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Regioselectively modified sulfated cellulose as prospective drug for treatment of malaria tropica

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Abstract Adhesion of *Plasmodium falciparum* infected erythrocytes (IE) to placental chondroitin-4-sulfate (CSA) has been linked to the severe disease outcome of pregnancy-associated malaria. Consequently, sulfated polysaccharides with inhibitory capacity may be considered for therapeutic strategies as anti-adhesive drugs. During *in vitro* screening a regioselectively modified cellulose sulfate (CS10) was selected as prime candidate for further investigations because it was able to inhibit adhesion to CSA expressed on CHO cells and placental tissue, to de-adhere already bound infected erythrocytes, and to bind to infected erythrocytes. Similar to the undersulfated placental CSA preferred by placental-binding infected erythrocytes, CS10 is characterized by a clustered sulfate pattern along the polymer chain. In further evaluation of its effects on *P. falciparum* interactions with host erythrocytes, we now show that CS10 inhibits the *in vitro* asexual growth of parasites in erythrocytes. Furthermore, we show that CS10 interferes with C1 of the classical complement pathway but not with MBL of the lectin pathway. In order to gain insights into the possible interactions of CS10 with known parasite receptors at the molecular level, we designed 3D-structures of characteristic stretches of CS10. CS10 fragments with clustered sulfate groups showed complex patterns of hydrophobic and hydrophilic patches most likely suitable for interactions with protein binding partners. The significance of CS10 interactions with the complement system as well as its anti-malarial effect for prospective drug application are discussed.

Keywords Cellulose sulfate · Sulfated polysaccharides · Pregnancy associated malaria · Complement system · Molecular modelling · Adhesion · *Plasmodium falciparum*

Abbreviations

CSA	chondroitin sulfate
CS10	cellulose sulfate 10 (as described)
IE	infected erythrocytes
MBL	mannan binding lectin
PAM	pregnancy associated malaria

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Introduction

Infections with the human malaria parasite *Plasmodium falciparum* often result in severe disease pathology with a high mortality rate, estimated to be between 1 and 2 million annually. The high pathogenicity of this parasite is partly due to adhesive interactions between infected erythrocytes (IE) and host endothelial cell receptors during the blood stage of the parasites' life cycle. These interactions include 1) the ability of mature trophozoite- and schizont-stage infected erythrocytes to adhere to microvascular endothelial cells [1] or to the syncytiotrophoblast and extracellular matrix components within the intervillous space of the placenta [2, 3], 2) to form rosettes with uninfected erythrocytes [4] and 3) to autoagglutinate [5]. Such adhesive interactions seem to contribute to the symptoms of severe malaria, cerebral malaria, and pregnancy-associated malaria (PAM). In recent years, PAM in particular has been a focus for vaccine and drug development [6]. Regardless of their previous level of exposure and immunity [7], pregnant women in malaria endemic areas undergoing their first or second pregnancy are more susceptible to this type of malaria. Women who have undergone multiple pregnancies (multigravidae) have a level of protection against PAM by antibodies recognizing placental binding parasitized erythrocytes [8]. Thus the parasite adhesin mediating adhesion in the placental is recognized as a good vaccine and drug target.

Two placental-receptors to which parasitized erythrocytes can adhere have been identified to date — chondroitin-4sulfate (C4S or CSA) and hyaluronic acid [9, 10]. CSA appears to be the major placental receptor, and CSA-binding parasites have been shown to bind preferentially to chondroitin sulfate isolated from human placenta with unusually low levels of sulfation. Two different sulfation patterns have been observed with 2 to 3% and 9 to 14% of the CS chain disaccharide units having 4-sulfation and the remainder of the chondroitin chain disaccharide units being non-sulfated [11]. In the experimental setting optimal binding of infected erythrocytes in the placenta requires ~30% 4-sulfated and ~70% non-sulfated disaccharide repeats, with a minimal binding motif of six disaccharide repeating units with two 4-sulfated and 4 non-sulfated disaccharide units [12].

