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Crystal structures of the Erp protein family members ErpP and ErpC from *Borrelia burgdorferi* reveal the reason for different affinities for complement regulator factor H

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ABSTRACT

Borrelia burgdorferi is the causative agent of Lyme disease, which can be acquired after the bite of an infected *Ixodes* tick. As a strategy to resist the innate immunity and to successfully spread and proliferate, *B. burgdorferi* expresses a set of outer membrane proteins that are capable of binding complement regulator factor H (CFH), factor H-like protein 1 (CFHL-1) and factor H-related proteins (CFHR) to avoid complement-mediated killing. *B. burgdorferi* B31 contains three proteins that belong to the Erp (OspE/F-related) protein family and are capable of binding CFH and some CFHRs, namely ErpA, ErpC and ErpP. We have determined the crystal structure of ErpP at 2.53 Å resolution and the crystal structure of ErpC at 2.15 Å resolution. Recently, the crystal structure of the Erp family member OspE from *B. burgdorferi* N40 was determined in complex with CFH domains 19–20, revealing the residues involved in the complex formation. Despite the high sequence conservation between ErpA, ErpC, ErpP and the homologous protein OspE (78–80%), the affinity for CFH and CFHRs differs markedly among the Erp family members, suggesting that ErpC may bind only CFHRs but not CFH. A comparison of the binding site in OspE with those of ErpC and ErpP revealed that the extended loop region, which is only observed in the potential binding site of ErpC, plays an important role by preventing the binding of CFH. These results can explain the inability of ErpC to bind CFH, whereas ErpP and ErpA still possess the ability to bind CFH.

1. Introduction

Lyme disease is an infectious disease caused by the spirochete *Borrelia burgdorferi* [1,2]. *B. burgdorferi* is propagated in nature through a complex enzootic life cycle that entails circulating between two different hosts—*Ixodes* ticks and warm-blooded vertebrates. When transferred to the mammalian host during the blood meal of an infected *Ixodes* tick, *Borrelia* must first evade the host's immune response to successfully spread and survive in the new host.

To resist the host's first line of defense, *B. burgdorferi* is able to bind complement regulators factor H (CFH), factor H-like protein 1 (CFHL-1) and several factor H-related proteins (CFHRs) [3–5]. The complement system is a major component of the innate immune system's ability to respond to infection and is associated with the removal of immune complex and damaged or anomalous self-cells [6]. The activation of the complement system is regulated by several plasma proteins that act in concert as inhibitors of the system by binding to self-surfaces through host-specific targets such as glycosaminoglycans. Normally, foreign particles or damaged self-cells do not bind these complement system inhibitor proteins and are thus targeted for complement system activation. This leads to the formation of the membrane attack complex followed by cell lysis in bacteria or apoptosis in damaged self-cells [7].

CFH and CFHL-1 are the main inhibitors of the alternative pathway of the human complement system [8]. CFH is a 155 kDa glycoprotein composed of 20 repetitive domains called the short consensus repeats (SCRs), which contain 60 amino acids each [9]. Alternative splicing of the *CFH* gene generates a shorter form of CFH protein called factor H-like protein 1 (CFHL-1). CFHL-1 is a 43 kDa protein consisting of seven SCR domains (SCR1-SCR7) that are also found in CFH [10,11]. Sequence and structural homology indicate that there are another five proteins (CFHR1–CFHR5) that are closely related to CFH. Compared with CFH, they are smaller, composed of 4 to 9 SCR domains and are known as factor H-related proteins (CFHR) [12,13]. The CFHR proteins show the highest sequence identity with SCR domains 19–20 from CFH [14]. For example, SCR domains 4 and 5 from CFHR-1 share 100 and 97% identity,

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but SCR domains 8 and 9 from CFHR-5 share 66 and 43% identity with CFH domains 19 and 20, respectively.

