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Epitope map and processing scheme for the 195,000-dalton surface glycoprotein of *Plasmodium falciparum* merozoites deduced from cloned overlapping segments of the gene

(malaria antigen/λgt11/genomic expression library/antibody purification)

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ABSTRACT **DNA fragments from human malaria parasites were cloned into λgt11 to produce a genomic DNA expression library. A pool of monoclonal antibodies (mAbs) recognizing three domains of the 195-kDa major merozoite surface glycoprotein (gp195) reacted with seven clones expressing malaria antigens. mAbs recognizing the 83-kDa product of gp195 reacted with the clones, but mAbs recognizing a glycosylated 45-kDa and a nonglycosylated 45-kDa domain did not. Restriction enzyme mapping revealed that the clones contained overlapping segments encoding about 70% of the gene beginning at the 5′ end and ending at an *Eco*RI restriction enzyme site 3.3 kilobase pairs downstream. The mAbs recognizing the 83-kDa domain reacted differently with the clones, allowing the mapping of three epitopes, one of which was repetitive. Affinity-purified antibodies were selected from immune monkey serum with recombinant expression proteins adsorbed to nitrocellulose filters. When used to probe electrophoretic immunoblots of parasite extracts, these antigen-selected antibodies reacted with specific sets of processed products of gp195, including those associated with the 83- and the nonglycosylated 45-kDa domains. This information, combined with the mAb epitope map, allowed a tentative scheme for processing gp195 from the Camp strain to be proposed.**

Merozoites from *Plasmodium falciparum* malaria parasites have, as major components of their surface, a 195-kDa glycoprotein (gp195) (1) and polypeptides derived from this protein as a result of processing by proteases (2, 3). Processing of gp195 produces at least three smaller products found on the surface of merozoites (3) and has been observed in at least six *P. falciparum* strains (1, 2, 4, 5). Processing occurs about the time merozoites are released from infected erythrocytes, and a series of transitory intermediates with molecular masses of about 153, 150, and 110 kDa appear (2). One of the final products has an M_r of 83 kDa (2, 4, 5) and possesses serotype-restricted epitopes (4, 5). Other products of 50 (1), 42, and 19 kDa (3) have been identified; the 50-kDa antigen is glycosylated (1) and may be homologous with the 42-kDa antigen. gp195 and its proteolytic products are recognized on intact merozoites by growth-inhibitory immune sera (6) and may be good candidates for vaccine development (1, 7–10).

The molecular cloning and expression of parasite antigens in bacterial systems allows the preparation of sufficient amounts of pure antigen to conduct immunological and immunochemical studies. The cloning from cDNA of the complete structural gene encoding gp195 has been reported (11), and cDNA clones encoding 0.21 (7) and 1.1 (12) kilobase pairs (kb) of the gp195 gene have been reported as well. We report the cloning of a 3.6-kb portion of gp195 genomic DNA from the Camp strain and propose a scheme for processing the Camp strain protein, locating major proteolytic products and the positions of epitopes recognized by monoclonal antibodies (mAbs).

MATERIALS AND METHODS

Culture of *P. falciparum*. Camp strain parasites (clone A) (13), maintained in asynchronous cultures according to standard techniques (14, 15), were synchronized by using two sorbitol treatments (16, 17).

Screening the Expression Library. Recombinant phage were prepared with mung bean nuclease-sheared DNA (13, 18, 19) ligated into alkaline phosphatase-treated λgt11 (20). Phage producing malaria antigens were identified with a pool of sera from *Aotus* monkey A076. This animal was clinically immune to *P. falciparum* Camp strain malaria, and its serum contained antibodies that inhibited parasite growth *in vitro* (14, 21). Hybridoma culture supernates were the sources of mAbs (7H10, 3B10, 3D3, 7B11, 7B2, and 7F1) specific for gp195.

Before use, the *Aotus* sera were depleted of antibodies against λgt11 and *Escherichia coli* by adsorption first with sonicated λgt11 lysogens grown in *E. coli* Y1089 (22) and second with a nitrocellulose filter plaque lift (see below) prepared from nearly confluent plaques of λgt11 grown on a lawn of *E. coli* Y1090.