Several negatively charged polysaccharides, such as heparins, chondroitin sulfate, dextran sulfates, fucoidan and the non-sulfated glycosaminoglycan hyaluronic acid, have been reported to inhibit invasion of erythrocytes by plasmodial merozoites and cytoadherence of parasite-infected erythrocytes to host cells [13, 14] and to disrupt rosette formation of parasite-infected with uninfected erythrocytes [15, 16]. We recently screened a panel of sulfated oligosaccharides with differing degrees and patterns of sulfation. From these, two types of carrageenans and a cellulose sulfate with unusually high sulfation in positions 2 and 3 and block-like arrangement of sulfate groups (CS10) showed the best inhibitory capacity and were able to de-adhere already bound IE to cells and placenta tissue [17]. When infected erythrocytes were incubated together with the glycans prior to host cell adhesion, only CSA and the cellulose sulfate CS10 blocked the adhesion in a dose-dependent manner. This would suggest a specific interaction with the infected erythrocyte adhesin. In summary, this special cellulose sulfate may be a suitable candidate for further drug development. In this report, we have investigated the ability of CS10 to inhibit adhesion of *P. falciparum*-infected erythrocytes to CD36, a major endothelial receptor to which most *P. falciparum* laboratory lines and field isolates can adhere [18]. We also investigated the possible anti-malarial effect of CS10 on the erythrocytic invasion and growth of the *P. falciparum in vitro*.

Complement activation and expression of the complement receptor 1 (CR1, CD35) as well as of the complement regulator CD 55 has been associated with the pathophysiology and clinical outcome of malaria [19]. As we could recently demonstrate that chondroitin sulfate proteoglycans of human B lymphocytes block C1 and the classical pathway of complement [20] and therapeutic targeting of complement has meanwhile been shown to be beneficial in the treatment of inflammatory disorders [for review 21], we wished to know the potential impact of CS10 in comparison to other sulfated polysaccharides on the classical and lectin pathway of the complement cascade.

Finally, knowledge of the complex structure of biologically active carbohydrates is indispensable both to understand interactions with protein receptors at the molecular level and to design targeted drugs which best fit to their counterparts. As a first step in addressing this, we generated three-dimensional structural models based on oligomeric sequences of CS10 fragments reflecting the block-like sulfate patterns that may be involved in interactions with parasite adhesins.

Materials and methods

Carbohydrates

Chondroitin sulfate A (CSA, chondroitin-4-sulfate) was obtained from Fluka and Sigma. Dextran sulfate from *leuconostoc* 500 kDa and 8 kDa, Fucoidan from *Fucus vesiculosus*, Carrageenans CSW-2 (Lambda), LP-42 (Iota), and MB 73F (Kappa) were kindly provided by CP Kelco, Denmark. Cellulose sulfates (CS2 and CS10) were kindly provided by Dr. W. Wagenknecht, Fraunhofer Institute for Applied Polymer Research, Potsdam, Germany. Regioselective sulfation of these compounds was carried out as

previously described [22]. CS2 was prepared from a nearly randomly acetylated cellulose-2.5-acetate. CS10 synthesis started with a cellulose triacetate (DP of about 300 corresponding to a molecular mass of 50 kDa with respect to the cellulose backbone) which then was partially deacetylated with 1,6-diaminohexane and subsequently sulfated [23, 24]. Structural analysis of the cellulose sulfates has been described [25, 26]. The chemical characteristics of the sulfated polysaccharides applied have been summarized and described in detail elsewhere [17].

P. falciparum and C32 cell culture

In vitro cultivation of *P. falciparum* infected erythrocytes was carried out in RPMI 1640 medium supplemented with 10% heat-inactivated pooled human serum and using O+ human erythrocytes [27]. Parasites were maintained in a synchronous state by sorbitol treatment [28] or gelatine floatation [29]. C32 melanoma cells (DSMZ, Braunschweig, Germany) were cultured in modified Dulbecos modified eagle's media (DMEM) (JRH Biosciences, Brooklyn, Victoria, Australia) containing 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco BRL). All parasite lines and cell cultures were mycoplasma-free as determined using a Mycoplasma Plus™ PCR Primer Set (Stratagene, La Jolla, CA).

Cytoadhesion assays

Cytoadhesion assays were carried out essentially as described previously [17] using gelatine-enriched trophozoite-infected erythrocytes. The parasite line 3D7 [30], which has a predominantly CD36-binding phenotype was used for cytoadhesion studies. CD36-specific binding was determined by pre-incubating cells with 5 μg/ml of monoclonal antibody FA6/152 (Beckman Coulter) for 1 h at 37°C prior to binding studies. Specific binding to CSA was determined by adding 100 μg/ml of soluble CSA to the binding assay. C32 cells were grown in 70 to 90% confluent monolayers in 24-well tissue culture plates. The number of IE bound to 100 cells was calculated for at least 500 cells. In each case three independent experiments were carried out.

