Published in final edited form as:

Mol Biochem Parasitol. 1997 December 1; 90(1): 33–41.

Anopheles stephensi salivary glands bear receptors for region I of the circumsporozoite protein of Plasmodium falciparum

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Abstract

In the mosquito, *Plasmodium* sporozoites rupture from oocysts found on the midgut wall, circulate in the hemolymph and invade salivary glands where they wait to be injected into a vertebrate host during a bloodmeal. The mechanisms by which sporozoites specifically attach to and invade salivary glands are not known but evidence suggests that it is a receptor-mediated process. Here we show that the major surface protein of sporozoites, the circumsporozoite protein (CS), binds preferentially to salivary glands when compared to other organs exposed to the circulating hemolymph. In addition, we show that a peptide encompassing region I, a highly conserved sequence found in all rodent and primate *Plasmodium* CS proteins, inhibits binding of CS to mosquito salivary glands.

Keywords

Plasmodium; Malaria; Salivary glands; Circumsporozoite protein; Mosquito; Sporozoite

1. Introduction

Three stages of the *Plasmodium* life cycle are invasive for cells: Ookinetes, merozoites and sporozoites. Sporozoites are unique in that they are invasive for both mosquito salivary glands and mammalian hepatocytes. Although some of the molecules involved in sporozoite invasion of hepatocytes have been identified, little is known on the molecular level about the events involved in salivary gland invasion by sporozoites.

Sporozoites are released from mature oocysts located between the midgut epithelium and the basal lamina, over a 3–4 day period, usually occurring between 10 and 14 days after the mosquitoes have received an infective bloodmeal ([1]). During this time, the parasites [2] and their major surface protein [3] are found adherent to salivary glands as well as to the mid and hindgut, alary muscles, ovaries, Malpighian tubules, and the crop. In all cases, however, adhesion of sporozoites and their major surface protein is always greatest to salivary glands. These observations suggest that sporozoites are passively transported by the hemolymph throughout the whole mosquito and preferentially accumulate in the salivary

glands. The mechanisms by which sporozoites localize to and invade salivary glands are not known, although their target cell specificity suggests that these are receptor-mediated events. Recent work demonstrating that antibodies to molecules on the salivary gland surface can inhibit sporozoite invasion supports this hypothesis [4].

A candidate parasite ligand for the surface of salivary glands is the major surface protein of sporozoites, the circumsporozoite protein (CS; [5]; Fig. 1). CS begins to be expressed on oocyst membranes before the appearance of sporozoites but is later associated with the surface of oocyst sporozoites [6,7]. Several fine structural studies have shown that oocyst sporozoites, like salivary gland sporozoites, are uniformly covered by CS [6–8]. In an attempt to better understand the molecular mechanisms responsible for sporozoite recognition of salivary glands, we set out to determine whether recombinant CS bound specifically to mosquito salivary glands.

2. Materials and methods

2.1. Recombinant proteins monoclonal antibodies and peptides

The *Escherichia coli*-derived recombinant CS27IVC represents the complete *Plasmodium falciparum* CS sequence from the T4 isolate, excepting the hydrophobic NH₂- and COOH-terminal amino acids (residues 1–26 and 412–424) and includes five histidine residues which were added to facilitate purification [9]. The recombinant CS used in these studies was kindly provided by Dr Bela Takacs (F. Hoffmann-La Roche, Basel, Switzerland). The monoclonal antibody (mAb) 2A10 is directed against an epitope contained in the (NANP)_n repeat domain of *P. falciparum* CS [10]. The yeast-derived recombinant construct TBV25H [11] represents the entire *P. falciparum* surface protein (Pfs25) coding region, excepting the N-terminal secretory signal sequence and the hydrophobic carboxy-terminus (residues 1–22 and 193–202), and includes six terminal histidines added for purification purposes. mAb 4B7 [12] recognizes an epitope in the third EGF-like domain of Pfs25. Both recombinant Pfs25 and mAb 4B7 were kindly provided by Dr David Kaslow (NIH, Bethesda, MD). Peptides were synthesized by Boc Chemistry, using the multiple peptide synthesis method describe by Houghten [13]. Cleavage from the resin was performed with low-high hydrofluoric acid and sequence was verified by amino acid analysis. Sequences are based on the *P. falciparum* CS strain NF54.