Screening the expression library for antigen production was performed essentially as described by Young *et al.* (20, 23) except for the following details. (*i*) Unamplified phage (5,000) were grown in *E. coli* Y1090 on each 15-cm plate. (*ii*) Filters were blocked with Tris-buffered saline (TBS; 10 mM Tris·HCl/150 mM NaCl, pH 8.2) containing 0.3% Tween-20, 3% bovine serum albumin, 5 mM $MgCl_2$, 0.01 unit of bovine pancreas DNase I per ml, and 0.02% NaN_3. (*iii*) The primary antibody reaction was amplified by using 1-hr reactions with affinity-purified second antibodies diluted to 0.5 μg/ml in TBS containing 0.05% Tween 20 (TBS-T). Rabbit IgG against *Aotus* monkey IgG was prepared by collecting that fraction of anti-*Aotus* IgG that bound to an *Aotus* IgG-Sepharose column but did not bind to a human IgG-Sepharose column. (*iv*) The signal-producing reagent was affinity-purified ^{125}I-labeled protein A (30 mCi/mg, Amersham; 1 Ci = 37 GBq) diluted in TBS-T to 75,000 cpm/ml.

Antigen-Selected Antibodies. Monospecific antibodies were affinity-purified from polyspecific immune sera by using a modification of the method of Hall *et al.* (7). Nitrocellulose filter plaque lifts were prepared from cloned recombinant phage as was normally done in assaying for antigen expression, except that the filters were left in contact with the phage for 6 hr, and sufficient phage (approximately 2×10^5 per

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Abbreviations: mAb, monoclonal antibody; kb, kilobase pair(s); gp195, 195-kDa major merozoite surface glycoprotein.

15-cm plate) were plated to give nearly confluent plaques at the end of the incubation period. Antibodies were selected by treating the filters with 10 ml of immune serum diluted 1:400 in TBS-T containing 0.02% NaN_3 overnight at room temperature on an orbital shaker. Filters were washed five times for 20 min each with 50 ml of TBS-T and one time with 0.15 M NaCl/0.05% Tween-20. Monospecific antibodies were eluted for 30 min with 10 ml of 0.2 M glycine·HCl/0.15 M NaCl/0.05% Tween-20, pH 2.8. The eluate was neutralized with 8 mg of Tris per ml of eluate.

Polyacrylamide Gel Electrophoresis. Discontinuous sodium dodecyl sulfate/polyacrylamide gel electrophoresis was performed with slab gels containing an 8% acrylamide separating gel and a 3% stacking gel (24, 25). 2-Mercaptoethanol was omitted from samples electrophoresed under nonreducing conditions.

Electrophoretic Immunoblots. Electroblotting (26) was essentially as described by Harn *et al.* (27). For blots of parasite antigens, schizonts were purified on Percoll gradients (28), and antigens from anti-protease-stabilized clusters of merozoites were solubilized as described (25). Blot strips containing antigen from 2×10^6 parasites were rocked overnight at room temperature in 5 ml of antibody diluted in TBS-T containing 0.02% NaN_3. Antibodies were diluted 1:10 (hybridoma culture supernates), 1:5 (antigen-selected antibodies), or 1:2,000 (immune serum). Strips were rocked for 1 hr at room temperature in 5 ml of ^{125}I-labeled sheep anti-mouse IgG (Amersham) diluted to 100,000 cpm/ml in TBS-T (for mAbs) or in 5 ml of affinity-purified rabbit IgG against *Aotus* IgG, diluted to 0.5 μg per ml in TBS-T, and rocked for 1 hr in 5 ml of ^{125}I-labeled protein A (Amersham) diluted to 75,000 cpm/ml in TBS-T (for antigen-selected antibodies or *Aotus* monkey serum).

Metabolic Labeling and Immunoprecipitation. Protease inhibitor-stabilized clusters of merozoites were labeled metabolically with [^{3}H]isoleucine or [^{3}H]glucosamine as described (25). Immunoprecipitation was performed with 100 μl of 10 times-concentrated hybridoma culture supernate and antigen extracts from 2.5×10^7 parasites. Immune complexes were precipitated by using 50 μl (packed volume) of goat anti-mouse IgG coupled to Sepharose CL-4B beads (Cappel), and the precipitates were washed and eluted in 2 times-concentrated $NaDodSO_4$ sample buffer (25) with or without 2-mercaptoethanol.

DNA Mapping of Clones. DNA inserts in phage clones were excised with *Eco*RI and were subcloned into plasmid pBR322 for restriction enzyme mapping. For cross-hybridization of the clones, DNA was labeled by nick-translation and hybridized to Southern blots of the cloned DNA (13) or to phage plaque lifts (29). Orientation of the inserts within the λgt11 vector was determined by *Hin*dIII and *Eco*RV digestion of phage DNA for clones a34, a79, a88, a98, and a119 and by *Hin*fI digestion of phage DNA followed by hybridization of the resulting fragments to labeled a88 DNA insert for clones a50 and a23.