Antimalarial assays

For *in vitro* anti-malarial studies, the multi-drug resistant clone Dd2 [31] was utilized. The effect of polysaccharides on inhibition of parasite growth was tested radiometrically using ^{3}H-hypoxanthine incorporation, as previously described [32]. *P. falciparum* infected erythrocytes at 0.25% parasitemia and 5% hematocrit were seeded in wells of a 96-well tissue culture plates in hypoxanthine-free RPMI 1640 media (Gibco) supplemented with 10% pooled human serum and 50 mg/ml gentamicin (Sigma). Carbohydrates, prepared in hypoxanthine-free RPMI 1640 media (Gibco) supplemented with 10% pooled human serum and 50 mg/ml gentamicin (Sigma), were tested in triplicate in at least three independent experiments. Growth inhibition was carried out over 72 h, starting with ring stages. Concentrations of drug that inhibited parasite growth by 50% compared to controls (EC_{50}) were determined by linear interpolation of inhibition curves. Statistical significance was determined using a two-tailed Student's *t*-Test.

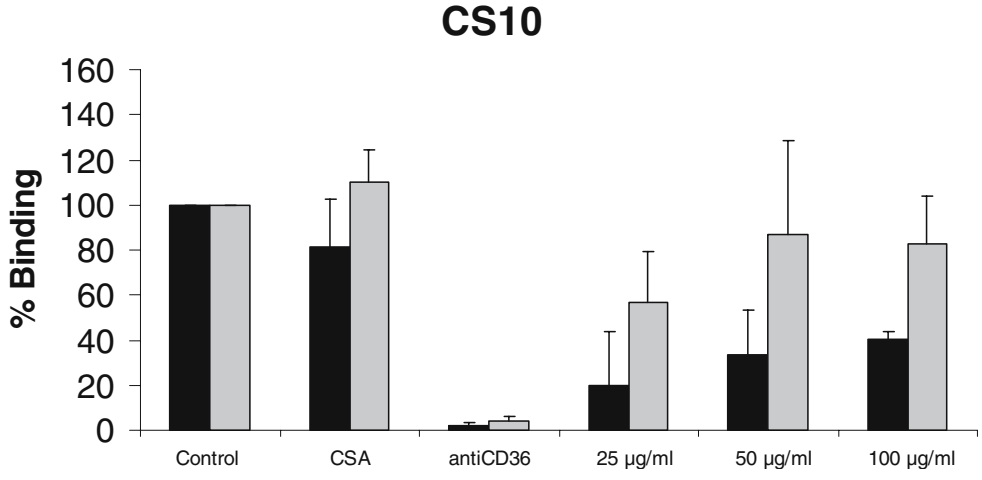

Fig. 1 Effect of cellulose sulfate CS10 on CD36-specific adhesion of *P. falciparum* infected erythrocytes to C32 melanoma cells. Inhibition of CD36-specific adhesion (*black*) and de-adhesion (*grey*) at different concentrations of cellulose sulfate (CS10) was evaluated using CD36-binding *P. falciparum* clone 3D7. Soluble chondroitin-4-sulfate (CSA; 100 μg/ml) was included as a negative control. Specific adhesion to CD36 was determined using anti-CD36 monoclonal antibody FA6/152 (5 μg/ml). Mean percentage of binding (±SD) compared to untreated controls is shown for three independent experiments

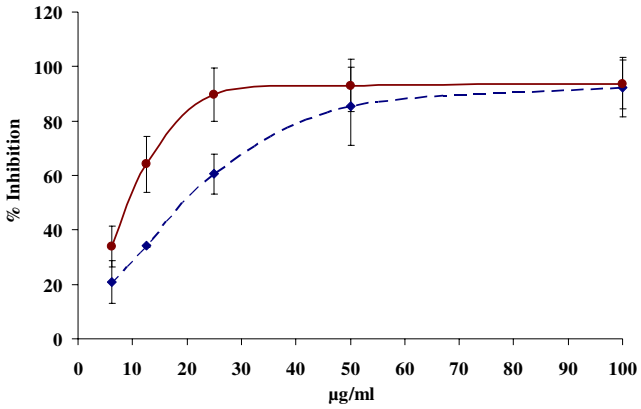

Fig. 2 Antimalarial effect of cellulose sulfate CS10. The antimalarial activity of CS10 (*bold line*) and fucoidan (*dashed line*) against the multi-drug resistant *P. falciparum* clone Dd2 was determined radiometrically using ^{3}H-hypoxanthine uptake. The average percentage of inhibition (± SD) for three independent experiments is shown