2.2. Mosquitoes

Anopheles stephensi mosquitoes were reared as previously described [14]. Unless otherwise stated sugar-fed female mosquitoes between 7 and 12 days post-emergence were used. When male salivary glands were tested, mosquitoes in the same age range were used. In the experiment in which the binding activity of salivary glands from blood-fed and sugar-fed mosquitoes was compared, mosquitoes were fed on an anesthetized hamster 4 days prior to the experiment. For dissection of organs, mosquitoes were anesthetized on ice, washed with 70% ethanol and then distilled water. Their organs were carefully isolated using a dissecting microscope and immobilized in wells created on microscope slides. They were rinsed twice within M199 pH 6.7 (Gibco BRL, Grand Island, NY) containing 1% bovine serum albumin (BSA; Sigma, St. Louis, MO) and then fixed with a solution of 4% paraformaldehyde

(Eastman Kodak, Rochester, NY) plus 1% glutaraldehyde (Grade 1, Sigma) for 10 min at room temperature. After fixation, the slides were washed 3 times (all washes were 5 min each) with M199/1% BSA, then transferred onto a slide warmer set at 30°C where they remained for the duration of the experiment. The organs were blocked for 1 h with M199/3% BSA and incubated with 10 μ l of either CS or Pfs25 in M199/1% BSA for 50 min. In the peptide inhibition experiments, the organs were first incubated with the peptide (250 μ g/ml) for 50 min; this solution was then removed and replaced with a solution of the peptide (250 μ g/ml) and CS (5 μ g/ml) for 50 min. After incubation with recombinant protein, or in the case of the peptide inhibition experiments with protein and peptide, the binding of CS was revealed with mAb 2A10 (10 μ g/ml) and the binding of Pfs25 was revealed with mAb 4B7 (1:40 or 1:200). The antibodies were incubated with the organs for 45 min, washed 3 times and then incubated with goat anti-mouse immunoglobulin (Ig) conjugated to β -galactosidase (1:200; American Qualex, San Clemente, CA) for 45 min. The temperature of the slide warmer was then increased to 37°C and slides were washed twice with M199/1% BSA and once with phosphate buffered saline (PBS) before the addition of 5-bromo-4-chloro-3-indolyl- β -D-galactoside (X-gal, 2.5 mg/ml in PBS; American Qualex). The reaction was stopped by washing with PBS after 10–20 min, when the positive-control salivary glands began to turn blue. The organs were transferred to a new slide where they were mounted in gel/mount (Fisher Scientific, Springfield, NJ) and observed and photographed using a Zeiss Photomicroscope III. In the peptide inhibition experiments, salivary glands were scored blindly as to the intensity of the blue color, using a scale of 0–3.

2.3. Injection experiments

Mosquitoes were lightly anesthetized on ice and injected in the membranous region between the mesothoracic and metathoracic spiracles using flame-drawn glass needles. Approximately 0.5 μ l of CS (1.79 mg/ml in saline) was injected into each mosquito. Control mosquitoes were injected with the same volume of M199/1% BSA. Between 95 and 100% of the mosquitoes survived. At 1 h later the live mosquitoes were anesthetized on ice for removal of organs. Organs were dissected from both control and CS-injected mosquitoes, washed, fixed as stated above and then stained with mAb 2A10 (10 μ g/ml) followed by goat anti-mouse Ig conjugated to β -galactosidase. They were then washed and incubated with X-gal.

