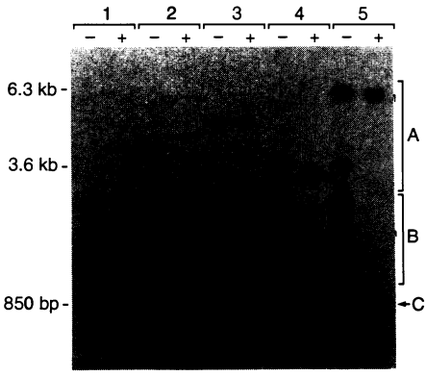

Fig. 4. Methylation status of the *FRA16A* $p(CCG)_n$ repeat assessed by Fnu 4HI digestion. Pst I–Rsa I double digests of chromosomal DNA from lymphocytes of normal (lanes 1 and 2) and *FRA16A* individuals (lanes 3 to 5) were treated with (+) or without (−) Fnu 4HI and subjected to Southern blot analysis with the 650–base pair Not I–Rsa I fragment from pf16A3. This probe detects an 850–base pair constant band (C) at the normal *FRA16A* locus, several cross-hybridizing bands located elsewhere in the genome (B), and the unstable *FRA16A* alleles in *FRA16A*-expressing individuals (A). Digestion with Fnu 4HI eliminated the normal (C) allele [and cross-hybridizing bands (B)]; however, only minor alteration is afforded to the *FRA16A* fragment (not all Fnu 4HI sites contain a methylatable CpG), indicating resistance to digestion of the majority of the sequence containing the $p(CCG)_n$ repeat because of methylation.

ylation associated with fragile sites does not appear to be a necessary condition for the mutation process, although it may be essential for the phenotypic manifestation of the disease (*19*) or the cytogenetic expression of the fragile site or both.

To explore the relation between the sequence composition of fragile sites and the chemistry of their induction, it will be of interest to determine the structure of (nonfolate-sensitive) fragile sites that are induced by a variety of chemicals and to determine whether methylation plays a role in their mutation or cytogenetic expression.

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7. YACs were isolated from the CEPH first-generation library by PCR screening for the presence of sequence-tagged site (STS) sequences from probes 1.79, VK20, and 16XE81. Total yeast DNA, including these YACs, was used as a template to generate *Alu*-PCR products for screening the Los Alamos human chromosome 16–specific cosmid library. Positive cosmids that mapped to the appropriate somatic cell hybrid breakpoint interval were subject to sequence analysis to develop further STSs. These STSs were used to screen the CEPH Mega Yac library. Positive YACs were mapped for the presence of rare-cutting restriction enzyme sites.
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10. PCR products were subject to SSCP PCR analysis according to conditions described by R. I. Richards and K. Friend [*J. Med. Genet.* **28**, 856 (1991)]. Normal Mendelian inheritance of this SSCP was demonstrated in a CEPH pedigree. Several *FRA16A* individuals were found to be heterozygous, indicating the presence of products from both chromosomes (*6*) and therefore excluding the region of instability from between the c and d PCR primers. Sequence analysis between the d primer and the Rsa I site (Fig. 1E) revealed a previously unidentified sequence deficient in repeat motifs (*6*).
11. The 2.7-kb Sac I–Not I fragment (encompassing the CpG island) from pf16A1 (Fig. 1D) was localized by FISH distal to *FRA16A* (*FRA16A* chromosomes signal scored 12 distal, 1 central, and 2 proximal). The 3.6-kb Not I–Sac I fragment [including the $p(CCG)_n$ repeat] from pf16A1 was localized by FISH proximal to *FRA16A* (*FRA16A* chromosomes signal scored 7 proximal, 1 central, and 1 distal). FISH analysis was not possible with whole YACs as probes because of the presence of duplicated sequences present on both sides of *FRA16A* (as evident by 1.79 hybridization) and also because of the presence of chromosome 16–specific repeat sequences.
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Receptor and Ligand Domains for Invasion of Erythrocytes by *Plasmodium falciparum*

