Glycoconjugate Journal (1997) **14:** 473–479

Saccharide anions as inhibitors of the malaria parasite

Diana L. Clark, Shidong Su and Eugene A. Davidson*

Department of Biochemistry and Molecular Biology, Georgetown University, 3900 Reservoir Road, Washington, DC 20007, USA

The asexual erythrocytic stage of *Plasmodium falciparum* was grown in culture in the presence or absence of glycoconjugate polyanions of varying structure, size and substitutions. Heparin, dextran sulfate, fucoidan and pentosan polysulfate had antimalarial IC_{50} values between one and 11 μg ml^{-1}. Constituent heparin disaccharides were ineffective against the malaria parasite and desulfation from either the O- or N-substitution sites of heparin or reduction of the uronic acid carboxyl group neutralized the antimalarial response to varying degrees. Immobilization of heparin onto agarose beads still permitted antimalarial activity suggesting that parasite uptake of the glycoconjugate is not required for inhibition. Accordingly, it is concluded that invasion of free parasites into the erythrocytes was inhibited rather than parasite maturation within the red cell. Merozoite surface antigen-1 was apparently prevented from binding to human erythrocytes in the presence of highly sulfated polyanions and, in a dose-dependent fashion, heparin.

Keywords*: glycoconjugate, heparin, malaria, merozoite surface antigen-1, *Plasmodium

***Abbreviations*: MSA-1, merozoite surface antigen-1**

Introduction

Heparin exhibits antimalarial activity when incubated with *Plasmodium falciparum* parasites growing in culture. This activity is independent of the anticoagulant activity of heparin. Different investigators report IC_{50} values ranging from micrograms heparin per milliliter parasite culture to milligrams heparin per milliliter culture [1, 2]. The cause of parasitemia reduction is unclear but has been attributed to either the prevention of successful schizont rupture or prevention of merozoite reinvasion after schizont rupture. The activity has been reported to be reversible if the culture is washed free of heparin before schizont maturation and release, but whether heparin interacts with the host erythrocyte surface or a cognate parasite protein on the merozoite surface has not been determined.

Heparin, being highly anionic, is more apt to interact with a parasite protein than with the anionic glycocalyx on the erythrocyte surface. One candidate protein in this context is merozoite surface antigen-1 (MSA-1). This protein is expressed on the merozoite surface and has been reported to have a role in erythrocyte invasion [3, 4]. MSA-1 binds to the uninfected erythrocyte cell surface in a sialic acid-dependent manner. This binding correlates with successful invasion of the erythrocyte by defined strains of the merozoite and is specific for only those erythrocytes able to support *P. falciparum* growth. The estimated size of MSA-1 in different clones or isolates varies between 185 and 205 kDa. It is proteolytically processed to a number of discrete fragments (83, 42, 33 and 19 kDa) during merozoite maturation and during or just before erythrocyte invasion. The function of this post-translational modification is unknown. It should be noted that other erythrocyte-binding proteins of the malaria parasite have been identified. In no case has an explicit connection been made between binding and the subsequent events that result in invasion of the red cell.

More specifically, MSA-1 binds only to human erythrocytes expressing glycophorin A, B or C, a heavily sialylated transmembrane protein [5]. Diminishing the availability of glycophorin by wheat germ agglutinin, trypsin treatment or chemical modification of erythrocytes renders the erythrocytes resistant to parasite invasion [3, 6]. This suggests that glycophorin on the erythrocyte surface and parasite MSA-1 both have roles in recognition and/or invasion by specific isolates of the malaria parasite. Strains of the parasite have been identified wherein neuraminidase treatment of erythrocytes has no effect on ability of the parasite to invade the red cell. Further, the erythrocytic receptor for

* Corresponding author. Tel: 202-687-1401; Fax: 202-687-7186.

0282–0080

other species of the malaria parasite (*P. vivax*, *P. knowlesi*) has been reported to be the Duffy blood group antigen [7, 8]. Thus, not all strains may recognize glycophorin or be dependent on the presence of sialic acid. It has been suggested, however, that most field isolates are dependent on sialic acid for erythrocyte recognition [25].

