin-related anonymous protein, for hepatocytes has also been mapped to a region of nonrepetitive sequence (38). The binding domains of RESA, MESA and merozoite surface protein 1 mentioned above are all found in nonrepetitive sequence (17, 29, 30). Although the 271-residue spectrin/surface protein 1 mentioned above are all found in nonrepetitive sequence elsewhere in the circumsporozoite protein (37).

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