By binding the host's complement regulators, the bacteria mimic the action of the host's self-cells and thus avoid the host's complement attack [15,16]. The proteins that bind CFH, CFHL-1 and/or CFHRs in *B. burgdorferi* are termed as complement regulator-acquiring surface proteins or CRASPs [3,17]. In *B. burgdorferi*, five CRASP proteins have been identified, i.e., CspA, CspZ, ErpA, ErpC, and ErpP, and belong to three different gene families [18,19]. CRASPs are not only different in their primary and tertiary structures, but also display different binding affinities for the complement regulators CFH, CFHL-1 and CFHRs [4,20]. CspA (also known as BbCRASP-1) is a member of the PFam54 gene family and is able to bind both CFH and CFHL-1 [21]. The crystal structure of CspA has been determined, and a putative binding site in the cleft between the monomers in the homodimer has been proposed as a binding site for the complement factors [22,23]. CspZ (also known as BbCRASP-2) is also able to bind CFH and CFHL-1 but is genetically distinct and does not share any sequence similarity with other CFH/CFHL-1 binding proteins [24,25]. The crystal structure of CspZ has been determined recently and shows a unique protein fold, not only among CFH/CFHL-1 binding proteins but also among any known proteins deposited in Protein Data Bank [26]. ErpP, ErpC and ErpA (also known as BbCRASP-3, BbCRASP-4 and BbCRASP-5) belong to the Erp (OspE/F-related) protein family and display a high sequence similarity [5,27,28]. ErpP, ErpC and ErpA have shown the ability to bind CFH and/or CFHR-1, CFHR-2, and CFHR-5 proteins [4,5,19].

Recently the crystal structure was determined for the Erp protein OspE from *B. burgdorferi* N40 in complex with CFH domains 19–20, revealing the residues involved in the complex formation [29]. The sequence identity between OspE from *B. burgdorferi* N40 and ErpP, ErpC and ErpA from *B. burgdorferi* B31 is approximately 80%. Note that the abbreviation "OspE" can be misleading because the members of the Erp protein family are occasionally collectively referred to as OspE proteins [30].

In the present study, we have solved the crystal structures of the ErpP protein at 2.53 Å resolution and the ErpC protein at 2.15 Å resolution. We compare the CFH binding site of OspE with the corresponding sites in ErpP and ErpC. Although the crystal structure of ErpC has been determined previously at 2.37 Å resolution, the loop region located near the binding site was not observed in the structure [31].

There are two reasons why we have solved the crystal structures of ErpP and ErpC proteins and compared the CFH binding site of OspE with those of homologous ErpP and ErpC proteins. First, we sought to explain why the *B. burgdorferi* ErpP, ErpC and ErpA proteins differ in their affinities for CFH and CFHR proteins, which suggests that unlike ErpA and ErpP, native ErpC protein is not able to bind CFH and primarily binds only CFHR1 and/or CFHR2 [4,5,17,20].

The second reason is due to the fact, that before the crystal structures for Erp proteins were solved, there have been various studies about the putative residues potentially involved in CFH and CFHR binding by Erp family proteins [28,32]. The residues and the respective binding sites found in those studies do not match the binding site found in the OspE–CFH complex. Therefore, considering that the binding site for CFH in ErpP, ErpC and ErpA is the same as that in OspE–CFH complex, we wanted to examine the potential role of residues thought to be involved in CFH and CFHR binding found in the previous studies.

2. Materials and methods

2.1. Cloning and recombinant expression of ErpP and ErpC

The genes encoding ErpP and ErpC from *B. burgdorferi* strain B31 were PCR-amplified from genomic DNA. For both genes, the predicted sequence coding for the signal peptide (residues 1–26) was excluded from the amplified product. The amplified products coding for ErpP and ErpC were cloned into the pETm_11 expression vector (EMBL) encoding an N-terminal 6xHis tag followed by a TEV (Tobacco Etch Virus) protease cleavage site. The plasmid was transformed into *Escherichia coli* RR1 cells and grown overnight at 37 °C on LB agar plates containing kanamycin. Colonies were inoculated into LB medium containing kanamycin at 37 °C for another 24 h. Plasmid DNA was isolated from the resulting culture and verified by DNA sequencing. The plasmid DNA was transformed into *E. coli* BL21(DE3) cells and grown in modified 2x TYP media supplemented with kanamycin (10 mg/ml), 133 mM phosphate buffer, pH 7.4, and glucose (4 g/l) with vigorous agitation at 25 °C. When the OD_{600} reached 0.8–1.0, cultures were induced with 0.2 mM IPTG and grown for an additional 16–20 h.