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A 175-kilodalton erythrocyte binding protein, EBA-175, of the parasite *Plasmodium falciparum* mediates the invasion of erythrocytes. The erythrocyte receptor for EBA-175 is dependent on sialic acid. The domain of EBA-175 that binds erythrocytes was identified as region II with the use of truncated portions of EBA-175 expressed on COS cells. Region II, which contains a cysteine-rich motif, and native EBA-175 bind specifically to glycophorin A, but not to glycophorin B, on the erythrocyte membrane. Erythrocyte recognition of EBA-175 requires both sialic acid and the peptide backbone of glycophorin A. The identification of both the receptor and ligand domains may suggest rational designs for receptor blockade and vaccines.

The erythrocytic stage of *P. falciparum* is responsible for the death of an estimated 2 million children annually. Invasion of erythrocytes by malaria parasites requires parasite ligands and erythrocyte receptors (*1*, *2*). One ligand of *P. falciparum* is an 175-kD protein (EBA-175), the binding of which is dependent on sialic acid (*N*-acetylneuraminic acid) on the erythrocyte membrane (*3*, *4*). Evidence from a number of studies indicates that EBA-175 is a ligand for invasion (*3*–*8*), and antibodies against EBA-175 block merozoite invasion of erythrocytes in vitro (*6*). Heretofore, the binding domain on EBA-175 was unknown and its erythrocyte receptor had not been definitively identified. We report here both the identification of the domain of EBA-175 for erythrocyte binding and the erythrocyte receptor requirements for this binding.

To define the binding domain of EBA-175, we expressed different regions of EBA-175 (Camp clone) defined by homology between *P. falciparum*, *P. vivax*, and the related monkey parasite *P. knowlesi* (*8*) on the surface of COS cells as chimeric proteins using the

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secretory signal and transmembrane segments of herpes simplex virus glycoprotein D [HSV gD] (9) (Fig. 1A). Expression on COS cells was determined by immunofluorescence (*10*). In an erythrocyte rosetting assay (*11*), only COS cells expressing region II (Fig. 1B) bound human erythrocytes in a rosette (Fig. 1C). When expression was followed by immunofluorescence and the rosetting assay, rosetting was seen only on COS cells that expressed region II. However, some COS cells expressing these regions did not form rosettes, possibly because of the different levels of expression obtained in this transient expression system. Regions I, III to V, and VI did not bind erythrocytes.

The specificity of binding of normal, mutant, and enzyme-treated erythrocytes to region II expressed on COS cells was identical to the pattern of binding of native EBA-175 to erythrocytes (Table 1). Importantly, both native EBA-175 and region II expressed on COS cells did not bind human erythrocytes that lacked sialic acid or that lacked glycophorin A [En(a−)]. Furthermore, the binding pattern of animal erythrocytes to native EBA-175 and to region II was identical. The absolute correlation of the binding patterns of region II and native EBA-175 indicates that region II contains the erythrocyte binding domain of EBA-175. This conclusion is supported by a recent study demonstrating that the homologous region II of *P. vivax* and *P. knowlesi* Duffy binding proteins is the only region to bind Duffy blood group–positive human erythrocytes (*12*).

Region II of EBA-175 consists of two copies of the 5′ cysteine-rich domain designated F_1 and F_2 (Fig. 1A). To investigate the significance of the doublet of the 5′ cysteine-rich domain, we expressed F_1 and F_2 separately on COS cells. Only region F_2 bound erythrocytes, the binding pattern of which was identical to that of the entire region II and to that of native EBA-175 (Table 1).

