FEBS Letters 588 (2014) 4694–4700

journal homepage: www.FEBSLetters.org

Human ficolin-2 recognition versatility extended: An update on the binding of ficolin-2 to sulfated/phosphated carbohydrates

Emmanuelle Laffly [1], Monique Lacroix [1], Lydie Martin, Emilie Vassal-Stermann, Nicole M. Thielens *, Christine Gaboriaud *

Univ. Grenoble Alpes, IBS, F-38044 Grenoble, France
CNRS, IBS, F-38044 Grenoble, France
CEA, IBS, F-38044 Grenoble, France

ARTICLE INFO

Article history:
Received 15 September 2014
Revised 26 October 2014
Accepted 27 October 2014
Available online 11 November 2014

Edited by Stuart Ferguson

Keywords:
Fibrinogen-like domain
Heparin
DNA
Recognition
Competition
Crystal structure
Ficolin

ABSTRACT

Ficolin-2 has been reported to bind to DNA and heparin, but the mechanism involved has not been thoroughly investigated. X-ray studies of the ficolin-2 fibrinogen-like domain in complex with several new ligands now show that sulfate and phosphate groups are prone to bind to the S3 binding site of the protein. Composed of Arg132, Asp133, Thr136 and Lys221, the S3 site was previously shown to mainly bind N-acetyl groups. Furthermore, DNA and heparin compete for binding to ficolin-2. Mutagenesis studies reveal that Arg132, and to a lesser extent Asp133, are important for this binding property. The versatility of the S3 site in binding N-acetyl, sulfate and phosphate groups is discussed through comparisons with homologous fibrinogen-like recognition proteins.

1. Introduction

Initially discovered as a N-acetylglucosamine (GlcNAc) recognition protein with structural and functional similarities to collectins [1], human ficolin-2 has then been shown to bind to a wider variety of non-self and self molecular patterns [2]. It plays a major role in the first line of defense, for example against pneumococcal infections [3,4]. Ficolin-2 also acts as a scavenger molecule, promoting non-inflammatory removal of dying host cells or mitochondria [5–7].

Three different ficolins are found in humans, namely ficolin-1, -2 and -3 (also called M-, L- and H-ficolin) and two in rodents, ficolin-A and -B, while homologous molecules were also discovered in invertebrate species [8]. The recognition properties of ficolins are mediated by their C-terminal trimeric fibrinogen-like (FBG) domains [9]. They assemble into higher oligomeric forms through their N-terminal collagen-like region, which, except for mouse ficolin-B, also binds mannan-binding lectin-associated serine protease-1 and -2 to trigger the lectin complement pathway [10].

Ficolins are characterized by their capacity to bind acetylated ligands, including carbohydrates such as GlcNAc and acetylated bovine serum albumin (AcBSA) [11–13]. It is now well established that ficolin-2 exhibits a wider binding versatility than the other two human ficolins [2], as exemplified by its capacity to bind to DNA [5], a ligand shared with mouse ficolin-B [14], and heparin [2]. Since DNA is present at the surface of apoptotic/necrotic cells, its interaction with ficolin-2 has been proposed to contribute to enhanced uptake and clearance of dying cells, thereby participating to the maintenance of tissue homeostasis [5]. Heparin is known to interact with several complement proteins and to regulate several steps in the complement cascade [15]. Interestingly, it has been proposed that properdin, a component of the alternative

Abbreviations: 6SGlcN, D-glucosamine-6-sulfate; BSA, bovine serum albumin; AcBSA, acetylated BSA; ESRF, European Synchrotron Radiation Facility; FBG, fibrinogen-like recognition domains; FIBCD1, Fibrinogen C Domain Containing 1 Protein; GlcNAc, N-acetylglucosamine; PCho, phosphocholine; PEG, polyethylene glycol; SOS, sucrose octasulfate; wt, wild-type

* Corresponding authors at: Institut de Biologie Structurale, UMR 5075 CNRS-CEA-Université Joseph Fourier, CAMPUS EPN, 71, avenue des Martyrs, CS 10090, 38044 Grenoble cedex 9, France. Fax: +33 4 76 50 18 90.