2.4. ELISA

Serial dilutions of Pfs25 or CS, starting at 10 μ g/ml in phosphate buffered saline (PBS) were added to 96-well plates (Falcon no. 3915, Becton Dickinson, Lincoln Park, NJ) in triplicate and incubated for 2 h at 37°C. The wells were washed twice with PBS and blocked with PBS/1% BSA for 1 h at 37°C. mAb 2A10 (10 μ g/ml) or mAb 4B7 (one set with 1:40 and another set with 1:200) were added to the appropriate wells for 1 h at 37°C, the wells were washed 3 times and anti-mouse Ig conjugated to [p105]-galactosidase (1:200) was added for 1 h at 37°C. The wells were washed 3 times and X-gal was added. The plates were read at 650 nm in an ELISA plate reader (Molecular Devices, Palo Alto, CA) 20 min and 1 h after the substrate was added.

3. Results

As shown in Fig. 2, CS bound to dissected, fixed salivary glands and not to midguts, Malpighian tubules or ovaries. Although not shown, the fat body and thoracic muscles were also tested and did not bind CS. The binding of CS is indicated by the blue color of the X-gal product and was strongest on the median lobe and the distal portion of the lateral lobes. The enzymatic reaction leading to the development of a blue precipitate is a time-dependent reaction and we stopped the reaction shortly after the salivary glands turned blue. At this time, the other organs were always negative. However, some blue would begin to appear on the midguts and ovaries if they were left to develop longer. This suggests that CS can bind to these organs, although with a much lower efficiency than to salivary glands. Negative-control salivary glands incubated with the detection system alone (data not shown), and salivary glands incubated with a control protein, Pfs25 followed by its homologous detection system (see below) remained completely unstained, even when left to develop for over 1 h.

As an additional specificity control, we performed this binding assay with Pfs25, a protein found on the surface of zygotes and ookinetes [15]. As shown in Fig. 2B, Pfs25 does not bind to salivary glands. To insure that the lack of binding of Pfs25 to salivary glands was not due to problems with the detection system, we performed an ELISA using CS and Pfs25 and their respective detection systems. We found that Pfs25 immobilized in wells bound to mAb 4B7 at the dilutions we used in our binding assay and the anti-mouse Ig conjugated to β -galactosidase bound to mAb 4B7 (data not shown).

Most of our experiments were performed with sugar-fed adult female *A. stephensi* mosquitoes. When the same experiment was performed with mosquitoes that had received a blood meal 4 days earlier, the same results were obtained (data not shown). We also investigated the binding of CS to male salivary glands. Shown in Fig. 3 are the results of a typical experiment. Although CS did bind to the male glands, binding was subjectively not as strong and not as defined; CS seemed to bind diffusely on the gland and in no particular pattern. In this experiment, the control female salivary gland stained identically to the one shown in Fig. 2A.

We then analyzed the fate of CS injected into the mosquito hemocoel in vivo. Ten mosquitoes were injected with 0.9 μ g of CS; all of the mosquitoes survived the injection and their salivary glands, midguts, Malpighian tubules and ovaries were harvested 1 h later to investigate the fate of the injected CS. All of the salivary glands from mosquitoes injected with CS were positive for CS binding, whereas the midguts, Malpighian tubules and ovaries were consistently negative (Fig. 4). Salivary glands from four control mosquitoes injected with M199/1%BSA were negative (data not shown). These findings exclude the possibility that the results of the previous experiments were artefactual due to fixation of the glands.

Finally, we used synthetic peptides to investigate which portion of the CS was responsible for binding to salivary glands. Table 1 shows the results from six experiments in which peptides representing different regions of CS were used as inhibitors of binding of the entire recombinant CS molecule to salivary glands. We used peptides representing the two conserved regions of the protein, region I and region II-plus, and the species-specific repeats