2.2. Protein purification and 6xHis tag cleavage

Cells were lysed by sonication, and the cell debris was removed by centrifugation at 10,000 rpm for 30 min. The supernatant was loaded onto a Ni-NTA agarose (Qiagen, Germany) column, washed, and bound protein was eluted by a high imidazole concentration. For the eluted fractions, buffer exchange was performed in 20 mM Tris–HCl pH 8.0 using an Amicon centrifugal filter unit (Millipore, Germany).

The 6xHis tag was removed by incubating the protein solution overnight with TEV protease. The protease, the digested 6xHis tag and any remnants of un-cleaved protein were removed by an additional round of Ni-NTA chromatography. The eluted protein fraction was concentrated to 9 mg ml^{-1} using an Amicon centrifugal filter unit (Millipore, Germany).

2.3. Crystallization of ErpP and ErpC proteins

The initial crystallization conditions for ErpP and ErpC were determined by mixing 0.4 μl of protein with 0.4 μl of reservoir solution from commercially available screens (Molecular Dimensions Ltd., UK) using the sitting drop vapor diffusion method. For the initial condition screening for the ErpC protein the Tecan Freedom EVO100 workstation was used (Tecan Group Ltd., Switzerland). The crystals were obtained in the conditions containing 28% PEG 3350 for ErpP and 20% PEG 6000, 10% isopropanol and 0.1 M HEPES, pH 7.5, for ErpC. As a cryoprotectant, the mother liquor was supplemented with 25% glycerol in both cases, and the crystals were flash-frozen in liquid nitrogen.

2.4. Data collection and structure determination

X-ray diffraction data for ErpP and ErpC were collected at the MAX-lab beamline I911-3 (Lund, Sweden). Reflections were indexed and scaled using MOSFLM [33] and SCALA [34] from the CCP4 suite [35]. Initial phases for ErpP and ErpC were obtained by molecular replacement using Phaser [36] and the crystal structure of the homologous protein OspE as a search model for ErpP (PDB ID: 4J38, chain A [29]), and the crystal structure of ErpP as a search model for ErpC (PDB ID: 4BOB). The initial protein model was built automatically in BUCCANEER [37] and minor re-building of the model was performed manually in COOT [38]. Water molecules were picked automatically in COOT and inspected manually. Crystallographic refinement was carried out using REFMAC5 [39].

A summary of the data collection, refinement and validation statistics for ErpP and ErpC is given in Table 1.

2.5. Accession numbers

The coordinates and the structure factors for the ErpP and ErpC proteins have been deposited in the Protein Data Bank with the accession numbers 4BOB for ErpP and 4BXM, 4BOD for ErpC.

Table 1
Statistics for data and structure quality.

Dataset	ErpP	ErpC
Space group	P $2_12_12_1$	$P4_1$
Unit cell dimensions		
a (Å)	27.03	62.68
b (Å)	55.13	62.68
c (Å)	81.37	76.27
Wavelength (Å)	1.0408	1.0000
Resolution (Å)	32.74–2.53	38.32–2.15
Highest resolution bin (Å)	2.67–2.53	2.27–2.15
No. of reflections	10,415	46,574
No. of unique reflections	3927	16,062
Completeness (%)	90.5 (86.8)	99.7 (100.0)
R_{merge}	0.11 (0.27)	0.05 (0.23)
$I/\sigma(I)$	6.7 (3.5)	13.4 (5.0)
Multiplicity	2.7 (2.7)	2.9 (2.9)
Refinement		
R_{work}	0.234 (0.407)	0.170 (0.111)
R_{free}	0.260 (0.660)	0.208 (0.171)
Average B-factor (Å^2)		
Overall	17.4	37.3
From Wilson plot	31.9	34.2
No. of atoms		
Protein	1035	2158
Water	0	47
RMS deviations from ideal		
Bond lengths (Å)	0.011	0.019
Bond angles (°)	1.707	1.955
Ramachandran outliers (%)		
Residues in most favored regions (%)	88.28	92.69
Residues in allowed regions (%)	11.72	6.92
Outliers (%)	0.00	0.38

Values in parentheses are for the highest resolution bin.