In this report we show that heparin, in a length and sulfation-dependent manner, inhibits parasite invasion of the erythrocyte as well as the binding of MSA-1 to the erythrocyte. This effect appears to be surface related and does not require heparin uptake since heparin immobilized on agarose beads behaves similarly. Other sulfated polysaccharides, dextran sulfate, fucoidan and pentosan polysulfate act analogously. In contrast, dermatan sulfate, chondroitin 6-sulfate and colominic acid (a sialic acid polymer) showed neither antimalarial activity nor inhibition of MSA-1 binding to erythrocytes although they are all polyanions. These studies suggest that there is specificity in the anion constellation on the erythrocyte that is associated with binding/recognition of the parasite and successful invasion.

Materials and methods

Reagents

Polyanionic glycoconjugates were obtained from Sigma Chemical (St Louis, MO). Modified heparins lacking sulfate moieties on either the N- or O-atoms were a kind gift from P. Shaklee (Glycomed, Alameda CA). Heparin disaccharides were prepared by digestion of heparin with heparinase and purification of the products by exclusion chromatography on Bio-Gel P4. Heparin was immobilized onto agarose beads by prior activation of the agarose with cyanogen bromide, coupling in the presence of bicarbonate and blocking unreacted sites with triethanolamine [9].

Culture of parasites

P. falciparum (isolate FCR-3) was maintained in complete medium: RPMI 1640 medium supplemented with 10% human serum, 25 mM HEPES, 367 μM hypoxanthine and 0.24% sodium bicarbonate at 5% hematocrit. The parasites were grown in an atmosphere of 5% O_2, 5% CO_2 and 90% N_2 with daily changes of medium [10].

Invasion assay

The invasion assay was performed as described [11]. Briefly, parasites were synchronized to the ring stage with 5% sorbitol (w/v). When the cells had matured to schizonts, the test compounds were added to the culture in a microtiter plate. The parasites were incubated an additional 24 h during which they burst their host erythrocyte and invaded fresh erythrocytes, forming young rings. The parasitemia was then determined as follows: The cells were fixed in 0.1% glutaraldehyde (v/v in phosphate buffered saline, PBS) and then counted by fluorescent activated cell sorting after staining with 0.005% propidium iodide (w/v, in PBS). The percentage inhibition was determined by dividing the parasitemia of test cultures by that found with control cultures without inhibitors, multiplying the result by 100, then subtracting the result from 100.

Metabolic radiolabeling

The parasites (4% parasitemia, 4% hematocrit) were synchronized twice with sorbitol, 48 h between synchronizations. When the parasites had matured to schizonts after the second sorbitol synchronization, the parasitemia was enriched to 50% by incubation with 0.6% (w/v) gelatin in complete medium for 30 min at 37° at 10% hematocrit. The upper layer, containing the schizonts, was returned to culture in complete medium lacking methionine (Select-Amine, Gibco-BRL) and supplemented with 1 mCi of [^{35}S]-methionine (1000 Ci mmol^{-1}) overnight. The unincorporated label was removed by centrifugation through an Amicon Centriprep (30 000 molecular weight cut-off) and the lysate was washed with PBS containing either 4.9 TIU aprotinin or PBSI (PBS, pH 8.0, 5 mM iodoacetamide, 2 mM PMSF, 5 mM EDTA, 2 mM $Na_2S_2O_5$ and 4.6 TIU aprotinin) before storage at −80° [5]. The labeled parasite lysate was used within 2 d to minimize subsequent degradation during storage.

Antigen solubilization

Radiolabeled parasite proteins were treated with five volumes of PBSI supplemented with 1% NP-40 and 0.1% deoxycholate for 1 h at 4° [12]. Sediment was removed by centrifuging (10 000 × **g**, 10 min) before immunoprecipitation.