(Fig. 1). As shown in Table 1 and Fig. 5E, a peptide that encompasses region I and includes 5 amino acids upstream of region I (region I, pep3; KPKHKKLKQPADGNPDPNAN) inhibited binding of CS to salivary glands whereas peptides representing the repeat region or region II-plus did not have inhibitory activity (Table 1 and Fig. 5B, D). Since the inhibitory peptide encompassed region I as well as 5 residues upstream of this region, we synthesized overlapping peptides (Fig. 1) to determine which portion of this peptide was responsible for the inhibitory activity. As shown in Table 1, a peptide beginning with region I and missing the 5 upstream residues (region I, pep 4; KLKQPADGNPDPNANPNVDP) and a peptide including the upstream residues but missing region I (region I, pep 2; GENDDGNNEDNEKLRLKPKHK) did not have inhibitory activity. In addition, the 5 amino acid peptide representing region I alone (region I, pep1; KLKQP) did not inhibit CS binding to salivary glands. Of note, region I, pep2 and region I, pep4 have a net negative charge whereas our inhibitory peptide, region I, pep3, has an overall charge of +3. To insure that inhibition of CS binding was not due to a nonspecific charge interaction, we synthesized a control peptide from an N-terminal region of the CS whose overall charge was +3 (control peptide, MNYYGKQENWYSLKKNSRSL) and this peptide did not have any inhibitory activity (Table 1 and Fig. 5C).

4. Discussion

Our findings, demonstrating that CS binds to salivary glands with greater avidity than to other mosquito organs exposed to the hemolymph, provide a rationale for the preferential binding of sporozoites to salivary glands. In our studies, binding of CS was most intense on the median lobe and the distal portion of the lateral lobes, those portions of the glands that are preferentially invaded by sporozoites [16]. Although male mosquitoes do not transmit malaria, CS bound to these glands, albeit in a less intense and more diffuse pattern when compared to female glands. The salivary glands of male mosquitoes do not show the same regional differences as the female glands and the secretory activity of the entire male gland is comparable to the proximal portion of the lateral lobes of the female glands [17]. It is not known, however, if the composition of the basal lamina of the male glands is similar to that of the distal or proximal portion of the lateral lobes of the female glands. Therefore, we cannot say whether CS binding to male glands is mediated by the same receptors as in the female glands.

We have also shown that a peptide encompassing the conserved region I of CS can inhibit binding of the protein to the glands. We found, however, that the short 5 amino acid region I sequence is necessary but not sufficient for inhibitory activity and that some of the residues upstream of this conserved region are also required. A comparison of the residues upstream of region I reveals that although this region is not conserved, it always contains a cluster of basic residues with 2 or 3 acidic residues (Table 2). Further studies will hopefully elucidate whether differences in this region of CS among different *Plasmodium* species are responsible for some of the species specificity between the parasite and its mosquito host [18,19].

A recent electron microscopic study demonstrates that salivary gland invasion by sporozoites is a multistep process involving sequential interactions with the basal lamina

and the plasma membrane of the target cell [20]. The first event in invasion is the adhesion of the sporozoite to the target cell. Electron micrographs of this step show the attachment of the parasite's cell coat to the filamentous structures of the basal lamina. If the specific binding of CS to salivary glands is important in salivary gland invasion, we hypothesize that it may be functioning at this early step, namely by mediating the initial adhesion of sporozoites to salivary glands. Although further studies are needed to elucidate the nature of the CS-receptor on the salivary gland, it could be a component of the basal lamina. There are tissue-specific components in the basal laminae of the various mosquito organs [21]; thus salivary gland specificity of CS binding could be explained if CS bound to one of these components.

It has been previously shown that CS is important for sporozoite invasion of hepatocytes (reviewed in [22]). CS, therefore, may be involved in sporozoite adhesion to both of its target organs, the salivary glands and the liver although a different region of the CS is involved in each case. CS binding to hepatocytes is region II-plus dependent [23] whereas our results suggest that binding to salivary glands involves the other conserved sequence of the protein, namely region I. It has long been known that salivary gland and oocyst sporozoites are not identical; oocyst sporozoites lack significant infectivity for the vertebrate host [24] and salivary gland sporozoites are unable to reinvade salivary glands [25]. Although CS may have a different conformation in these two sporozoite populations, exposing different regions of the protein at different stages, this difference may also be due to an, as of yet undefined, maturation process, such as the acquisition of gliding motility [26].