Having identified region II as the parasite domain on EBA-175 for binding erythrocytes, we next investigated the receptor domain on the erythrocyte for binding region II of EBA-175. It was previously known that EBA-175 could not bind to erythrocytes that were treated with neuraminidase (*3*) or that lacked both glycophorin A and glycophorin B (M^kM^k erythrocytes) (*4*). The structure of sialic acid was critical because removal of the 9-O-acetyl group from the sialic acid of mouse erythrocytes, which converted the mouse sialic acid to the human form, enhanced binding of EBA-175 (*7*). However, the concentration of sialic acid (α2-3) lactose or other oligosaccharides containing sialic acid (α2-3) required to inhibit the binding was 100-fold more than that of glycophorin A (*4*). Our finding that erythrocytes without glycophorin A [En(a−)] did not bind to region II (Table 1) raised the possibility that this specificity was not dependent on sialic acids alone, because En(a−) erythrocytes express glycophorin B, which contains the same 11 O-linked oligosaccharides clustered in this region as does glycophorin A (*13, 14*) (Fig. 2A). Each oligosaccharide in glycophorins A and B contains two sialic acids linked by α2-3 and α2-6 glycosidic bonds. The question then arose: If EBA-175 recognizes sialic acid alone, why did En(a−) erythrocytes (which express sialic acid on glycophorin B) not form rosettes? It was possible that erythrocytes that express glycophorin B, but not glycophorin A, could not bind region II of EBA-175 because the copy number of glycophorin B is 10% that of glycophorin A (1×10^5 and 1×10^6 per cell, respectively) (*15*).

To evaluate the relative binding efficiency of the glycophorins A and B, we measured inhibition of erythrocyte binding to region II expressed on COS cells with soluble glycophorin A and glycophorin B. In one experiment, glycophorin B at a sialic acid concentration of 427 μM did not inhibit binding of erythrocytes to COS cells that expressed region II, whereas glycophorin A inhibited binding by 80% and 50% at sialic acid concentrations of 510 μM and 255 μM, respectively. Similar results were observed when glycophorins A and B were used to block the binding of native EBA-175 to erythrocytes. Glycophorin A showed a 50% inhibition at a concentration of 52 to 64 μM sialic acid (Fig. 2B and Table 2). The 50% inhibition concentration for glycophorin B was greater than 677 μM sialic acid in two experiments. One possible explanation is that although sialic acids are required for binding, sialic acids are not sufficient for optimal binding.

A second possible explanation for the differences between glycophorins A and B is that the single N-linked oligosaccharide at amino acid 26, which is present on glycophorin A but not on glycophorin B (Fig.

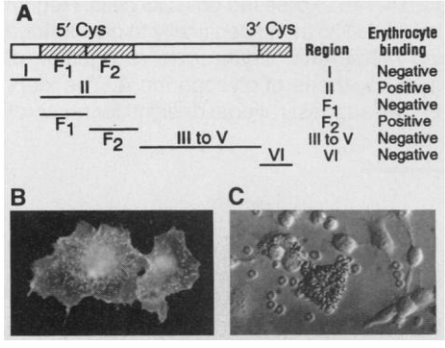

Fig. 1. Regions II and F_2 (Camp) expressed in COS cells bind human erythrocytes. (**A**) Schematic drawing of regions I to VI of the extracellular portion of EBA-175 (*8*). Hatched areas represent the cysteine-rich regions (5′ Cys and 3′ Cys). Lines below the schematic represent regions expressed separately on the surface of COS cells. The data relate the rosetting (positive) or the lack of rosetting (negative) of human erythrocytes on COS cells expressing each region (Table 1). (**B**) Immunofluorescent localization of region II expressed on the surface of COS cells transfected with the gene fragment encoding region II. (**C**) Human erythrocytes forming a rosette over COS cells expressing region II.

Table 1. Regions II and F_2 bind erythrocytes with the specificity of EBA-175. All assays described here were performed with the Camp clone of *P. falciparum*. Binding was scored as positive when COS cells covered tightly with adherent erythrocytes (rosettes) were observed (Fig. 1C). Binding was scored as negative when no rosettes were seen. The binding for region II was always positive in all 12 experiments performed, with a range of 30 to 150 rosettes per 3.5-cm well. The binding to F_2 was variable (10 of 17 experiments were positive), with a range of 10 to 50 rosettes per positive well. ND, not done.