Immunoprecipitation

Solubilized parasite lysate (diluted with PBSI to 1 ml) was incubated with 10 μl antiserum and incubated for 2 h. The antiserum, directed against recombinant MSA-1, was a kind gift from Dr George Hui. Formalin-fixed *Staphylococcus aureus* (100 μl of 10% solution) was washed with washing buffer (PBS, pH 8.0, 0.1% SDS, 0.1% BSA and 0.5% NP-40) and resuspended in the solubilized parasite lysate-primary antibody mixture. After incubating overnight at 4°, the sediment was extensively washed as follows: twice in washing buffer, twice in PBS-0.5 M NaCl-0.1% NP-40 and once in PBS. Bound proteins were eluted by boiling in SDS sample buffer. The *S. aureus* sediment was pelleted before SDS-PAGE and fluorography.

SDS-PAGE and fluorography

Electrophoresis was performed with Laemmli buffers in 7.5% polyacrylamide gels. To visualize the molecular weight standards, the gels were first stained with Coomassie blue and destained. The gels were rehydrated in water before soaking with fluor (Fluoro-Hance, Research Products

International, Corp.), subsequent drying and film exposure. Exposure time was typically 3 d.

Results and discussion

Effects of anionic polysaccharides on *P. falciparum* parasitemia

To investigate the effect of heparin and other polyanionic polysaccharides on *P. falciparum* proliferation, the glycoconjugates were incubated with the asexual erythrocytic stage of the parasite. Synchronized parasite cultures at the late trophozoite/early schizont stage of development were incubated with polysaccharides differing in saccharide composition and degree of sulfation. The parasites were allowed to mature and release merozoites. The ability of these merozoites to invade uninfected erythrocytes was determined by measuring the resultant parasitemia. Those erythrocytes containing parasites were positively stained with propidium iodide and their increased fluorescence detected by fluorescent activated cell (FACS) analysis. Only the densely sulfated polysaccharides heparin, dextran sulfate, pentosan polysulfate and fucoidan were effective in reducing the parasitemia. These polysaccharides had IC_{50} values of 2, 7.5, 11 and 22 $\mu g\,ml^{-1}$ respectively (Table 1). The less densely substituted polysaccharides, chondroitin-6-sulfate, dermatan sulfate and colominic acid had little or no antimalarial activity when tested in concentrations up to 10-fold higher than the aforementioned sulfated glycoconjugates.

The antimalarial efficacy of heparin described here agrees closely with an earlier finding. Kulane reports an IC_{50} value for heparin of 5 $\mu g\,ml^{-1}$ [2] while Butcher reports an IC_{50} almost three orders of magnitude greater (1000 $\mu g\,ml^{-1}$). It is unclear what is responsible for the discrepancy in the IC_{50} values. The difference may lie in assay conditions, parasitemia determination or heparin source. As heparin is a heterogeneous population of polymers, different heparin sources may be enriched with species of varying composition and sulfation. Although the investigators employed different strains of *P. falciparum* in their studies, Kulane detected no difference in heparin antimalarial activity among four different *P. falciparum* strains [FCR3, FCR3 (-RESA), F32 and R + PA1] [2], suggesting that the antimalarial activity of heparin is not strain-specific.

To investigate the contribution of nonspecific ion effects, all anionic polysaccharides were additionally tested at equivalent molar sulfate (or carboxylate in the case of colominic acid) concentration. Otherwise, the requirement for densely anionic polysaccharides, rather than chondroitin sulfate, dermatan sulfate or colominic acid, could simply be attributed to differences in total anion concentration between compounds. Those polysaccharides with lower anion densities were ineffective in inhibiting parasite invasion even when present at the same total anion concentration as ten times the IC_{50} value of heparin (25 $\mu g\,m^{-1}$). Dextran sulfate, fucoidan and pentosan polysulfate inhibited parasite invasion 50, 61 and 42% respectively whereas heparin inhibited 86% at this concentration. This suggests that the inhibition of invasion is not due only to nonspecific ionic interactions between sulfates and a parasite protein, but instead, a particular conformation of the anion(s) is required for effective inhibition. Calcium supplementation of incubations had no influence on the inhibitory activity of heparin (data not shown).