Complement inhibition assays

In order to investigate whether sulfated polysaccharides interfere with the enzymatic cascade reaction of the complement pathway, increasing amounts of the respective polysaccharides were incubated with purified C1 (Sigma) or mannan-binding lectin (MBL), kindly provided by Dr. J.C. Jensenius, Aarhus, Denmark. Inhibition of C1 activity was measured in a hemolytic assay as described earlier [20]. Inhibition of the MBL pathway was determined by C4 binding assay. In brief, human serum with defined amounts of MBL was preincubated with either sulfated polysaccharides, buffer, or mannan as positive control before it was added to wells of mannan-coated ELISA plates. MBL-induced lectin pathway activity was determined as the amount of bound C4 as quantified by specific ELISA [33].

Determination of carbohydrate 3D structures

The 3D structures of CS10 fragments were generated with the SWEET-II (http://www.glycosciences.de/sweet2) [34]. For the graphical display the plugin Chime was used. The water accessible surface was colour-coded with the relative hydrophobicity scheme as defined by RASMOL. Analysis affinity of defined amino acids for monosaccharides or sulfate groups was done as described [35] using the GlycVicinity database (http://www.glycosciences.de/glyvicinity).

Results

Inhibition of CD36-dependent adhesion of IE by CS10

In order to determine the effect of cellulose sulfate CS10 on CD36-specific adhesion of *P. falciparum* infected erythrocytes, we carried out cytoadhesion assays between 3D7 infected erythrocytes displaying a CD36 binding phenotype, and C32 melanoma cells expressing CD36 and other known *P. falciparum* adhesion receptors [36]. When infected erythrocytes were pre-incubated with different