In summary, we show that CS, the major surface protein of oocyst sporozoites, binds specifically to its target organ, the mosquito salivary glands. This interaction involves the conserved region I. Future studies will hopefully elucidate the identity of the CS-receptors on the salivary gland and the role of this binding event in the life cycle of the parasite.

Acknowledgments

The authors would like to thank Drs Victor Nussenzweig and Kenneth Vernick for helpful discussions and review of the manuscript, Jean Nonon for rearing the mosquitoes and Pedro Clavijo for peptide synthesis. We thank Drs David Kaslow and Bela Takacs for their generous gifts of recombinant Pfs25 and CS respectively. SPS was supported by the Fondation Medic pour la Recherche Medicale, Switzerland and by the Rotary Foundation. PS was supported by NIH grant no. K11AI0117501.

References

1. Simonetti AB. The biology of malarial parasite in the mosquito-A review. *Mem Inst Oswaldo Cruz*. 1996; 91:519–41. [PubMed: 9137738]
2. Golenda CF, Starkweather WH, Wirtz RA. The distribution of circumsporozoite protein (CS) in *Anopheles stephensi* mosquitoes infected with *Plasmodium falciparum* malaria. *J Histochem Cytochem*. 1990; 38:475–81. [PubMed: 2181019]
3. Robert V, Verhave JP, Ponnudurai T, Louwe L, Scholtens P, Carnevale P. Study of the distribution of circumsporozoite antigen in *Anopheles gambiae* infected with *Plasmodium falciparum*, using the enzyme-linked immunosorbent assay. *Trans R Soc Trop Med Hyg*. 1988; 82:389–91. [PubMed: 3068853]