The specificity for polysaccharides with higher sulfate content is identical to that reported earlier by Butcher who also reports no activity with chondroitin 4- or 6-sulfate [1]. Curiously, murine *P. berghei* sporozoite invasion of hepatocytes is also inhibited by heparin and other densely sulfated polysaccharides albeit at concentrations 100-fold greater than reported here. In the murine model, the sporozoite circumsporozoite protein is reportedly prevented from binding and invading host hepatocytes possibly via a receptor like interaction with heparan sulfate present at the plasma membrane of the hepatocyte [13]. The same susceptibility to anionic polysaccharides suggests an evolutionarily conserved mechanism of recognition or invasion

Table 1. Inhibition of *P. falciparum* proliferation by anionic polysaccharides.

Polysaccharide	*Structure*	*IC_{50} [$\mu g\,ml^{-1}$]*	*% Inhib*
Heparin	$[(\text{iduronic glucuronic-GlcNAc})\text{-}S_3]_n$	2.0	86.0
Dextran sulfate	$[\text{Glc-}S_3]_n$	7.5	50.1
Pentosan polysulfate	$[\text{Xylan]-}S_2]_n$	11	60.9
Fucoidan	$[\text{Fucose-}S_2]_n$	22	42.3
Dermatan sulfate	$[\text{Iduronic-GalNAc-S}]_n$	> 200	− 2.28
Chondroitin sulfate C	$[\text{Glucuronic-GalNAc-S}]_n$	> 1000	6.91
Colominic acid	$[\text{Sialic acid}]_n$	> 1000	− 1.33

Polysaccharides were tested in the *P. falciparum* invasion assay at $\mu g\,ml^{-1}$ concentrations to determine IC_{50} values and also at the equivalent sulfate content as 25 $\mu g\,ml^{-1}$ heparin to determine the role of total anion concentration. Data shown is average of duplicates and representative of repeated experiments.

among species and developmental stages of the parasite although the only anionic species available on the erythrocyte would be carboxyl groups.

In a different study, the rosetting of infected erythrocytes with uninfected red cells was shown to be inhibited by heparin and other sulfated molecules including fucoidan but not by the chondroitin sulfates, analogous to the results reported here [14].

Heparin is a more potent inhibitor than dextran sulfate and fucoidan although all three compounds exhibit antimalarial activity. There is a unique specificity of sugar or anion binding in proteins involved in adhesion or cell-to-cell interactions. These proteins include thrombospondin [15], von Willebrand factor [16], bindin [17] and laminin [15]. Thrombospondin binds fucoidan better than dextran sulfate and heparin [15], whereas von Willebrand factor prefers dextran sulfate to fucoidan [16]. The polysaccharide binding preferences of each system may reflect which glycoconjugate offers a conformation that most closely mimics the native ligand.

Effects of modified heparins on parasitemia

To confirm that the sulfate moieties play an essential role in the antimalarial activity of heparin, they were specifically removed from either the N- or O-positions on the polysaccharide. Additionally, the role of the carboxylate ion was investigated with a chemically modified heparin having the carboxyl groups reduced to alcohols (Figure 1). As predicted, when tested in the invasion assay, N-desulfated heparin lost almost all of its potency to diminish parasitemia and O-desulfated and carboxylate-reduced heparin retained less than half of their efficacy. Clearly, the anionic character of heparin is critical for activity and the sulfate moieties play a larger role than the carboxylate anions in the antimalarial activity of heparin.