Erythrocyte and treatment*	Binding agent		Region F_2‡
	EBA-175†	Region II‡	
Human (positive for glycophorins A and B)			
No treatment	+	+	+
Neuraminidase	−	−	−
Trypsin	−	−	−
Human glycophorin A–negative [En(a−)]	−	−	ND
Human glycophorin B–negative (S−s−U−)	+	+	ND
Mouse DBA/2			
No treatment	+	+	+
Neuraminidase	−	−	−
Rhesus	+	+	ND
Aotus	−	−	ND
Guinea pig	−	−	ND

*Enzymatic treatments were performed as described (*24*). †A + signifies that EBA-175 binds to erythrocytes; a − indicates no binding. The assay was performed as described (*25*). ‡A + indicates rosetting of erythrocytes on COS cells; a − indicates no rosetting.

2A) (2), is required for optimal binding. *N*-Glycanase treatment of erythrocytes, which cleaves N-linked oligosaccharides, did not affect rosette formation of COS cells expressing region II nor the binding of EBA-175 to erythrocytes, which demonstrates that this N-linked sugar is not required for binding.

A third possible explanation for the differences we observed between glycophorins A and B is that a specific peptide sequence of glycophorin A is also required for binding. Glycophorins A and B (of the blood group N phenotype) are identical for the first 25 amino acids (*13*). Any differences must occur beyond amino acid 25. We therefore studied the ability of glycopeptides of glycophorin A (Fig. 2A) to inhibit EBA-175 binding to erythrocytes (*16*). This work was conducted with native EBA-175 instead of region II expressed on COS cells because the assays with native EBA-175 required much less of our limited supply of glycopeptides.

Glycopeptide MCH1(1–64) inhibits binding at sialic acid concentrations similar to those required by glycophorin A (Fig. 2A and Table 2). Because glycopeptide MCH1(1–64) lacks the transmembrane segment, it cannot form dimers (*17*); dimerization is not required for binding. We next attempted to delineate the binding region within glycopeptide MCH1(1–64). With glycopeptides of glycophorin A that included the 11 NH_2-terminal O-linked sugars and single N-linked complex chain [MCH2(1–34) and MT1(1–31, 1–39)] (Fig. 2A), 50% inhibition of EBA-175 binding was not achieved at sialic acid concentrations greater than 4050 μM and 3500 μM, respectively (Table 2). Furthermore, glycopeptide NCH3(35–64), which includes the four COOH-terminal O-linked oligosaccharides (Fig. 2A), achieved 50% inhibition of EBA-175 binding to erythrocytes only at a sialic concentration of 2308 μM, or a glycopeptide concentration of 240 μM, which is almost two orders of magnitude more than the concentration of glycopeptide MCH1(1–64) that is required to give 50% inhibition of binding. Next, a mixture of glycopeptides MCH2(1–34) and NCH3(35–64) was studied. With this glycopeptide mixture, 50% inhibition of EBA-175 binding was not achieved at sialic concentrations greater than 100 μM (Table 2).

The fact that glycopeptide MCH1(1–64), but not the mixture of glycopeptides MCH2(1–34) and NCH3(35–64), blocked the binding of EBA-175 to erythrocytes at sialic acid and glycopeptide concentrations similar to glycophorin A is the first direct evidence that in addition to sialic acid, the amino acid sequence specific for glycophorin A is necessary for the binding of EBA-175 to glycophorin A. The peptide may be involved in direct binding to EBA-175 or may contribute a unique conformation of the sialic acid residues. The requirement for both peptide and sialic acid may be similar to the binding of P-selectin on endothelial cells to its ligand on leukocytes. The binding site consists of the heavily glycosylated mucin domain, but the protein core dictates the specificity of the interaction (*18*). Sialic acid and the peptide sequence are also crucial for the binding of M and N antibodies to MN blood group antigen on glycophorin A. Neuraminidase treatment of erythrocytes destroys M and N antigenicity, although the molecular differences between blood groups M and N are determined by differences in amino acids at positions 1 and 5 of glycophorin A (*19*). In all three of these examples, the role of the peptide in the interaction is unknown.