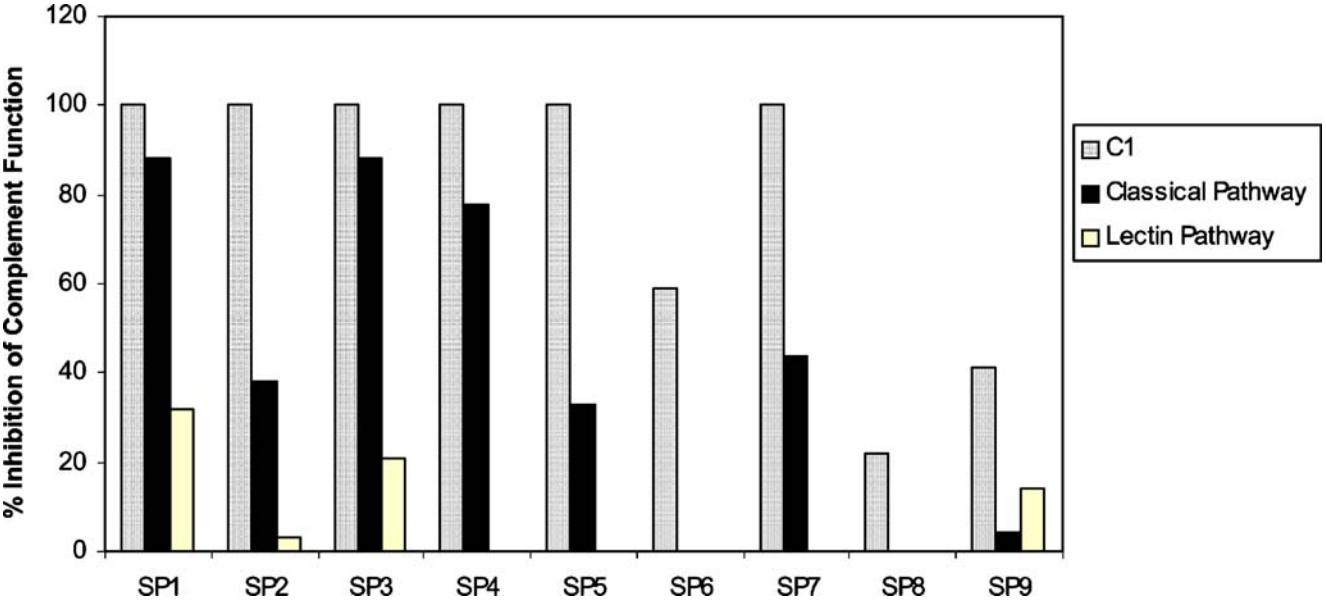

Fig. 3 Impact of sulfated polysaccharides on complement. C1, MBl, or fresh serum (at defined functional activity) were incubated with sulfated polysaccharides (SP) at different concentrations (0.64 to 80 µg/ml) and inhibition was assessed as a reduction of functional activity of either C1 or the classical or the lectin pathway of complement. SP1 = 500 kDa dextran sulfate, SP2 = 8 kDa dextran sulphate, SP3 = fucoidan, SP4 = λ-carrageenan CSW-2, SP5 = ι-carrageenan LP-42, SP6 = κ-carrageenan MB73F, SP7 = CS10, SP8 = chondroitin sulfate CS10 (SP7) exerted a strong inhibitory activity upon binding to C1, partly also reflected by reduced classical pathway activity, whereas MBL/lectin pathway function was not affected. The data are representative for two independently performed experiments

Table 1 Frequency of amino acids in the spatial vicinity of all glucose and sulphated residues

	Residue	Asp	Glu	Lys	Asn	Arg	Gln	His	Gly	Ser	Ala	Pro	Thr	Tyr	Val	Cys	Met	Trp	Phe	Leu	Ile	Total
Glc	Count	1272	1361	656	1278	1331	667	772	1120	523	828	318	675	1671	471	84	257	1804	841	730	361	17020
	(%)	**7.5**	**8.0**	3.9	7.5	**7.8**	3.9	4.5	6.6	3.1	4.9	1.9	4.0	**9.8**	2.8	0.5	1.5	**10.6**	4.9	4.3	2.1	100
SO3	Count	2	19	252	88	185	33	26	34	30	37	13	25	43	19	3	4	30	15	10	7	875
	(%)	0.2	2.2	**28.8**	**10.1**	**21.1**	3.8	3.0	3.9	3.4	4.2	1.5	2.9	4.9	2.2	0.3	0.5	3.4	1.7	1.1	0.8	100

The analysis was performed with the *Glyvicinity web interface* (http://www.glycosciences.de/glyvicinity/) and is based on the PDB-release from April 27, 2005. All PDB entries having a resolution better than 3 Å are evaluated. All residues where the distance of at least one pair of atoms is closer than 4 Å are taken into account.

concentrations of CS10 before incubating with C32 cells, significant inhibition of binding compared to controls was observed (25 μg/ml, $P = 0.0212$; 50 μg/ml, $P = 0.0455$; 100 μg/ml, $P = 0.0423$). No significant inhibition was observed compared to controls in de-adhesion assays at any of the concentrations tested ($P > 0.1$). In contrast, inclusion of an anti-CD36 monoclonal antibody in the adhesion assay completely abrogated direct adhesion to C23 cells ($P = 0.0434$) and de-adhered most infected erythrocytes ($P = 0.011$; Fig. 1). Inclusion of 100 μg/ml soluble CSA in either the adhesion or de-adhesion assay had no significant effect on adhesion of 3D7-infected erythrocytes to C32 cells, as would be expected given their CD36-binding phenotype (Fig. 1).

Anti-malarial effect of CS10

In order to determine the impact of CS10 on the growth of the *P. falciparum* parasites *in vitro*, we measured uptake of ^{3}H-hypoxanthine. As shown in Fig. 2, CS10 was able to inhibit the growth of the multi-drug resistant *P. falciparum* clone Dd2 with an EC_{50} of 1.2 μg/ml (+/−0.2 μg/ml). As a control, we assayed the complex polysaccharide fucoidan and the well known anti-malarial drug chloroquine, which gave EC_{50s} of 2.4 μg/ml (+/−0.3 μg/ml) and 0.032 μg/ml (+/−0.00128 μg/ml), respectively.

Impact of sulfated polysaccharides on complement function

In order to determine whether sulfated polysaccharides, in particular CS10, may affect the human complement system with regard to their potential application as drugs, we analyzed the impact of these compounds both on the classical and the lectin pathway of complement activation. In a first approach we added sulfated polysaccharides to human serum and measured the total hemolytic activity of the classical complement pathway. As shown in Fig. 3 a considerable, dose-dependent inhibition was observed by 500 kDa dextran sulfate, fucoidan and λ-carrageenan CSW-2; less inhibition was achieved by addition of 8 kDa dextran sulfate ι-carrageenan LP-42 and CS10. No blocking effect was seen with κ-carrageenan and CS2, two compounds which also displayed no anti-adhesive properties in the malaria assays. Interestingly, blocking activity of CSA for the classical pathway was also very low.