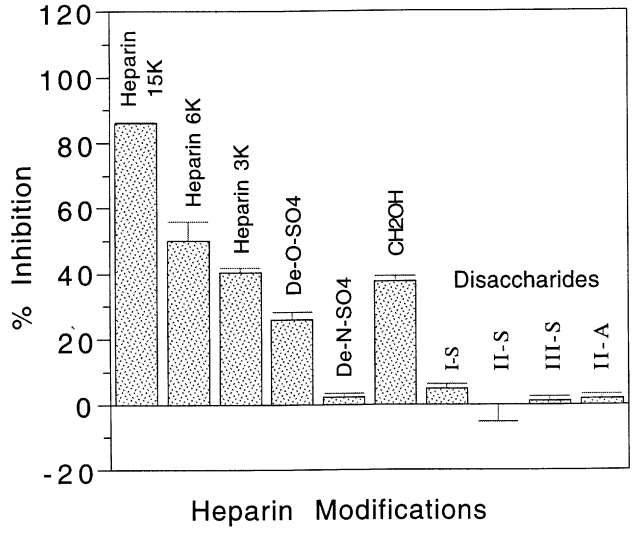

Figure 1. The antimalarial activity of heparin is sulfation and length-dependent. Modified heparins and fragments were assayed in the invasion assay at equivalent molar sulfate concentration, or in the case of desulfated material, at a carboxylate ion concentration equivalent to the sulfate concentration employed for heparin. Disaccharides were isolated after enzymatic digestion of heparin and were monosulfated (I-S), disulfated (II-S), trisulfated (III-S) and unsulfated (II-A).

In addition to anion density, the antimalarial activity is dependent on polymer length. Lower molecular weight heparins ($M_r = 6000$ or 3000) were less effective than the higher molecular weight standard heparin ($M_r = 15\,000$) even when present at equivalent anion concentration (Figure 1). Disaccharides obtained by enzymatic digestion of heparin were ineffective although tested at the same total anion concentration as the 15 000 molecular weight heparin (Figure 1). Kulane also reported a lower molecular weight fraction of heparin (of undetermined size) having 200 times less antimalarial activity than a higher molecular weight population of heparin [2]. Size-specificity is a characteristic of most heparin-binding proteins. The requirement for higher molecular weight heparin is also evident in heparin binding to fibronectin [18], endothelial cells [19] and the antithrombin III-thrombin-heparin ternary complex [16]. The number of heparin-binding sites in the protein or the secondary conformations available to a longer heparin chain length may be important in determining the length specificity for heparin antimalarial activity.

Differences in polysaccharide size alone cannot be solely responsible for the specificity in inhibiting *P. falciparum* proliferation. Pentosan polysulfate is the same size as low molecular weight heparin but is twice as effective while chondroitin 4-sulfate is the same size as heparin but is ineffective. The lack of activity of heparin disaccharides may also reflect the structural alteration that occurs as a result of enzymatic cleavage or the fact that heparin does not have a strict repeat structure and would give rise to several different disaccharide products.

Since a failure of the parasite to replicate once inside the erythrocyte could also be interpreted as inhibition, heparin was immobilized to prevent cellular entry and then this material examined for inhibition of invasion. The data show that the heparin-agarose is an inhibitor although not quite as effective as free heparin in solution (Figure 2).

Effect of anionic polysaccharides on parasite proteins binding to erythrocytes

To investigate if heparin's antimalarial activity may be due to the disruption of the pathogen/host interaction, as is seen in *P. berghei* sporozoites, herpes simplex virus [20] and human immunodeficiency virus [21], parasite proteins that bind to erythrocytes were investigated in the absence or presence of 25 $\mu g\,ml^{-1}$ heparin and other polysaccharides. Metabolically radiolabeled parasite proteins were bound to uninfected erythrocytes, the cells washed and eluted with

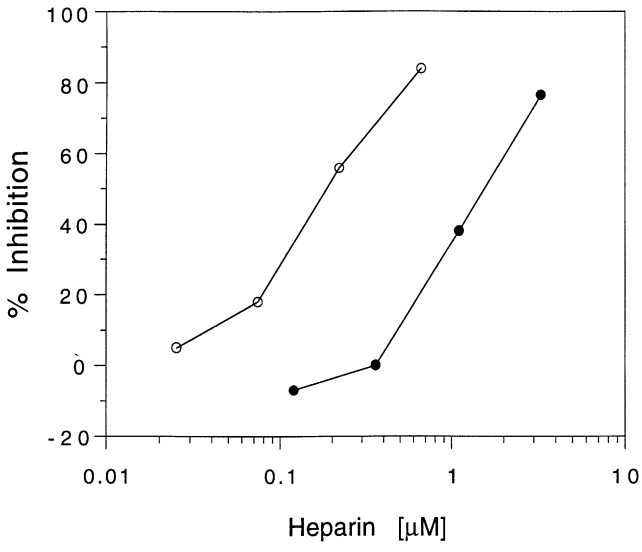

Figure 2. Immobilized heparin inhibits parasite invasion. Heparin was immobilized on agarose and tested in the invasion assay. Open circles, free heparin (MW 15 000) in solution, filled circles, immobilized heparin.