RESULTS

Screening 35,000 plaques from the genomic DNA expression library with immune monkey serum identified 147 clones expressing antigens. Screening duplicate filters was useful for confirming positive clones, which usually stood out more clearly against the background of negative plaques on the second filter than on the first, and allowed selection of phage that gave weak positive signals.

A pool of mAbs reacting with different epitopes of gp195 was used to screen 47 clones giving the strongest signals with the monkey serum. Seven clones reacted with the mAbs. When tested individually, three of six mAbs against different gp195 epitopes reacted specifically with expression proteins produced by the clones (Table 1). mAb 3D3 reacted with expression proteins from clones a23, a50, a88, a98, and a119; mAb 7B11 reacted with clones a34, a50, a79, a88, a98, and a119; and mAb 7B2 reacted with clone a50. mAb 3B10 reacted with all clones and the negative control (λgt11 grown in Y1090), indicating reaction with a component of the bacteriophage or bacterial host. mAbs 7H10 and 7F1 did not react with any of the clones.

Antigen-selected antibodies, purified from immune serum with expression proteins from clones adsorbed to nitrocellulose, were used to probe electroblots (Fig. 1 *Left*) of parasite extracts electrophoresed under reducing (lanes R) or nonreducing (lanes NR) conditions. All seven clones selected antibodies that reacted with parasite antigens of 195, 152 (doublet), 112 (doublet), 83, and 73 kDa (Fig. 1 *Left* and Table 1). In addition clone a50 selected antibodies that reacted with a 67-kDa antigen, and clones a34, a79, a88, a98, and a119 selected antibodies that reacted with a 67-kDa antigen and a 45-kDa antigen. Clone a79 also selected antibodies that reacted with a 70-kDa antigen. The electrophoretic mobilities of all of these antigens were the same in gels electrophoresed under reduced or nonreduced conditions.

The results of probing electroblots of parasite extracts with mAbs specific for gp195 are shown in Fig. 1 *Right*, and results of immunoprecipitation of antigens metabolically labeled with [^{3}H]isoleucine or [^{3}H]glucosamine are shown in Fig. 2. In electroblots, mAbs 7B2 (not shown), 3D3, and 7B11 reacted with gp195, its processing intermediates of 152 kDa (doublet) and 112 kDa (doublet), and the products of 83 kDa and 73 kDa. In addition, mAbs 7B11 and 7B2 reacted with a 67-kDa processed product not recognized by 3D3. Among the antigens recognized by these mAbs (Fig. 1 *Right*), only gp195 was glycosylated (Fig. 2, compare lanes c and g). Two 45-kDa antigens were recognized. mAb 3B10 immunoprecipitated a glycosylated 45-kDa product (Fig. 2, compare lanes b and f) that had a greater electrophoretic mobility under nonreduced conditions than under reduced conditions (Fig. 2, compare lanes b and j). mAb 7F1 reacted with this same 45-kDa antigen in electroblots (Fig. 1 *Right*). In contrast, mAb 7H10 gave a strong reaction in electroblots with a 45-kDa product that electrophoresed as a sharp band under reduced conditions (Fig. 1 *Right*) but did not react with this product in electroblots of antigens electrophoresed under nonreduced conditions (not shown). mAb 7H10 precipitated two 45-kDa products from parasite extracts. One of these products was labeled with [^{3}H]isoleucine but not with [^{3}H]glucosamine (Fig. 2, compare lanes i and m) and had the same electrophoretic mobility under reducing and nonreducing conditions (Fig. 2, compare lanes e and m). The second 45-kDa product precipitated by mAb 7H10 had the same characteristics as the

Table 1. Characterization of inserts encoding gp195

No.	Insert size, kb	mAb reaction	Specificities of antigen-selected antibodies,* kDa
a23	0.5	3D3†	195, 152, 112, 83, 73
a34	2.6	7B11‡	195, 152, 112, 83, 73, 67, 45
a39	0.4	3D3	195, 152, 112, 83, 73
a50	1.5	3D3, 7B11, 7B2‡	195, 152, 112, 83, 73, 67
a79	2.0	7B11	195, 152, 112, 83, 73, 70, 67, 45
a88	3.6	3D3, 7B11	195, 152, 112, 83, 73, 67, 45
a98	3.6	3D3, 7B11	195, 152, 112, 83, 73, 67, 45
a119	3.4	3D3, 7B11	195, 152, 112, 83, 73, 67, 45

*Antibodies were affinity-purified with expression products of the recombinant clones.
†3D3 reacts with 195-, 152-, 112-, 83- and 73-kDa antigens.
‡7B11 and 7B2 react with a 67-kDa antigen in addition to antigens with which 3D3 reacted.

