Molecular & Biochemical Parasitology 173 (2010) 115–122

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Functional and immunological characterization of the var2CSA-DBL5ε domain of a placental *Plasmodium falciparum* isolate

Stéphane Gangnard[a,1], Nicaise G. Tuikue Ndam[b,c,1], Sedami Gnidehou[b], Michael Quiviger[b], Alexandre Juillerat[a], Grazyna Faure[a], Bruno Baron[d], Firmine Viwami[b], Philippe Deloron[b], Graham A. Bentley[a,*]

[a] Institut Pasteur, Unité d'Immunologie Structurale, CNRS URA2185, Paris, France
[b] Institut de Recherche pour le Développement, Mother and Child Health in the Tropics Research Unit UMR216, Paris, France
[c] Institut des Sciences Biomédicale et Appliquées, IRD UMR216, Cotonou, Benin
[d] Institut Pasteur, Plate-Forme de Biophysique des Macromolécules et de leurs Interactions, CNRS URA2185, Paris, France

ARTICLE INFO

Article history:
Received 8 February 2010
Received in revised form 15 April 2010
Accepted 17 May 2010
Available online 24 May 2010

Keywords:
Pregnancy-associated malaria
PfEMP1
var2CSA
DBL domain
Recombinant protein

ABSTRACT

Pregnancy-associated malaria (PAM) arises from sequestration of *Plasmodium falciparum*-parasitized erythrocytes (PE) in the placenta, leading to chronic symptoms in the expectant mother and serious consequences for fetal development. Placental sequestration has been linked to binding of chondroitin sulphate A (CSA) by the var2CSA variant of PfEMP1 expressed on the PE surface, and a substantial body of evidence shows that the immune response to var2CSA gives an effective protection against PAM. We have expressed the var2CSA-DBL5ε domain, derived from a placental isolate from Senegal, as soluble product in *Escherichia coli* and have shown using different criteria that the recombinant protein is obtained with the native conformation. Using surface plasmon resonance techniques, we have examined binding of DBL5ε to placental chondroitin sulphate proteoglyan and CSA; however, the recombinant protein also binds to other sulphated oligosaccharides, with higher affinity in some cases, indicating that the single domain lacks the specificity for CSA shown by the complete extra-cellular region of var2CSA and placental parasites. Recombinant DBL5ε was specifically recognized by sera from malaria-exposed Senegalese women in a parity-dependent manner but by sera not from children or males from the same endemic region. Conversely, DBL5ε induced antibodies in mice that recognized placental isolates from Benin but not isolates from children. The presence of universal epitopes thus supports DBL5ε as an interesting component of var2CSA to be considered for vaccine development.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

In malaria-endemic regions, individuals acquire protective immunity through the continuous exposure to *Plasmodium falciparum* from early childhood [1]. Nonetheless, pregnant women can develop new infections with a high risk of serious complications, especially during their first pregnancy, as the placenta provides a novel sequestration site for erythrocytes infected with certain parasite phenotypes not yet encountered by the immune system [2]. Indeed, examination of the placenta after delivery reveals a massive accumulation of parasitized erythrocytes (PE) in the intervillous space, leading, for example, to maternal anemia and impairment of fetal development, the major outcomes of pregnancy-associated malaria (PAM) [3]. Field studies have shown that primagravid women are vulnerable to placental infections, but after one or more pregnancies, women acquire an immune protection against PAM that correlates directly with gravidity and the presence of antibodies directed against placental PE [4,5]. This immune response transcends geographic location [4], suggesting that epitopes are conserved or are cross-reactive between different placental isolates.

The receptor for placental parasitized erythrocytes (PE) is chondroitin sulphate A (CSA) [2], the glycan component of chondroitin sulphate proteoglycan (CSPG) on the syncytiotrophoblast lining of the intervillous space. Compelling evidence suggests that var2CSA, a highly conserved variant of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family exclusively expressed by placental PE, is the main variable surface antigen of parasites binding to placental CSPG [6,7]. The presence of conserved epitopes underlines

Abbreviations: CSA, chondroitin sulphate A; CSB, dermatan sulphate; CSPG, chondroitin sulphate proteoglycan; CD, circular dichroism; PAM, pregnancy-associated malaria; PE, parasitized erythrocytes; PfEMP1, *P. falciparum* erythrocyte membrane protein 1; SPR, surface plasmon resonance.