To further analyze the impact of the sulfated polysaccharides on the pathway initiating complement proteins, such as C1 (classical pathway) and mannan binding lectin (MBL, lectin pathway), increasing amounts of the compounds were incubated with either purified C1 or MBL. Inhibition of C1 activity was measured in a hemolytic assay as described [20]. Most of the compounds were able to inhibit C1 hemolytic activity (Fig. 3) although with differ-

Table 2 Distribution of differently substituted monomers and dimers along the CS10 polymer

Substitution of Glc	Monomer composition [Mol %]	Number of sulfate groups/ dimer	Dimer composition [Mol%]
Glc	28.9	0	13,5 (8,4)[a]
2-S	13.5	1	14,6 (15.3)
3-S	6.9	2	14,9 (25,8)
6-S	6.1	3	17,2 (24,2)
2,3-S	25.5	4	18,6 (16,9)
2,6-S	5.0	5	14,7 (7,9)
3,6-S	1.9	6	6,6 (1,5)
2,3,6-S	12.2		

[a] In brackets calculated values for statistical (random) distribution of the given dimers are shown.

ences in efficacy. Again, κ-carrageenan and CS2 showed lowest values.

Inhibition of the MBL-lectin pathway was determined by C4 binding assay as described in Materials and methods. In contrast to the classical, pathway the lectin pathway was inhibited only by few of the compounds such as 500 kDa dextran sulphate, fucoidan, and to a lesser extent by CSA and 8 kDa dextran sulfate. CS10 had no blocking activity for the lectin pathway (Fig. 3).

Design of 3D-structures of CS10 fragments

Depending on the respective sequence of sulfated and non-sulfated residues, cellulose sulfate constitutes a linear, extended structure, where hydrophilic patches around the negatively charged sulfate ions alternate with more hydrophobic areas mainly found above and below the pyranose ring of unsubstituted glucose units. Analysis of the frequency of amino acids found in the spatial vicinity of sulfated sugar residues and glucose shows remarkable differences. Whereas for sulfated sugars, which nearly exclusively interact with the polar, positively charged residues Lys, Arg, and Asn, the aromatic residues Tyr and Trp are predominantly found in the spatial vicinity of glucose residues (Table 1). Taking into account that Trp is the amino acid with the lowest natural abundance, it can be assumed that this specific type of hydrophobic interaction is essential for the recognition of glucose units through proteins. Consequently, peptide domains with higher content of Trp may be candidates for glucose binding.

Table 3 Composition of 3D-designed decamers

Substitution of Glc	Mol %	#	M_1	M_2	M_3
Glc:	28.9%	3	1,3,7	8,6,9	2,8,10
2-S	13.5%	1	2	1	9
3-S	6.9%	1	8	2	5
6-S	6.1%	1	9	10	4
2,3-S	25.5%	2	5,10	7,5	1,3
2,6-S	5.0%	1	4	4	6
3,6-S	1.9%				
2,3,6-S	12.2%	1	6	3	7

In a first approach to evaluate the possible binding sites on CS10 for interacting proteins like IE adhesions, we calculated the 3D structure of several fragments of the CS10 molecule based on the chemical data available [25, 26]. As outlined in Table 2 for CS10, 8 monosaccharide components are known to differ in their composition and content of sulfate groups. Dimer composition with low and very high grade of sulfation are much more frequent than that calculated for a random distribution of sulfate groups. These 8 monomers are on an average distributed along the cellulose polymer chain with different probabilities as obtained by neighbourhood analysis from fragments received by random degradation. Here we differentiated between non-sulfated up to hexa-substituted dimers.