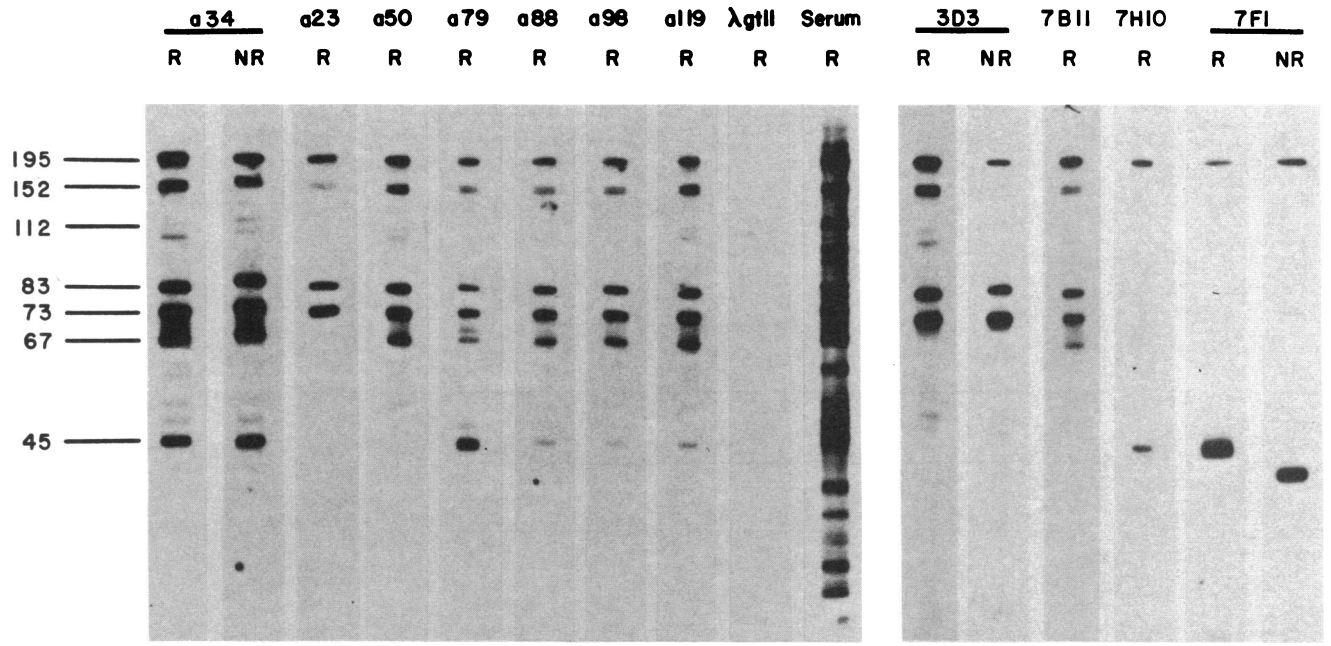

FIG. 1. Electroblots of *P. falciparum* antigens probed with antibodies selected by using recombinant expression proteins (*Left*) or with mAbs (*Right*). Electroblot strips containing antigen from 2×10^6 parasites electrophoresed under reduced (lanes R) or nonreduced (lanes NR) conditions were probed with antibodies selected with clones expressing gp195, with antibodies selected from Y1090 host infected with control phage (λgt11), with immune serum from *Aotus* monkey A076 (serum), or with mAbs. Sizes of specific antigen bands are shown in kDa.

glycosylated 45-kDa product precipitated by mAb 3B10 (Fig. 2, compare lanes a and b, e and f, i and j, and m and n).

DNA inserts within the seven clones were aligned by restriction enzyme mapping (Fig. 3*A*). Insert sizes ranged from 0.5 kb for clone a23 to 3.6 kb for clones a88 and a98. The inserts in clones a23, a34, a50, a88, and a98 had the same relative orientation within the vector, while the inserts in a79 and a119 had the opposite orientation. Electroblots of lysogen extracts revealed that the 129- and 161-kDa fusion proteins produced by clones a23 and a50, respectively, were