Invasion of erythrocytes is critical for the survival of *P. falciparum*. Glycophorin A, the erythrocyte receptor for EBA-175, is required for optimal invasion by all *P. falciparum* clones (*1*). Our definition of the receptor binding domain on EBA-175 and the receptor domain on glycophorin A may suggest rational designs for receptor-blocking therapy and vaccines.

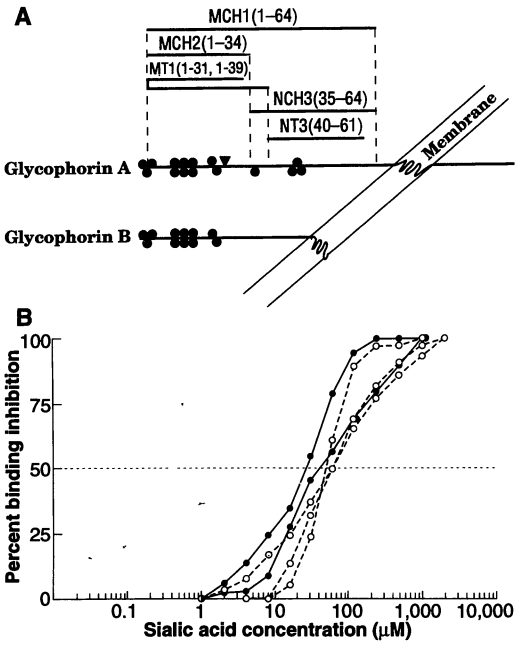

Fig. 2. Glycopeptide 1–64 of glycophorin A and glycophorin A inhibit binding of EBA-175 to erythrocytes. (**A**) Schematic representation of glycophorins A and B with O-linked (closed circles) and N-linked (closed triangle) oligosaccharides on the extracellular domains and tryptic and chymotryptic glycopeptides used in EBA-175 binding inhibition assays. (**B**) Inhibition of binding of EBA-175 to erythrocytes by glycophorin A (open dots) (performed separately three times) or by chymotryptic glycopeptide MCH1(1–64) (closed dots) (performed separately twice). A four-parameter logistic regression function was fitted to the data by a nonlinear least squares algorithm. The NLIN procedure from the SAS library was used (*22*). The dotted line shows the sialic acid concentration giving 50% inhibition. Isolation and purification of glycophorins and glycopeptides were as described (*23*).

Table 2. Inhibition of EBA-175 binding to erythrocytes. The 50% inhibition values for glycophorin A and glycopeptide MCH1(1–64) were derived from Fig. 2B. Inhibition of EBA-175 binding to erythrocytes never reached 50% for glycophorin B and other glycopeptides used, except for one experiment with NCH3(35–64). The values for these glycopeptides and for glycophorin B are shown at the highest concentration used in each individual assay. The 50% inhibition is expressed as the concentration of sialic acid and glycoprotein or glycopeptide (in parentheses). The concentration of sialic acid used was determined from a quantitative sialic acid determination (*21*). The concentration of glycophorins or glycopeptides was determined from the concentration of alanine in a quantitative amino acid analysis, with the exception of glycopeptide MT1(1–31, 1–39), where calculations were based on histidine and valine. The nomenclature of glycophorin A glycopeptides is as follows: M, blood group activity M; N, blood group activity N; CH, chymotryptic glycopeptide; T, tryptic glycopeptide.

Glycophorin or glycopeptide	50% inhibition concentration (μM)
Glycophorin A	63 (1.4), 64 (1.4), 52 (1.2)
Glycopeptide MCH1(1–64)	28 (1.0), 42 (1.5)
Glycopeptide MCH2(1–34)	>1803 (>100), >4050 (>225)
Glycopeptide NCH3(35–64)	>577 (>60), 2308 (240)
Glycopeptide MCH2(1–34) + NCH3(35–64)	(>100)*
Glycopeptide MT1(1–31, 1–39)	>1740 (>76), >3500 (>152)
Glycopeptide NT3(40–61)	>411 (>77), >1640 (>90)
Glycophorin B	>677 (>45), >1350 (>90)

*Glycoprotein concentration of each glycopeptide used in this mix.