4. Barreau C, Touray M, Pimenta PF, Miller LH, Vernick KD. *Plasmodium gallinaceum*: sporozoite invasion of *Aedes aegypti* salivary glands is inhibited by anti-gland antibodies and by lectins. *Exp Parasitol*. 1995; 81:332–43. [PubMed: 7498430]
5. Sinnis P, Nussenzweig V. Preventing sporozoite invasion of hepatocytes.. In: Hoffman, SL., editor. *Malaria Vaccine Development: A Multi-Immune Response Approach*. ASM Press; Washington D.C.: 1996. p. 15-33.
6. Nagasawa H, Procell PM, Atkinson CT, Campbell GH, Collins WE, Aikawa M. Localization of circumsporozoite protein of *Plasmodium o[54]ale* in midgut oocysts. *Infect Immun*. 1987; 55:2928–32. [PubMed: 3679537]
7. Nagasawa H, Aikawa M, Procell PM, Campbell GH, Collins WE, Campbell CC. *Plasmodium malariae*: distribution of circumsporozoite protein in midgut oocysts and salivary gland sporozoites. *Exp Parasitol*. 1988; 66:27–34. [PubMed: 3284757]
8. Boulanger N, Matile H, Betschart B. Formation of the circumsporozoite protein of *Plasmodium falciparum* in *Anopheles stephensi*. *Acta Trop*. 1988; 45:55–65. [PubMed: 2896447]
9. Takacs BJ, Girard MF. Preparation of clinical grade proteins produced by recombinant DNA technologies. *J Immunol Methods*. 1991; 143:231–40. [PubMed: 1940392]
10. Nardin EH, Nussenzweig V, Nussenzweig RS, Collins WE, Harinasuta KT, Tapchaisri P, Chomcharn Y. Circum sporozoite proteins of human malaria parasites *Plasmodium falciparum* and *Plasmodium [p54]i[p54]ax*. *J Exp Med*. 1982; 156:20–30. [PubMed: 7045272]
11. Kaslow DC, Shiloach J. Production, purification and immunogenicity of a malaria transmission-blocking vaccine candidate: TBV25H expressed in yeast and purified using nickel-NTA agarose. *Biotechnology*. 1994; 12:494–9. [PubMed: 7764708]
12. Barr PJ, Green KM, Gibson HI, Bathurst IC, Quakyi IA, Kaslow DC. Recombinant Pfs25 protein of *Plasmodium falciparum* elicits malaria transmission-blocking immunity in experimental animals. *J Exp Med*. 1991; 174:1203–8. [PubMed: 1940798]
13. Houghten RA. General method for the rapid solid-phase synthesis of a large number of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc Natl Acad Sci USA*. 1985; 82:5131–5. [PubMed: 2410914]
14. Vanderberg JP. *Plasmodium berghei*: quantitation of sporozoites injected by mosquitoes feeding on a rodent host. *Exp Parasitol*. 1977; 42:169–81. [PubMed: 324786]
15. Vermeulen AN, Ponnudurai T, Beckers PJA, Verhave JP, Smits MA, Meuwissen JHET. Sequential expression of antigens on sexual stages of *Plasmodium falciparum* accessible to transmission-blocking antibodies in the mosquito. *J Exp Med*. 1985; 162:1460–5. [PubMed: 2865324]
16. Sterling CR, Aikawa M, Vanderberg JP. The passage of *Plasmodium berghei* sporozoites through the salivary glands of *Anopheles stephensi*: an electron microscope study. *J Parasitol*. 1973; 59:593–605. [PubMed: 4578977]
17. Wright KA. The anatomy of salivary glands of *Anopheles stephensi* Liston. *Can J Zool*. 1969; 47:579–87.
18. Rosenberg R. Inability of *Plasmodium knowlesi* sporozoites to invade *Anopheles freeborni* salivary glands. *Am J Trop Med Hyg*. 1985; 34:687–91. [PubMed: 4025683]
19. McCutchan TF, Kissinger JC, Touray MG, et al. Comparison of circumsporozoite proteins from avian and mammalian malarias: biological and phylogenetic implications. *Proc Natl Acad Sci USA*. 1996; 93:11889–94. [PubMed: 8876233]
20. Pimenta PF, Touray M, Miller L. The journey of malaria sporozoites in the mosquito salivary gland. *J Euk Microbiol*. 1994; 41:608–24. [PubMed: 7866385]
21. Clements, AN. *The Biology of Mosquitoes*. Chapman and Hall; New York: 1992.
22. Sinnis P, Sim BKL. Cell invasion by the vertebrate stages of *Plasmodium*. *Trends Microbiol*. 1997; 5:52–8. [PubMed: 9108930]
23. Cerami C, Frevert U, Sinnis P, Takacs B, Clavijo P, Santos MJ, Nussenzweig V. The basolateral domain of the hepatocyte plasma membrane bears receptors for the circumsporozoite protein of *Plasmodium falciparum* sporozoites. *Cell*. 1992; 70:1021–35. [PubMed: 1326407]
24. Vanderberg JP. Development of infectivity by the *Plasmodium berghei* sporozoite. *J Parasitol*. 1975; 61:43–50. [PubMed: 1090717]

25. Touray MG, Warburg A, Laughinghouse A, Krettli AU, Miller LH. Developmentally regulated infectivity of malaria sporozoites for mosquito salivary glands and the vertebrate host. *J Exp Med*. 1992; 175:1607–12. [PubMed: 1588284]
26. Vanderberg JP. Studies on the motility of Plasmodium sporozoites. *J Protozool*. 1974; 21:527–37. [PubMed: 4138523]

Fig. 1.

(A) Schematic representation of CS showing the centrally located species-specific repeats; N-terminal and C-terminal to the repeats are region I and region II-plus, respectively. These regions contain motifs that are highly conserved in CS proteins from all species of *Plasmodium*. The amino acid sequences of these conserved regions from *P. falciparum* CS are shown. (B) Peptides used in this study were chosen to represent the three regions of *P. falciparum* CS shown above, namely the repeats, region I and region II-plus. Overlapping peptides from the region immediately N-terminal to the repeats were used to determine which residues from this region were required for inhibition of CS binding to salivary glands. The control peptide represents a region 40 residues upstream from region I. Bold letters indicate the positively-charged amino acids.