E-mail addresses: nicole.thielens@ibs.fr (N.M. Thielens), christine.gaboriaud@ibs.fr (C. Gaboriaud).

[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.febslet.2014.10.042

complement pathway, interacts with apoptotic cells through surface sulfated glycosaminoglycans chains [16], but it is not known if it is also the case for ficolin-2.

Structural analyses have provided some clues to this distinctive feature by revealing the lack of a functional S1 acetyl binding site, and the presence of three additional sub-sites, called S2, S3 and S4, allowing interaction with small acetylated ligands in various orientations (S2 and S3) and with elongated glycans (S3 and S4) [9]. The binding specificity of ficolin-2 for heparin and DNA has not yet been investigated in detail.

A novel member of the family of FBG domain containing proteins, called Fibrinogen C Domain Containing Protein 1 (FIBCD1) with binding specificity for chitin, a polymer of β1-4 linked glucose units, has been identified recently [17]. Resolution of the X-ray crystal structure of its FBG domain in complex with N-acetylmannosamine confirmed the presence of the canonical S1 acetyl binding site [18]. Interestingly, the FIBCD1 site corresponding to the acetyl-binding site S3 of ficolin-2 contained a sulfate ion and the proximity of the acetyl and sulfate sites suggested that FIBCD1 might bind glycosaminoglycans such as chondroitin or dermatan sulfate [18].

In the present study, structural analyses of complexes of the ficolin-2 FBG domain with sulfated ligands allowed us to show similar interactions of distinct chemical groups with the acetyl binding site S3 while site-directed mutagenesis identified the predominant role of Arg132 in these interactions.

2. Materials and methods

2.1. Reagents

Heparin and heparin-biotin sodium salt (mw 15 kDa) from porcine intestinal mucosa, glycerol phosphate disodium salt hydrate, phosphocholine chloride calcium salt tetrahydrate (PCho), D-glucosamine-6-sulfate (6SGlcN), bovine serum albumin (BSA, ref A 7906), acetylated BSA (AcBSA) and gelatin were from Sigma–Aldrich, IL, USA (Saint Quentin Fallavier, France). Calf thymus DNA (~2000 bp, corresponding to a molecular mass ~1 300 000 Da assuming a mass of 650 Da per bp) was from Invitrogen (Cergy-Pontoise, France). Sucrose octasulfate sodium salt (SOS) was purchased from US Biological (Salem, MA, USA).

2.2. Production and purification of human ficolin-2 variants

Recombinant human ficolin-2 was produced in Chinese hamster ovary cells and purified using a one-step affinity chromatography on N-acetylcysteine-Sepharose [13]. The concentration of ficolin-2 was estimated using an absorbance coefficient at 280 nm ($A_{1\%,\ 1\ cm}$) of 17.6 and the molarity estimated using a Mr value of 406 300, assuming that the proteins mainly associates as a tetramer of trimers.

The expression plasmids coding for the R132A, D133A, T136A and K221A mutants of ficolin-2 were generated using the QuickChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies, Massy, France) according to the manufacturer's protocol. A pcDNA3.1(+) vector coding for full-length ficolin-2 was used as a template [13]. Mutagenic oligonucleotides were purchased from Eurogentec (Seraing, Belgium). The sequences of all constructs were verified by dsDNA sequencing (GATC Biotech, Mulhouse, France). The mutated proteins were produced by transient transfection of CHO cells using Lipofectamine 2000 (Invitrogen), as described by the manufacturer. Cells were cultured for 4 days in DMEM:F12 medium (Invitrogen) containing 10% heat-inactivated fetal calf serum and 50 μg/ml ascorbic acid (Sigma–Aldrich). The ficolin-2 mutants were purified from the cell culture supernatant and their molar concentrations estimated as described for the wild-type (wt) protein [13]. Analysis of all variants by SDS–PAGE and Coomassie blue staining or western blotting yielded typical patterns, including a single band with an apparent Mr of 34 000 Da under reducing conditions and two major bands, one of Mr 34 000 and the other of high molecular weight, under non-reducing conditions (Fig. S1), as described previously for other ficolin-2 variants [13].