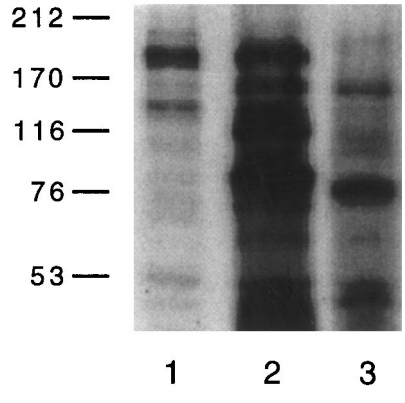

Figure 3. MSA-1 binds to erythrocytes. Metabolically radiolabeled merozoite lysate were incubated with uninfected erythrocytes. One dominant band was specifically eluted from the cell surface (lane 1). The merozoite lysate was immunoprecipitated with antiserum directed against MSA-1 or preimmune serum (lanes 2 with 3 respectively) and electrophoresed adjacent to the eluted protein.

buffer containing 1 M NaCl. After electrophoresis and fluorography, a 200 kDa parasite protein and a smaller molecular weight (120 kDa) protein were detected (Figure 3). We observed that storage of radiolabeled lysate prior to erythrocyte binding caused the smaller molecular weight band to become more prominent, suggesting that it might be a degradation product of the 200 kDa band [10]. In addition, the presence of either a single, diffuse band, a doublet or a triplet at 200 kDa depended on the protease inhibitors employed in parasite lysate preparation. The addition of a single protease inhibitor, aprotinin, minimized the number of bands observed.

An analogous binding study was performed with heparin-agarose. In this case, almost all of the parasite proteins bound to the immobilized heparin (data not shown). Thus, specific binding of MSA-1 could not be demonstrated although it among other proteins is able to bind to immobilized heparin. This result may, in part, be reflective of the relatively high isoelectric point of parasite proteins and the likelihood that they contain regions of net positive charge which would naturally interact with heparin.

To determine if the 200 kDa parasite protein is merozoite surface antigen-1 (MSA-1), a protein of the same apparent molecular weight reported to bind to erythrocytes [3, 4], an immunoprecipitation of the parasite lysate was peformed using serum directed against recombinant MSA-1 (a kind gift from Dr George Hui). Five bands were immunoprecipitated from the lysate (Figure 2). These bands correspond in apparent molecular weight to MSA-1 and its processed fragments (200, 120, 83, 42 and 33 kDa) [22]. The smallest processed fragment of MSA-1, 19 kDa, was removed from the metabolically radiolabeled sample when the parasite lysate was concentrated and the unincorporated [^{35}S]-methionine was removed from the lysate by passage through a filter with 30 000 molecular weight cut-off. A non-specific 150 kDa protein was immunoprecipitated from both antiserum and normal rabbit serum. The 200 kDa parasite protein eluted from erythrocytes was electrophoresed in the lane adjacent to the immunoprecipitation and migrates exactly as the unprocessed MSA-1. Detection of MSA-1 by Western blotting was not successful, apparently due to poor transfer of MSA-1 to the membrane prior to probing with the antibody.

The binding of the presumed MSA-1 to erythrocytes was inhibited in a dose dependent fashion by heparin (Figure 4) and also by dextran sulfate, pentosan polysulfate and fucoidan, the same compounds inhibiting erythrocyte invasion (Figure 5). Desulfated heparin and colominic acid failed to prevent the protein from binding when present at