* Corresponding author. Tel.: +33 01 45 68 86 10; fax: +33 01 40 61 30 74.
E-mail address: graham.bentley@pasteur.fr (G.A. Bentley).

[1] These authors contributed equally.

0166-6851/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbiopara.2010.05.014

the potential of var2CSA as a basis for vaccine development against PAM.

Var2CSA is a large transmembrane protein (∼350 kDa) expressed on the PE surface. The extra-cellular region can be divided into six Duffy-binding like domains (DBL) and four inter-domain regions [8]. Several DBL domains have been implicated in binding to CSA although molecular details of the interaction are not currently well delineated [9–12]. Indeed, recent studies suggest that the binding site depends on a higher order organization of the DBL domains and the interdomain regions of var2CSA [13,14]. Antibodies raised by immunization with recombinant DBL domains are cross-reactive with different placental parasite strains and can inhibit binding of PE to CSA [15,16]. In addition, recombinant var2CSA DBL domains are recognized by protective antibodies from multigravid women, which are directed mainly to DBL3X and DBL5ε [17].

Here, we report the production of the recombinant DBL5ε domain, derived from a placental isolate CYK39 from Senegal, as a soluble protein using an *Escherichia coli* expression system. As this isolate shows a small polyclonal character, we denote this domain as DBL5ε-CYK39a. Using biochemical and biophysical criteria, we show that recombinant DBL5ε-CYK39a (rDBL5ε-CYK39a) is produced with a homogeneous native protein fold and that it binds not only to CSA but also to other sulphated glycosaminoglycans, revealing a limited binding specificity of the isolated domain. Recombinant DBL5ε-CYK39a is specifically recognized by IgG from malaria-exposed pregnant women in a parity-dependent manner and induces antibodies that recognize several placental parasite isolates and the CSA-binding FCR3 laboratory strain. These results show that var2CSA-DBL5ε holds promise for PAM vaccine development.

2. Materials and methods

2.1. *E. coli expression of rDBL5ε-CYK39a*

The gene encoding rDBL5ε-CYK39a was amplified by two consecutive PCR from the plasmid 5ε-NTN39-1 of the baculovirus vector, pAcGP67-A (unpublished results). The PCR product was cloned into a modified pET21b plasmid (pET21SG) in which an EcoRI site had been introduced in-frame with the NdeI site, replacing the NheI site. Enzymes used for cloning were EcoRI and SalI. Recombinant DBL5ε-CYK39a was expressed as a C-terminal hexa-His-tagged protein in the Rosetta-Gami strain of *E. coli* (Novagen). Expression was carried out at 20 °C for 20 h, cells were centrifuged and resuspended in 20 mM Tris–HCl, 150 mM NaCl, pH 7.5, and lysis was performed with an Emulsiflex homogeniser (Avestin). The protein was purified using a metal affinity column (TALON, Clontech), followed by gel filtration (S75 16/60, GE Healthcare).

2.2. *Glycans and glycan biotinylation*

The following glycans were used for competitive binding experiments: CSA from bovine trachea (C8529, Sigma–Aldrich), chondroitin sulphate C (CSC) from shark cartilage (C4384, Sigma–Aldrich), dermatan sulphate (CSB) from porcine intestinal mucosa (C3788, Sigma–Aldrich), heparan sulphate (HS) from porcine intestinal mucosa (H9902, Sigma–Aldrich), hyaluronic acid (HA) from rooster comb (H5388, Sigma–Aldrich) and heparin sodium salt (51551, Fluka). CSPG was from human placenta (Malaria Research and Reference Reagent Resource (MR4) Centre) and from bovine articular cartilage (decorin, D8428, Sigma–Aldrich). Heparin was biotinylated on the carboxylated groups for fixation to streptavidin on the SA sensor chip. The glycan (5 mg mL^{-1} in MES buffer 0.1 M, pH 4.5) was incubated with EDC (Fluka) and EZ-link biotin hydrazide (Pierce) at final concentration of 1.3 and 0.5 mM, respectively. The mixture was incubated for 1 h at 22 °C. The biotinylated heparin was then dialyzed over night against TBS buffer.