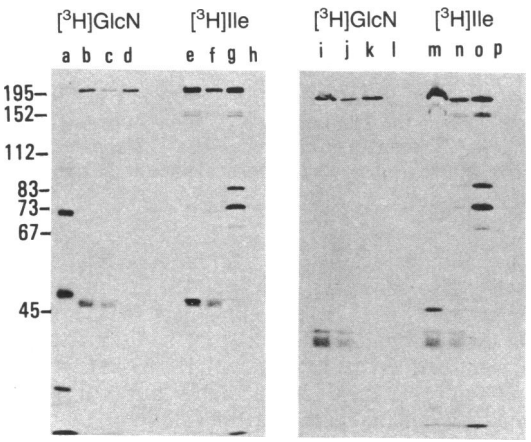

FIG. 2. mAb precipitation of *P. falciparum* antigens metabolically labeled with [^{3}H]isoleucine or [^{3}H]glucosamine. Precipitates from 2.5×10^7 parasites were electrophoresed under reducing (lanes a–h) or nonreducing (lanes i–p) conditions. [^{3}H]Glucosamine-labeled antigens (lanes a–d and i–l) and [^{3}H]isoleucine-labeled antigens (lanes e–h and m–p) were precipitated by mAbs 7H10 (lanes a, e, i, and m), 3B10 (lanes b, f, j, and n), and 7B11 (lanes c, g, k, and o); a negative control is shown in lanes d, h, l, and p. Molecular mass markers were run in the first lane and included myosin (200 kDa), phosphorylase *b* (92 kDa), bovine serum albumin (68 kDa), ovalbumin (46 kDa), and carbonic anhydrase (30 kDa). Specific antigens are identified on the left side of the figure.

concordant with the sizes of the parasite DNA inserts in these clones (unpublished data). The fusion proteins from clones a23 and a50 reacted with anti-β-galactosidase antiserum and with immune monkey serum and mAbs, thereby defining the direction of transcription of the gp195 gene in the parasite (left to right in Fig. 3). Clones a34, a88, and a98 gave no detectable fusion proteins, but these clones as well as clones a79 and a119 produced gp195 expression proteins having lower molecular mass than that of β-galactosidase (116 kDa). Additional evidence for the reverse orientation of the inserts in clones a79 and a119 is that they produced fusion proteins of 124 kDa recognized by antiserum against β-galactosidase but not by the immune monkey serum or the mAbs (unpublished data). λgt11 clones expressing parasite DNA inserts transcribed from promoters other than the β-galactosidase promoter have been found among clones encoding the *P. falciparum* circumsporozoite protein (30).

Fig. 3*B* identifies the sites on the gp195 gene where the epitopes recognized by the mAbs are encoded. mAb 3D3 reacts with a serotype-restricted repetitive epitope‡ present in all of the clones except a34 and a79. The 3D3 epitope is encoded by 171 base pairs of repetitive DNA as shown by DNA sequencing of the insert in clone a88 (not shown). The repeat begins 98 base pairs downstream from the *Hin*dIII restriction enzyme site and occupies the same position as that of strain-variable repetitive DNA observed in the gp195 genes from the Wellcome (11) and SGE2 (12) parasite strains. mAb 7B11 reacted with clones a50 and a79, and these clones overlap only in the 0.5-kb region shown in Fig. 3*B*. The 7B2 epitope was mapped by noting that this mAb, which reacts with the 67-kDa proteolytic product of gp195, reacted with the expression product produced by clone a50.

Fig. 3*C* is a hypothetical processing scheme for Camp strain gp195. This model is in agreement with all of our data and all published results, but is not completely proven. The first processing step removes the 45-kDa glycosylated prod-

‡Lyon, J. A., Haynes, J. D., Hadley, T. J. & Diggs, C. L., Joint Meeting of the Royal and American Societies of Tropical Medicine and Hygiene, December 4, 1984, Baltimore, MD, abstr. 166.