To estimate the sequence dependence for the arrangement of hydrophobic and hydrophilic areas of cellulose sulfate, we designed 3D structures of several decamers with an arrangement of sulfate groups as outlined in

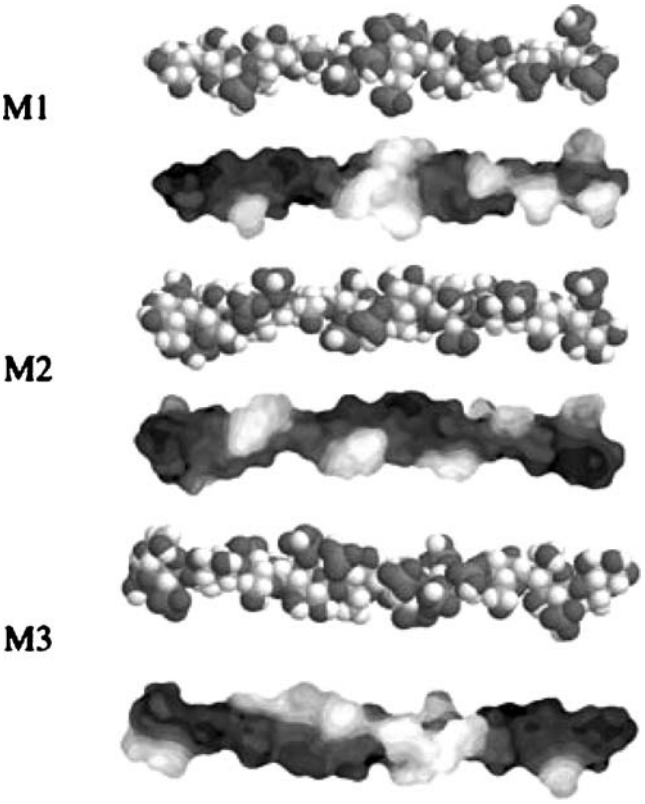

Fig. 4 3D models of CS10 fragments. Molecular models were designed using the program Sweet-II as outlined in Materials and methods. Fragments shown are defined as shown in Table 3. The color code of the relative hydrophobicity scheme was transferred in *graded grey tones*, hydrophobic regions are in *dark grey*, whereas at the opposite hydrophilic domains (sulfate groups) are displayed in *light grey*

Table 3. The structures obtained are shown in Fig. 4. The frequency of the sugar units corresponds roughly to the analytically determined mean composition. The sequences were chosen arbitrarily. Each decamer contains 3 unsubstituted glucose units, one unit in each case of a 2, 3, and 6 monosulfated residue, three 2,3 disubstituted units and one 2,3,6 sulfated residue.

Figure 4 shows that, depending on the respective sequence, characteristic patterns of hydrophobic and hydrophilic regions exist, and provide various alternatives to be specifically recognized by an interacting protein. However, without knowing the spatial structure of interacting protein partner and its bindings site, it is impossible to predict which pattern of hydrophobic and hydrophilic patches is required to form a strong complex.

Discussion

In previous work we screened a panel of sulfated polysaccharides for their ability to inhibit CSA-specific adhesion of *P. falciparum*-infected erythrocytes (IEs) and found that two carrageenans (CSW-2 and LP-42) and a cellulose sulfate (CS10) inhibit binding to CSA expressed on CHO cells and de-adhered already bound IE in a dose-dependent manner [17]. In contrast to the other sulfated polysaccharides tested, only CS10 and CSA remained bound to IE after washing, and continued to inhibit binding of IE to CHO cells.

Here we have further investigated the anti-parasite properties of CS10 as both an adhesion inhibitor and an anti-malarial compound. With the exception of adhesion to CSA in pregnancy-associated malaria, two other adhesive interactions between *P. falciparum*-infected erythrocytes and the placenta are of particular importance: adhesion to CD36 and ICAM-1 [18]. Adhesion to ICAM-1 in the brain microvasculature has been associated with cerebral malaria complications [18, 37] and adhesion of *P. falciparum*-infected erythrocytes to CD36 is one of the most common binding phenotypes of both laboratory parasite lines and field isolates. Interestingly, while adhesion to CD36 and other host receptors may be mediated by a single parasite adhesin, adhesion to CSA and CD36 are mutually exclusive [38]. While it has been suggested that adhesion to CD36 may contribute to malaria disease severity via sequestration in the blood microvasculature, this binding phenotype has more recently been associated with non-severe disease [39] and may actually protect the infected host via sequestration in non-vital sites such as the skin and muscle [40, 41]. Interfering with CD36 adhesion may promote adhesion to other parasite receptors, such as ICAM-1, and lead to more severe disease outcomes such as cerebral malaria [42]. Thus, in pursuing CS10 as a potential lead in the development of anti-adhesive therapies for placental adhesion, we have investigated the ability of CS10 to interfere with CD36 adhesion. While some inhibition of adhesion was observed when CS10 was included in the binding assay, this was not dose dependent. We observed no significant de-adhesion of already bound IE. Therefore, low inhibitory activity for CD36-mediated IE adhesion may be advantageous with regard to clinical application of CS10. Taken together with our previous findings on the ability of CS10 to interfere with CSA binding at concentration equivalent to CSA (~50% inhibition at 3 μg/ml), these data provide further evidence of the potential of CS10 or similar molecules as a malaria disease therapy especially suitable for treatment of PAM.