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9. The various gene fragments of EBA-175 were cloned into the Pvu II and Apa I sites of HSV gD in plasmid pRE4 (*20*). Cloning into these restriction sites resulted in constructs of chimeric EBA-175–HSV gD signal sequence and a COOH-terminal hydrophobic cytoplasmic tail, which allowed targeting of the malarial proteins to the surface of transfected COS-7 cells (*12*). The gene fragments encoding the various regions of EBA-175 were amplified with the polymerase chain reaction (PCR). Oligonucleotide primers used were 5′-ATCGATCAGCTGAAAGCTAGGAATGAATAT-3′ and 5′-ATCGATGGGCCCATTACTTAAAACT-TCGTT-3′ for amplification of region I; 5′-ATCGATCAGCTGGGAAGAAATACTTCATCT-3′ and 5′-ATCGATGGGCCCCGAAGTTTGTTCAT-TATT-3′ for amplification of region II; 5′-ATC-GATCAGCTGCCAGAAGTAAAGGATGTA-3′ and 5′-ATCGATGGGCCCTTTATTTATTTCTGCT-AA-3′ for amplification of regions III to V; and 5′-ATCGATCAGCTGAACAGAAATGATAGTA-CA-3′ and 5′-ATCGATGGGCCCTGAAAAAGCC-TCCTTTCT-3′ for amplification of region VI. The reverse primer for region F_1 was 5′-ATC-GATGGGCCCCTTAATTGTATTGTCGTT-3′ and the forward primer for region F_2 was 5′-ATCGATCAGCTGGAAAAGCGTGAACATATT-3′. The PCR products were size-fractionated on low-melt agarose, gel-purified with GENECLEAN II (BIO 101), restricted with Pvu II and Apa I, and ligated into pRE4. The 5′ and 3′ ends of each insert were sequenced, and an open reading frame read-through established. Fresh monolayers of COS-7 cells (American Type Culture Collection CRL 1651) cultured in Dulbecco's modified Eagle's medium and 10% fetal bovine serum (FBS) in 3.5-cm-diameter wells were transfected with 3 to 5 μg of Qiagen-purified, phenol chloroform–extracted plasmids with the use of calcium phosphate precipitation [F. L. Graham and A. J. van der Eb, *Virology* **52**, 456 (1973)].
10. To follow expression, we grew COS cells on cover slips in 3.5-cm-diameter wells, then transfected them and used them in immunofluorescence assays 40 to 60 hours after transfection. Immunofluorescence on 2% formaldehyde-fixed COS cells with HSV gD monoclonal antibodies ID3 and DL6 (*20*) (specific for amino acids 11 to 19 and amino acids 272 to 279 of HSV gD, respectively, contained in the resultant chimeric HSV gD–EBA-175 constructs) was performed by standard methods (*6*).
11. Rosetting assays were performed on COS cells 40 to 60 hours after transfection in 3.5-cm-diameter wells. Two hundred microliters of 10% hematocrit erythrocytes in media were added to the 2 ml of media in transfected wells, mixed, and incubated for 2 hours at 37°C. The COS cells were washed three times with phosphate-buffered saline to remove nonadherent erythrocytes.
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16. Metabolic labeling of EBA-175 was performed as described [J. D. Haynes *et al.*, *J. Exp. Med.* **167**, 1873 (1988)], with the exception that the label used was Trans-^{35}S (ICN Radiochemicals, Irvine, CA). Forty microliters of labeled EBA-175 (3.75×10^5 schizont equivalents/ml) was pre-incubated with glycophorin A or B or glycopeptides in a final volume of 100 μl with RPMI-1640 with 10% FBS for 1 hour at room temperature. This mixture was then added to 5×10^8 packed erythrocytes and rocked for 20 min at room temperature for binding to occur. SDS–polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed on eluates of erythrocytes eluted with 9 μl of RPMI, 1.5 M NaCl, 10% FBS, and 2 mM phenylmethylsulfonyl fluoride. Unenhanced gels were dried and quantitated with a Fuji phosphorescent image plate analyzer.
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22. The NLIN procedure is in *SAS/STAT User's Guide Version 6* (SAS Institute, Cary, NC, ed. 2, 1989), vol. 2, pp. 1135–1193.
23. Methods for isolation, digestion, and purification of glycophorins and glycopeptides were as described [E. Lisowska, L. Messeter, M. Duk, M. Czerhinski, A. Lundblad, *Mol. Immunol.* **24**, 605 (1987); K. Wasniowska *et al.*, *Glycoconjugate J.* **2**, 163 (1985)]. Crude glycophorins were isolated by phenol extraction of membranes of human erythrocytes. Glycophorin A was purified by gel filtration in the presence of SDS, and column fractions containing bands corresponding to monomeric and dimeric glycophorin A were pooled, dialyzed against 50% ethanol to remove SDS, dialyzed against distilled water, and lyophilized. Column fractions enriched in glycophorin B were pooled for further purification of glycophorin B with the use of reverse-phase, high-pressure liquid chromatography (LKB, Bromme, Sweden). Tryptic and chymotryptic fragments of glycophorin A were obtained by digestion with TPCK-trypsin (Sigma) or TLCK-chymotrypsin (Sigma) and purified. The purity of products was analyzed by SDS-PAGE followed by PAS (periodate acid Schiff) staining and immunoblotting with monoclonal antibody to N (mAb 453) and showed clean single bands, with appropriate mobilities. Glycophorin A and the glycopeptides of glycophorin A contained no detectable contaminants. The tryptic peptide (MT1) contained two peptides (1–31 and 1–39), with 1–39 predominating. Glycophorin B, when probed with antibodies to N, in an immunoblot showed a band below the glycophorin A dimer that was probably a heterodimer of glycophorins A and B. The heterodimer was not detected on PAS-stained gels, which indicates a minimal contamination of glycophorin A in the glycophorin B preparation. Amounts of glycophorins and glycopeptides obtained were also analyzed by quantitative amino acid analysis (Table 2), and sialic acid contents were determined by the thiobarbituric acid method (*21*). Molalities of the glycopeptides and sialic acid were the expected concentration for each glycopeptide, which indicates that the glycopeptides of glycophorin A were unaltered during enzyme treatment and purification.
24. Erythrocytes for binding assays were washed three times with 10 volumes of RPMI-1640 before use. Enzymatic treatment of erythrocyte was performed as described with neuraminidase and trypsin (*3*) and peptide *N*-glycosidase (Oxford Glycosystems) (12 U) for 16 hours at 37°C.
25. This assay was identical to that described (*16*), except that the step for pre-incubation with inhibitor was omitted.
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Structure of the RGD Protein Decorsin: Conserved Motif and Distinct Function in Leech Proteins That Affect Blood Clotting