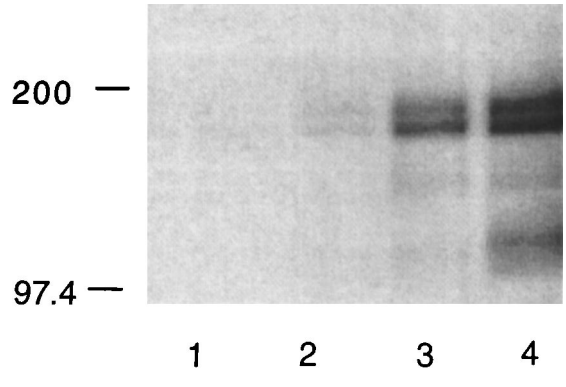

Figure 4. Heparin inhibits MSA-1 binding to erythrocytes in a dose-dependent manner. Merozoite lysate was incubated with either 100, 50, 10 or 0 μg ml^{-1} heparin (lanes 1, 2, 3, 4 respectively) prior to incubation with uninfected erythrocytes.

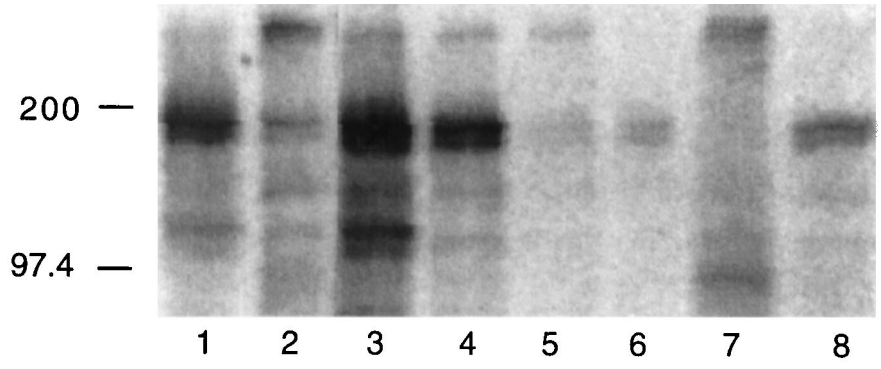

Figure 5. Antimalarial polysaccharides inhibit MSA-1 from binding to erythrocytes. Metabolically radiolabeled merozoite lysate was incubated with anionic polysaccharides prior to incubation with erythrocytes and specific elution. (Lane 1, no inhibitor; lane 2, 50 μg ml^{-1} heparin; lane 3, Colominic acid; lane 4, De-O-sulfated-heparin; lane 5, Fucoidan; lane 6, Pentosan Polysulfate; lane 7, Dextran sulfate; lane 8, Chondroitin sulfate C). All polysaccharides were tested at equivalent anion concentration as 25 μg ml^{-1} heparin.

the same total anion concentration as heparin. These compounds are also ineffective in inhibition of invasion. Thus, there is a direct correlation between successful protein binding and erythrocyte invasion by the parasite.

MSA-1 of specific parasite strains binds to erythrocytes in a sialic acid-dependent manner [3], putatively to glycophorin. Erythrocytes lacking glycophorin are resistant to invasion by these parasites [5] and removal of the extracellular portion of glycophorin with trypsin treatment protects erythrocytes from invasion [23]. The glycophorins (A, B, C) are transmembrane proteins; any members of the group appear to interact with the malaria parasite and all have, in common, a heavily sialylated, and thus highly anionic, extracellular domain and are able to inhibit MSA-1 binding and invasion. Heparin as well as dextran sulfate and fucoidan inhibit rosetting of *P. falciparum* infected erythrocytes with uninfected erythrocytes. Analogously, neither chondroitin sulfate nor hyaluronic acid are effective in this system. The mechanism of inhibition is unknown [14].

Heparin, dextran sulfate and pentosan polysulfate may effectively mimic the anionic character of the erythrocyte surface receptor and thus competitively inhibit MSA-1 binding to the erythrocyte. If MSA-1 preferentially interacted with the exogenous anionic polysaccharides and not glycophorin on the erythrocyte surface and MSA-1 is required for successful erythrocyte invasion, heparin and other anionic polysaccharides would prevent invasion by preventing MSA-1 from binding to the erythrocyte surface.