3. Results and discussion

3.1. Overall structure of ErpP and ErpC proteins

For both proteins, the region corresponding to the signaling sequence (residues 1–26) that is required to locate the protein on the outer surface of bacteria was excluded from the expression constructs. The crystal structure of ErpP was built for residues 51–186; the N-terminal residues 27–50 were not observed in the electron density. The loop region between β-strands β7 and β8 (residues 163–167) was not modeled because of poor electron density. The structure of ErpC was built for residues 38–179, excluding residues 154 and 155 in the loop region between β-strands β1 and β2.

The crystal structure of ErpP is very similar to the crystal structures of the homologous proteins ErpC (r.m.s.d. of 0.61 Å) and OspE (r.m.s.d. of 0.78 Å) that have been described previously and is composed of an alpha + beta domain structure made from eight antiparallel β-strands and two short α-helices [29,31] (Fig. 1a and c). Although the tertiary structures of the homologous proteins can be superposed almost exactly, a slight degree of variation is observed in the loop regions, particularly in the N-terminal region. The variation was also observed in NMR studies for the OspE N-terminal region, which is thought to serve as a connection between the structural domain and the lipid anchor that connects the protein to the cell surface [29] (Fig. 1b).

3.2. Residues in Erp family proteins potentially important for CFH binding

The crystal structure for OspE from *B. burgdorferi* N40 in complex with CFH SCR domains 19–20 has been solved. Because the homologous proteins ErpP and ErpC of *B. burgdorferi* B31 share high sequence and structural identity with OspE from *B. burgdorferi* N40, it is reasonable to believe that the binding of CFH by ErpP and ErpC occurs in the same manner. Therefore the potential binding sites in ErpP and ErpC can be compared with that found in OspE–CFH complex to identify the reason of the differences in CFH affinities among the members. But before the crystal structures of any Erp family protein and OspE–CFH complex were determined to reveal the exact residues involved in binding, several studies were conducted to identify potential residues and regions in Erp family proteins that are involved in the binding of CFH [28,32,40–42]. Revision of the residues identified in those previous studies shows that they are different from those involved in OspE–CFH complex formation. With the crystal structures now in hand, it would be interesting at first to examine the residues that previous studies identified as being important in CFH binding to determine their potential role in the interaction and to eliminate the possibility that ErpC