Fig. 2.

CS binds to mosquito salivary glands and not to other organs exposed to the hemolymph. Organs are dissected from mosquitoes, fixed and stained with recombinant CS protein (A, C, D, E) or Pfs25 (B). The organs are washed and binding of the protein is revealed with a mAb specific for either CS or Pfs25, followed by anti-mouse Ig conjugated to β -galactosidase and X-gal. Protein binding is indicated by a blue color. As shown, salivary glands (A) bind CS where as the midgut (C), Malpighian tubules (D) and ovary (E) do not. Salivary glands incubated with a control protein, Pfs25 (B) do not turn blue. This experiment was repeated 6 times and a representative experiment is shown. Bar=0.1 mm.

Fig. 3.

Male salivary glands bind CS. Salivary glands were dissected from male and female (not shown) mosquitoes, fixed and incubated with CS. Binding was revealed with mAb 2A10 followed by anti-mouse Ig conjugated to β -galactosidase and X-gal. Shown is a representative male salivary gland. Two experiments were performed, each with four male salivary glands, with identical results. Bar =0.1 mm.

Fig. 4.

CS binds specifically to salivary glands after injection into the mosquito's hemocoel. Approximately $0.9\ \mu\text{g}$ of CS was injected into the hemocoel of ten mosquitoes. After 1 h the organs were dissected from the mosquitoes, mounted, fixed and incubated with mAb 2A10 followed by anti-mouse Ig conjugated to β -galactosidase and X-gal. CS binding is indicated by the blue color. Salivary glands (A) bind CS whereas midguts and Malpighian tubules (B) and ovaries (C) do not. The blue in panel C is on the external cuticle of the mosquito and probably represents nonspecific binding of one of the antibodies. Shown are representative organs from one experiment. This experiment was repeated with identical results. Bar=0.1 mm.

Fig. 5.

Peptides encompassing region I inhibit CS binding to salivary glands. Mosquito salivary glands were fixed, blocked and preincubated with either no peptide (A), repeat peptide (B), control peptide (C), region II-plus (D) or region I-pep3 (E). The peptide solution was removed and the glands were then incubated with CS in the presence of the peptide, washed and CS binding was revealed as stated above. Bar=0.1 mm.

Table 1

Peptide inhibition of CS binding to salivary glands

Peptide	% Glands showing inhibition of CS binding		Number of salivary glands observed
	Median lobe ^a	Lateral lobe ^b	
None	5	0	38
Region II-plus	14	0	14
Repeat	0	0	14
Control Peptide	0	0	26
Region I-Pep1	0	0	9
Region I-Pep2	0	14	7
Region I-Pep3	94	88	34
Region I-Pep4	0	0	9

The results from six experiments are combined in this table. In each experiment, salivary glands were blindly scored as to the intensity of blue color on their lateral and medial lobes: 0 = no blue, + = faint blue, ++ = blue, +++ = dark blue.

^aOf the median lobes which stained 97% were scored as ++ or +++ (less than 3% were scored as +). Therefore, we considered inhibition of staining to be a score of 0 or +.

^bOf the lateral lobes which stained 98% were scored as + or ++, (less than 2% were scored as +++). Therefore, we considered inhibition of staining to be a score of 0.

Table 2

The amino acid sequences upstream of region I from different species of *Plasmodium*

Species	Sequence ^a
<i>P. falciparum</i>	EDNE KLRKPKHK KLKQP
<i>P. vivax</i>	G KKE APKNPREN KLKQP
<i>P. malariae</i>	E RKKK PTKA VEN KLKQP
<i>P. cynomolgi</i>	E KKV EP KK PREN KLKQP
<i>P. yoelli</i>	E KK ADPPKEAQN KLKQP
<i>P. berghei</i>	KNEKKN KIERNN KLKQP

^a Basic residues are shown in bold.