Sulfated proteoglycans are involved in a number of adhesive interaction between parasitized erythrocytes and host receptors, including binding of sporozoites to hepatocytes [43]. Negatively charged surface molecules may also contribute to the successful invasion of merozoites into erythrocytes [14]. This prompted us to explore the possibility that CS10 may possess anti-malarial activity in addition to anti-adhesive properties. When we examined the effect of this cellulose sulfate on the growth of a multi-drug resistant parasite *in vitro*, we obtained some anti-malarial activity [EC_{50} of 1.2 μg/ml (+/−0.2 μg/ml)]. This activity was comparable to that obtained for a control polysaccharide, fucoidan [EC_{50} 2.4 μg/ml (+/−0.3 μg/ml)], and these data are similar to those previously reported for other polysaccharides [44, 45]. Although it is unlikely that CS10 would ever be considered as a first-line antimalarial, the moderate antimalarial activity combined with its promising anti-adhesive capacity against CSA-, but not CD36-binding parasites, supports further investigation.

Several studies indicate a strong relationship between components of the complement cascade and the outcome of severe forms of malaria such as cerebral- and pregnancy-associated malaria [19, 46, 47]. Erythrocyte complement-regulatory proteins, in particular complement receptor 1 (CR1, CD35), are considered to be important in the pathogenesis of severe malaria [46]. The levels of expression of CR1 and the complement regulator CD55 on erythrocytes may have an impact on immune response to malaria exposure, and further age-dependent alterations in levels of erythrocyte complement-regulatory proteins may contribute to the differences in epidemiology of severe malaria-associated anaemia and cerebral malaria. Complement receptor 1 is suspected to be involved in rosette formation which may contribute to vascular obstructions during cerebral malaria.

Sulfated polysaccharides are able to interact with distinct components of the complement cascade [20]. We have recently shown that secreted chondroitin sulfate proteoglycan of human B lymphocytes binds to C1q, the binding subunit of the first component of complement [20]. This binding interfered with the formation of the C1 complex

with subsequent inhibition of the classical complement pathway activity. Therefore, we wanted to see whether CS10 and other sulfated polysaccharides applied in our study have an effect on the classical and the lectin pathways of the complement cascade. As shown in Fig. 3, CS10 (SP7) has an effect on the classical pathway although weaker than from high molecular dextran sulfate and fucoidan. Interestingly the lectin pathway is not affected by CS10. As both the classical and the alternative pathways of the complement system are profoundly activated in complicated malaria, CS10-mediated C inhibition may be of benefit for the clinical outcome [47]. More importantly, by blocking C1 and the classical pathway of complement, CS10 may have a protective effect on anti-erythrocyte or immune complex-induced complement-mediated destruction of infected erythrocytes [48].

From our recent studies it is evident that the anti-adhesive properties of CS10 are related to its unique sulfation pattern since randomly sulfated CS2 did not show any activity. CS10 as a product of polymer analogous transformations is a very complex mixture with no certain sequence but has been characterized by statistically calculations in comparison to experimental data. Based on these results we selected three sequences which shall represent this unique block-like pattern of CS10 in contrast to a randomly modified cellulose sulfate.

This pattern enables cooperative binding with complementary protein motifs, which are known to enhance strongly non-covalent molecular chain–chain interactions, responsible for adhesion phenomena. Since CS10 seems to display a specific binding to a receptor expressed on CSA-binding IE and given the frequency of defined amino acids found in close spatial vicinity of either glucose or sulfate groups as shown in Table 1, a systematic computational procedure for erythrocyte-expressed proteins from which their spatial structures are known will provide information about regions on the protein surface which may specifically interact with CS10. Data obtained by this modeling approach may give hints how to design best-fit regioselectively modified polysaccharides which can be applied as additive drugs for treatment of severe cases of malaria.

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