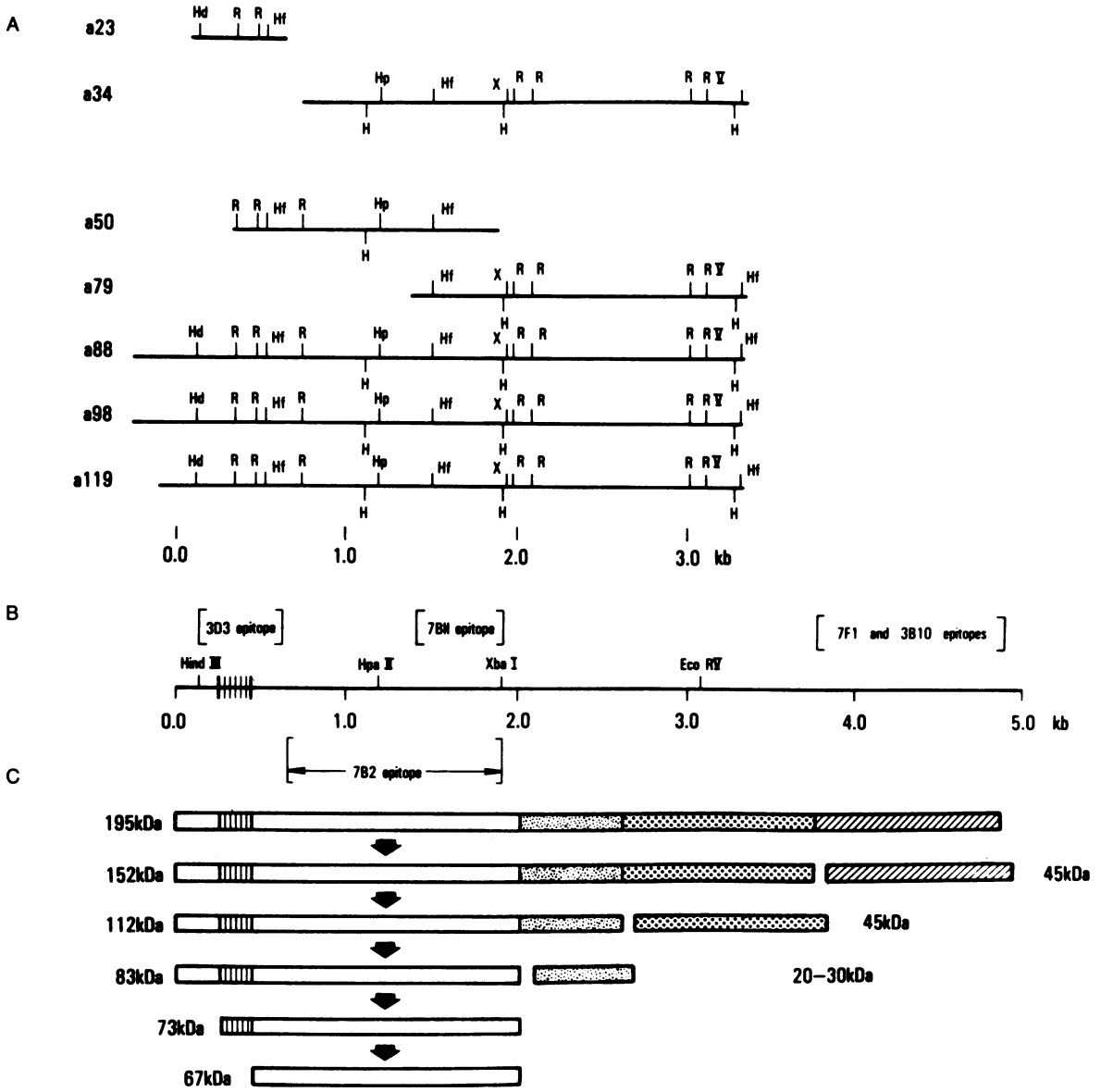

FIG. 3. (*A*) Restriction enzyme map of gp195 clones. Hd, *Hin*dIII; R, *Rsa* I; Hf, *Hin*fI; H, *Hae* III; Hp, *Hpa* II; X, *Xba* I; and RV, *Eco*RV. Transcription of the gene is from left to right. The order of the two *Rsa* I fragments between the *Xba* I and *Eco*RV sites is unknown. (*B*) gp195 epitope map. mAb epitopes were mapped to regions between brackets. (*C*) Hypothetical scheme of gp195 processing. The apparent molecular masses of the initial polypeptide and processing products are indicated. The repetitive epitope is indicated by vertical lines at the amino-terminal end of p73. All three parts of Fig. 3 are aligned and drawn to the same scale.