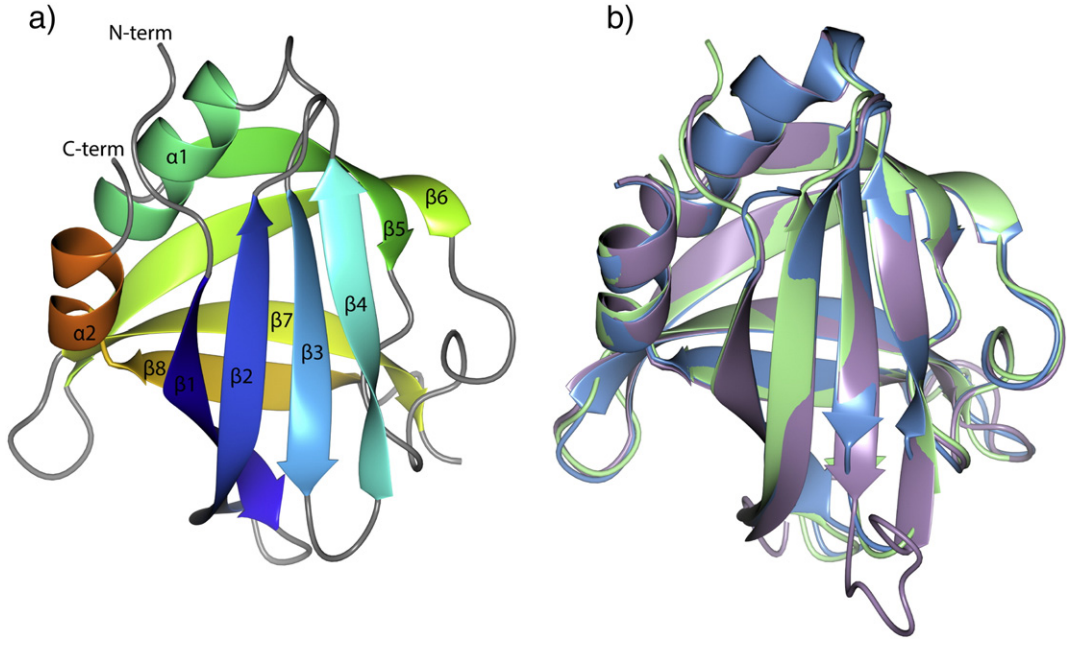

Fig. 1. Overall ErpP structure and superposition with the homologous proteins ErpC and OspE. a) Crystal structure of ErpP protein with the secondary structure elements labeled from the N-terminus and colored using a rainbow, ranging from blue at the N-terminus to red at the C-terminus. b) Superposed crystal structures of ErpP, ErpC from *B. burgdorferi* B31 and OspE from *B. burgdorferi* N40. The ErpC is colored in lilac, ErpP is in green and OspE is in blue.

and ErpP could bind CFH differently than OspE. In most of those studies, the OspE protein from *B. burgdorferi* N40 was involved for comparison, which makes it easier to assess whether the residues found in previous studies have any direct role in binding.

One of those studies determined that there are four proteins in *Borrelia spielmanii*, designated as Erp60, Erp61, Erp62 and Erp63, that share almost 100% sequence identity with each other and approximately 70% identity with the Erp family of proteins from *B. burgdorferi* [32]. Despite the high similarity and unlike the others, the Erp63 protein was not able to bind CFH/CFHRs; it was possible to conclude that the mutation of His79 (His94 in ErpP) to Arg79 in Erp63 is responsible for its inability to bind CFH and CFHR proteins. As the sequence identity among ErpP, ErpC from *B. burgdorferi* and Erp63 from *B. spielmanii* is approximately 70%, and the Erp family members from *B. burgdorferi* share approximately 80% sequence identity, so it is almost certain that the overall fold is the same among the proteins. In the crystal structure of the OspE–CFH complex, the histidine residue (His81) is located in the binding site of OspE–CFH complex. Though it is not directly involved in the complex formation, the mutation to an elongated side chain of Arg79 in Erp63 could disrupt the binding by a steric clash with the surrounding residues (Fig. 2a). In ErpC, the histidine is changed into a tyrosine but occupies the same position as the histidine residue in OspE and ErpP. Because histidine is not directly involved in OspE–CFH complex formation, it is not expected that a substitution from histidine to tyrosine in ErpC will lead to a complete loss of CFH binding.

Several other studies have devoted a great deal of effort to determine the exact regions and residues involved in CFH binding by the OspE proteins. In particular, it has been established that the N- and C-terminal parts play important roles in CFH binding by Erp proteins [28,40–42]. The deletion of only nine residues from the C-terminus abolishes the binding of CFH, suggesting that the C-terminal part is directly involved in CFH binding [28]. However, peptides representing the C-terminus of OspE protein do not bind to CFH in surface plasmon resonance assays, suggesting that a specific protein conformation may be required for proper binding [42]. It can also be speculated that other binding sites are involved in addition to the C-terminal region and were not detected in those studies or that the deletion of the residues at the C-terminus disrupts the overall structure of the protein. As observed from the crystal structures of the ErpP, ErpC and OspE proteins, the hypothesis that the deletion of the C-terminus disrupts the overall fold and thus the binding of CFH is very likely. The C-terminal region deleted in the studies corresponds to α-helix α2, which shields the hydrophobic interior of the molecule from the solvent. Therefore, the deletion of α-helix α2 could promote the incorrect folding or aggregation of the protein, resulting in its inability to bind CFH. The same effect was observed in the case with N-terminal deletion [41]. From the crystal structure, it is apparent that the outcome would be similar to that of C-terminal deletion because the C- and N-terminal parts are located near each other in the tertiary structure (Fig. 2b).

The direct involvement of the C-terminal in binding to CFH by some Erp family proteins is unlikely, given that the solved OspE–CFH complex clearly shows that the C-terminus is not involved in the binding of CFH, at least in the case of OspE.