2.3. Solid-phase binding assays

A solid-phase binding assay was performed to measure binding of the ficolin-2 variants to immobilized heparin. Clear 96-well microtiter plates (heparin binding plates, Becton–Dickinson, Le Pont de Claix, France) were coated with 2.5 μg/well heparin or gelatin as a control in 100 μl of PBS overnight at room temperature (RT). Wells were blocked with 0.2% gelatin in PBS for 2 h at 37 °C, washed with PBS containing 0.05% Tween 20 (PBS-T) and 0.5 μg of ficolin-2 diluted in PBS-T were added and incubated for 2.5 h at 37 °C. After washing with PBS-T, ficolin-2 binding was detected by incubation for 1 h at 37 °C with a rabbit polyclonal antibody against ficolin-2 (ST1689, Merck Millipore, Guyancourt, France) diluted 1/1000 in PBS-T. After washing with PBS-T, HRP-conjugated anti-rabbit IgG (Sigma–Aldrich) diluted 1/20 000 in PBS-T was added and incubated for 1 h at 37 °C. After washing with PBS-T, plates were developed with tetramethylbenzidine, the reaction was sopped with 0.5 M H_2SO_4 and absorbance was read at 450 nm.

2.4. Surface plasmon resonance studies

Analyses were performed at 25 °C using a Biacore 3000 instrument (GE Healthcare). Streptavidin (approximately 4000 RU) was immobilized on two flow cells of a CM4 sensor chip (GE Healthcare) as described previously [19]. Biotinylated heparin was captured on the streptavidin surface in 10 mM HEPES, 145 mM NaCl, 0.005% surfactant P20, pH 7.4 (HBS-P) until a coupling level of 250–300 RU was obtained. AcBSA and BSA were diluted to 25 μg/ml in 10 mM sodium formate, pH 3.0 and 10 mM sodium acetate, pH 4.0, respectively, and immobilized (650–950 RU) on a CM5 sensor chip in HBS-P using the amine coupling chemistry according to the manufacturer instructions (GE Healthcare Life Sciences, Velizy-Villacoublay, France). Ficolin-2 binding was measured in HBS-P at a flow rate of 20 μl/min. The heparin and AcBSA surfaces were regenerated by pulse injections of 1 M NaCl and 1 M sodium acetate (pH 7.2), respectively. The signal recorded on reference flow cells without captured heparin or with immobilized BSA were subtracted from that obtained on biotinylated heparin or AcBSA, respectively.

For competition assays, ficolin-2 was incubated for 15 min at room temperature in the presence of various concentrations of competitor before injection. The signal recorded for injection of the competitor alone was subtracted from the data.

2.5. Crystallization, data collection and structure determination

Crystallization of recombinant ficolin-2 FBG domain, ligand soaking and structure determination were performed as described before [9]. Briefly, crystals were grown using the hanging drop method by mixing equal volumes (2 μl) of the protein solution (5.7 mg/ml in 145 mM NaCl, 50 mM triethanolamine-HCl, pH 7.4) and of a reservoir solution composed of 15% (w/v) polyethylene glycol (PEG) 8000, 200 mM calcium acetate and 0.1 M HEPES, pH 7.0. Different ligand concentrations and soaking times were tried, but the best results in terms of diffraction resolution were selected here. They correspond to two soaking experiments, one using 150 mM SOS for 10 min and the other 300 mM 6SGlcN for 4 h.