2.3. *Limited proteolysis*

Recombinant DBL5ε-CYK39a (0.5 mg mL^{-1} in 20 mM Tris–HCl, 150 mM NaCl, pH 7.4) was incubated with 1/30 (w/w) of trypsin for various times (5, 10, 20, 40 and 60 min). The reaction was stopped by addition of a loading buffer containing 0.1% SDS and 5 mM DTT followed by heat inactivation. The samples were analyzed by SDS/PAGE 12%. N-terminal sequencing of peptide products was performed by Edman degradation (Applied Biosystem ABI 494 protein sequencer).

2.4. *Circular dichroism*

CD spectra were measured (Aviv215 spectropolarimeter, Aviv Biomedical) with protein samples at 0.4–0.8 mg mL^{-1} for the far UV and 0.8 mg mL^{-1} for the near UV in 10 mM $Na_3(PO4)_2$, 0.25 M NaF at pH 7.4. Far UV CD spectra and buffer baselines were acquired using a cylindrical cell with a 0.01 cm path length. The scan was repeated consecutively 5 times from 180 to 260 nm (0.5 nm step) with an averaging time of 1 s per step and merged to produce an averaged spectrum. This spectrum was corrected using buffer baselines and normalized to the molar peptide bond concentration and path length as mean molar differential coefficient per residue. Secondary structure estimations were derived from the normalized spectra using the CDSSTR method included in the CDPro software [18]. Near UV CD spectra and buffer baselines were measured between 250 and 350 nm with a constant 0.5 nm step and 1 s per step averaging time, using a 1 cm path length rectangular cell. Each spectrum was determined by averaging five consecutive scans. These were then corrected using buffer baselines, averaged and normalized to the molar concentration of peptide chain.

2.5. *Chemical modification of rDBL5ε-CYK39a*

Alkylation of lysines was performed at 1 mg mL^{-1} (protein) in 50 mM HEPES 150 mM NaCl, pH 7.5. Twenty μL of 1 M borane–dimethylamine complex ABC (Fluka), and 40 μL 1 M formaldehyde (Riedel-de Haën) were added under stirring to the protein solution. The mixture was incubated for 2 h at 4 °C; this procedure was repeated once. A final 10 μL of ABC was added and the mixture was incubated overnight at 4 °C. The sample was centrifuged at 9000 rpm at 4 °C for 20 min and purified by gel filtration (S75 10/300, 20 mM Tris, 500 or 150 mM NaCl, pH 7.4).

Recombinant DBL5ε-CYK39a was denaturated by incubation in 7.2 M urea and 0.2% SDS (final concentration) for 1 h at room temperature, followed by heating at 95 °C for 10 min. DTT was added twice at a final concentration of 1 mM during a 2 h incubation at room temperature, followed by two additions of 1 mM iodoacetamide for 2 h at room temperature.

2.6. *Surface plasmon resonance analysis*

Interaction of soluble rDBL5ε-CYK39a with placental CSPG (MR4 Centre) and biotinylated heparin was studied by real-time surface plasmon resonance (SPR) techniques using the Biacore® 2000 system (Biacore AB). CSPG was covalently immobilized *via* primary amino groups on a CM5 sensor chip as described elsewhere [19]. Biotinylated heparin was immobilized on a SA sensor chip (Biacore AB).

Experiments were run in TBS buffer (50 mM Tris–HCl, 150 mM NaCl, pH7.4) containing 0.005% (w/v) surfactant P20. Association

Download English Version:

https://daneshyari.com/en/article/5915753

Download Persian Version:

https://daneshyari.com/article/5915753

Daneshyari.com