uct recognized by mAbs 3B10 and 7F1, leaving the nonglycosylated 152-kDa intermediate (1, 2) (Fig. 2). The nonglycosylated 45-kDa fragment, recognized by antigen-selected antibodies from all clones except a23 and a50 (Fig. 1), lies downstream from the N-terminal 83-kDa product encoded at the beginning of the gene (11). Cleavage of the nonglycosylated 45-kDa product from the C-terminal end of the 152-kDa intermediate leaves a 110–112 kDa intermediate. Removal of 20–30 kDa of protein from the C-terminal part of the 110- to 112-kDa intermediate produces the 83-kDa product, which, in the Camp strain, is sequentially processed into products of 73 and 67 kDa (results of pulse-label cold-chase experiments not shown). The proposed alignment for the 83-, 73-, and 67-kDa products is based on the observations that mAb 3D3 reacts with a repetitive epitope present on the 83- and 73-kDa products but not on the 67-kDa product (Fig. 1). The 20- to 30-kDa peptide cleaved from the C-terminal end of the 110- to 112-kDa intermediate may not be immunogenic or may be degraded rapidly because we did not observe antibody specificities against this region. A similar result was obtained by Holder *et al.*, who used the fusion protein from a cDNA clone (pME1), encoding part of the N-terminal 83-kDa domain and about 40 kDa of additional protein, to produce antibodies that reacted with the 83-kDa product and the 150- and 110-kDa intermediates but not with other products of Wellcome strain gp195 (11).

DISCUSSION

Some procedures for constructing genomic DNA expression libraries use preparative gel electrophoresis to purify DNA fragments of a selected size. One advantage to cloning fragments of heterogeneous size is that groups of overlapping clones with various DNA insert sizes may be readily obtained, and these may be useful in determining protein structure as shown for gp195. Of course, DNA from any cloned gene can be sheared and subcloned into an expression vector (31), but the approach described in this paper makes

this step unnecessary and, in principle, should give nearly identical results if a large enough library is screened.

Antigen-selected antibodies were useful for confirming that the clones encoded gp195. Using parasite proteins expressed in *E. coli* (7) to purify polyclonal monospecific antibodies from immune sera is much faster than immunizing small animals with expression proteins, allowing early characterization of cloned inserts. Adsorbing expression proteins to nitrocellulose directly from phage plaques offers the added advantage of eliminating the need to purify fusion proteins to prepare the adsorbent, thus allowing antigen-selected antibodies to be prepared from expression products synthesized as unstable fusion proteins or not as fusion proteins at all.

Of the six gp195 mAbs tested against the clones, only 3D3, 7B11, and 7B2 gave gp195-specific reactions. 7B2 recognizes an epitope that is reversibly denatured during antigen purification,[§] and this mAb reacted only with clone a50, which expressed the greatest amount of parasite antigen, as detected by Coomassie blue staining (unpublished data). mAbs 7H10 and 7F1, which recognize different 45-kDa products of gp195, did not react with expression proteins from any of the clones, suggesting that the epitopes recognized by these mAbs may be encoded by the portion of the gp195 gene lying downstream of the 3′ end of clones a34, a79, a88, a98, and a119. The 3′ end of these clones is a chromosomal *Eco*RI site, cleaved during construction of our library because the DNA was not treated with *Eco*RI methylase. The reading frame of the fragment downstream from this *Eco*RI site does not match the reading frame of the λgt11 β-galactosidase gene (refs. 11 and 20 and unpublished results). Other possible reasons for explaining why we did not identify clones expressing this region include: (*i*) the concentrations or affinities of *Aotus* antibodies recognizing the C-terminal portion of gp195 may have been too low to allow detection of nonfusion proteins, and (*ii*) antibodies against this region of the protein might recognize primarily carbohydrate determinants.

Although our clone a88 and clone pME1 from Holder *et al.* (11) encode similar portions of gp195, antibodies selected with the a88 expression products reacted with antigenic determinants downstream from the 83-kDa domain, but immune serum raised against partially purified fusion proteins from pME1 did not. This difference may be the result of the methods used to obtain the antibodies or to differences in gp195 and its products between the two parasite strains used.

It is likely that parasite-derived proteases process *P. falciparum* gp195 as shown for the equivalent antigen in *P. knowlesi* (32). The biological significance of processing these proteins is unknown. One possibility is that processing converts an inactive form of the protein to an activated species that is involved in erythrocyte invasion. Another is that processing modulates immune responses against this antigen. The cloning of different segments of the gene should allow identification of functional and immunologically protective regions of the protein.

[§]Camus, D., Lyon, J., Reaud-Jareed, T., Haynes, D. & Diggs, C. L., Joint Meeting of the Royal and American Societies of Tropical Medicine and Hygiene, December 3, 1984, Baltimore, MD, abstr. 13.