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The structure of the leech protein decorsin, a potent 39-residue antagonist of glycoprotein IIb-IIIa and inhibitor of platelet aggregation, was determined by nuclear magnetic resonance. In contrast to other disintegrins, the Arg-Gly-Asp (RGD)–containing region of decorsin is well defined. The three-dimensional structure of decorsin is similar to that of hirudin, an anticoagulant leech protein that potently inhibits thrombin. Amino acid sequence comparisons suggest that ornatin, another glycoprotein IIb-IIIa antagonist, and antistasin, a potent Factor Xa inhibitor and anticoagulant found in leeches, share the same structural motif. Although decorsin, hirudin, and antistasin all affect the blood clotting process and appear similar in structure, their mechanisms of action and epitopes important for binding to their respective targets are distinct.

Blood-sucking leeches are anatomically and physiologically adapted to their ecto-parasitic life-style (*1*). Leech salivary glands secrete several proteins that affect blood clotting, a complex process involving platelet adhesion and aggregation, the coagulation and fibrinolytic systems, and the endothelium (*2*). Hirudin, from the leech *Hirudo medicinalis*, potently inhibits thrombin, an important serine protease in the coagulation cascade (*3*). Other leech proteins that affect the hemostatic process include the bdellins, which inhibit plasmin (*4*);

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