This model suggests that MSA-1 of appropriate strains of the malaria parasite is able to recognize and bind anions of two different natures (carboxylate on sialic acid and sulfate on heparin). A sialic acid-binding, lectin-like protein, E-selectin, reportedly binds with greater affinity to a sulfated ligand than the natural sialylated ligand [24]. The preference for sulfate anions compared to carboxylate anions, as seen with E-selectin, may also be present in MSA-1.

It is surprising that colominic acid does not effectively inhibit MSA-1 binding. The anionic component of the glycocalyx of erythrocytes is composed largely of sialic acid residues from glycophorin and, presumably, MSA-1 interacts with these residues. Colominic acid is comprised of poly α2-8-linked sialic acid whereas erythrocyte surface sialic acid is primarily α2-3 or α2-6 linked. Perhaps a different conformation due to the alternate linkages is responsible for the differential binding. The anionic selectivity exhibited for inhibition of parasite invasion (*eg* heparin but not chondroitin sulfate, glycophorin but not colominic acid) also implies that a specific conformation is recognized in the interaction between the parasite and the erythrocyte surface and that only certain polyanions are able to present an effective charge constellation.

References

1 Butcher GA, Parish CR, Cowden WB (1988) *Trans Royal Soc Trop Med Hyg* **82**: 558–59.
2 Kulane A, Ekre H-P, Perlmann P, Rombo L, Wahlgren M, Wahlin B (1992) *Am J Trop Med Hyg* **46**: 589–94.
3 Perkins ME, Rocco LJ (1988) *J Immunol* **141**: 3190–3196.
4 Camus D, Hadley TJ (1985) *Science* **230**: 553–56.
5 Pasvol G, Wainscoat JS, Weatherall DJ (1982) *Nature* **297**: 64–67.
6 Breuer WV, Ginsburg H, Cabatchnik ZI (1983) *Biochimica Biophys Acta* **755**: 263–71.
7 Miller LH, Mason SJ, Dvorak JA, McGinnis MH, Rothman IK (1975) *Science* **189**: 561–63.
8 Miller LH, Mason SJ, Clyde DF, McGinnis MH (1976) *N Eng J Med* **295**: 302–4.
9 Pirrotta V, Bickle TA (1980) *Methods Enzymol* **65**: 89–95.
10 Su S, (1993) *J Immunol* **151**: 2309–17.
11 Clark DL, Chrisey LA, Campbell JR, Davidson EA (1994) *Mol Biochem Parisitol* **63**: 129–34.
12 Pirson PJ, Perkins ME (1985) *J Immunol* **134**: 1946–51.

13 Pancake SJ, Holt GD, Mellouk S, Hoffman SL (1992) *J Cell Biol* **117**: 1351–56.
14 Rowe A, Berendt AR, Marsh K, Newbold CI (1994) *Exp Parasitol* **79(4)**: 506–16.
15 Roberts DD, Rao CN, Magnani JL, Spitalhik SL, Liotta LA, Ginsburg V (1985) *Proc Natl Acad Sci* **82**: 1306–10.
16 Hoylaerts M, Owen WG, Collen D (1984) *J Mol Biochem* **259**: 5670–77.
17 DeAngelis P, Glabe CG (1987) *J Biol Chem* **262**: 13946–52.
18 Ogamo A, Nagai A, Nagasawa K (1985) *Biochim Biophys Acta* **841**: 30–41.
19 Barzu T (1986) *Biochem J* **238**: 847–54.
20 WuDunn D, Spear PG (1989) *J Virol* **63**: 52–58.
21 DeClerq E (1989) *Antimicrob Chemother* **23**: 35–46.
22 Holder AA (1988) *Prog Allergy* **41**: 72–96.
23 Perkins ME (1984) *J Exp Med* **160**: 788–98.
24 Yuen C-T, Bezouska K, O'Brien J, Stoll M, Lemoine R, Lubineau A, Kiso M, Hasegawa A, Bockovich NJ, Nicolaou KC, Feizi T (1994) *J Biol Chem* **269**: 1595–98.
25 Perkins ME, Holt E (1988) *Mol Biochem Parasitol* **27**: 23–30.

Received 24 April 1996 and accepted 28 August 1996