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1. Pirson, P. J. & Perkins, M. E. (1985) *J. Immunol.* **134,** 1946–1951.
2. Holder, A. A. & Freeman, R. R. (1982) *J. Exp. Med.* **156,** 1528–1538.
3. Holder, A. A. & Freeman, R. R. (1984) *J. Exp. Med.* **160,** 624–629.
4. McBride, J. S., Newbold, C. I. & Anand, R. (1985) *J. Exp. Med.* **161,** 160–180.
5. Hall, R., McBride, J., Morgan, G., Tait, A., Zolg, J. W., Walliker, D. & Scaife, J. (1983) *Mol. Biochem. Parasitol.* **7,** 247–265.
6. Lyon, J. A., Haynes, J. D., Diggs, C. L., Chulay, J. D. & Pratt-Rossiter, J. M. (1985) *J. Immunol.* **136,** 2252–2257.
7. Hall, R., Hyde, J. E., Goman, M., Simmons, D. L., Hope, I. A., Mackay, M., Scaife, J., Merkli, B., Richle, R. & Stocker, J. (1984) *Nature (London)* **311,** 379–382.
8. Holder, A. A., Freeman, R. R. & Newbold, C. I. (1984) *Mol. Biochem. Parasitol.* **9,** 191–196.
9. Epstein, N., Miller, L. H., Kaushel, D. C., Udeinya, I. J., Rener, J., Howard, R. J., Asofsky, R., Aikawa, M. & Hess, R. (1981) *J. Immunol.* **127,** 212–217.
10. Majarian, W. R., Daly, T. M., Weidanz, P. & Long, C. A. (1984) *J. Immunol.* **132,** 3131–3137.
11. Holder, A. A., Lockyer, M. J., Odink, K. G., Sandhu, J. S., Riveros-Moreno, V., Nicholls, S. C., Hillaman, Y., Davey, L. S., Tizard, M. L. V., Schwarz, R. T. & Freeman, R. R. (1985) *Nature (London)* **317,** 270–273.
12. Cheung, A., Shaw, A. R., Leban, J. & Perrin, L. H. (1985) *EMBO J.* **4,** 1007–1012.
13. Weber, J. L. & Hockmeyer, W. T. (1985) *Mol. Biochem. Parasitol.* **15,** 305–316.
14. Chulay, J. D., Haynes, J. D. & Diggs, C. L. (1981) *J. Infect. Dis.* **144,** 270–278.
15. Trager, W. & Jensen, J. B. (1976) *Science* **193,** 673–675.
16. Lambros, C. & Vanderberg, J. P. (1979) *J. Parasitol.* **65,** 418–420.
17. Vernes, A., Haynes, J. D., Tapchaisari, P., Williams, J. L., Dutoit, E. & Diggs, C. L. (1984) *Am. J. Trop. Med. Hyg.* **33,** 197–201.
18. McCutchan, T. F., Hansen, J. L., Dame, J. B. & Mullins, J. A. (1984) *Science* **225,** 625–628.
19. Lanar, D. E., Pearce, E. J. & Sher, A. (1985) *Mol. Biochem. Parasitol.* **17,** 45–60.
20. Young, R. A., Bloom, B. R., Grosskinsky, C. M., Ivanyi, J., Thomas, D. & Davis, R. W. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2583–2587.
21. Chulay, J. D., Aikawa, M., Diggs, C. & Haynes, J. D. (1981) *Am. J. Trop. Med. Hyg.* **30,** 12–19.
22. Kemp, D. J., Coppel, R. L., Cowman, A. F., Saint, R. B., Brown, G. V. & Anders, R. F. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3787–3791.
23. Young, R. A. & Davis, R. W. (1986) in *Genetic Engineering: Principles and Methods,* eds. Setlow, J. K. & Hollander, A. (Pella, New York), Vol. 7, pp. 29–41.
24. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
25. Lyon, J. A. & Haynes, J. D. (1986) *J. Immunol.* **136,** 2245–2251.
26. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
27. Harn, D., Mitsuyama, M. & David, J. R. (1984) *J. Exp. Med.* **159,** 1371–1387.
28. Aley, S. B., Sherwood, J. A. & Howard, R. J. (1984) *J. Exp. Med.* **160,** 1585–1590.
29. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
30. Dame, J. B., Williams, J. L., McCutchan, T. F., Weber, J. L., Wirtz, R. A., Hockmeyer, W. T., Maloy, W. L., Haynes, J. D., Schnieder, I., Roberts, D., Sanders, G. S., Reddy, E. P., Diggs, C. L. & Miller, L. H. (1984) *Science* **225,** 593–599.
31. Nunberg, J. H., Rodgers, G., Gilbert, J. H. & Snead, R. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3675–3679.
32. David, P. H., Hadley, T. J., Aikawa, M. & Miller, L. H. (1984) *Mol. Biochem. Parasitol.* **11,** 267–282.