3.3. The CFH binding site in Erp family member proteins

The Erp family proteins from different borrelial strains, which bind CFH and CFHR proteins, interact with the SCR20 domain of CFH [17,28,29,42]. Binding of the CFH SCR20 domain by OspE with superposed ErpP and ErpC structures is shown in Fig. 3a. Interestingly, the binding affinity for CFH, CFHR1, CFHR2 and CFHR5 proteins differs among the Erp family members ErpP, ErpC and ErpA [4,5,20]. Furthermore, though

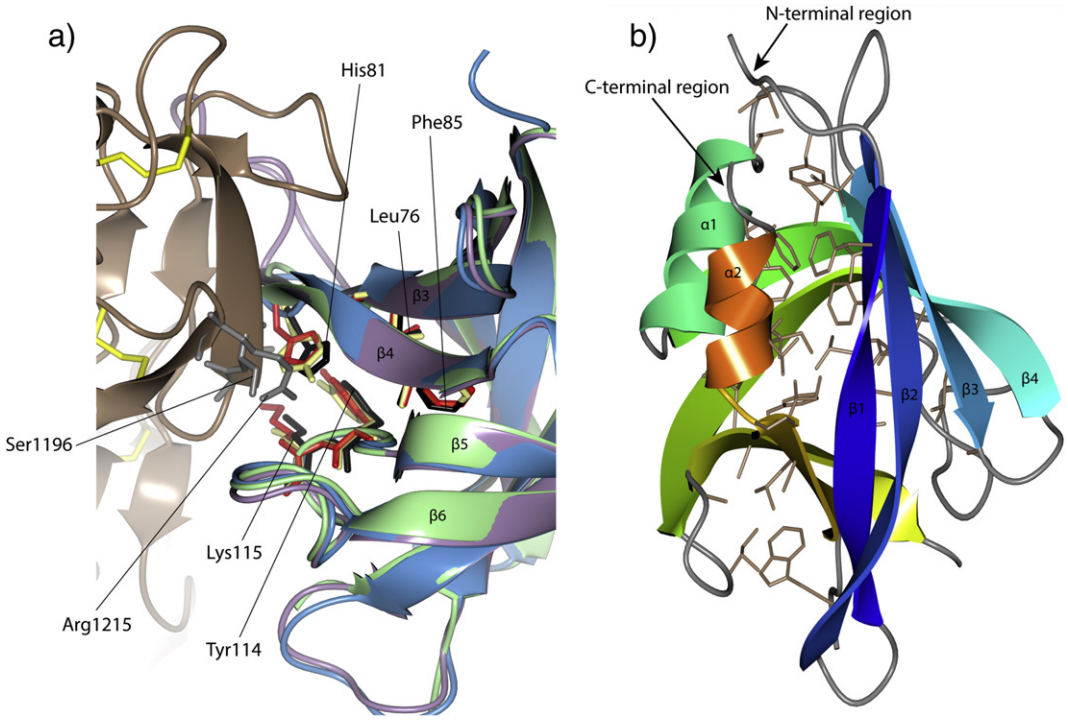

Fig. 2. Factors influencing the binding of CFH/CFHR proteins by Erp family proteins. a) Superposed crystal structures of OspE from the OspE–CFH complex with ErpP and ErpC showing the location of the histidine residue (His81 in OspE) found to be important to ensure the binding of CFH/CFHR as the mutation to arginine in the homologous protein Erp63 from *B. spielmanii* disrupts the binding of complement factors [32]. The substitution to a more elongated side chain of Arg79 in Erp63 may cause steric clashes with neighboring residues, thus explaining the loss of function because the residue is not directly involved in binding of CFH. Some of the neighboring residues are illustrated in Erp family proteins (corresponds to Leu76, Phe85, Tyr114, Lys115, in OspE) and CFH (Glu1195, Ser1196, Arg1215). The ErpC is colored in lilac, ErpP in green and OspE in blue. The SCR20 domain from CFH as observed in OspE–CFH complex is shown in a pale brown color [29]. The side chains are yellow for ErpC, red for ErpP, black for OspE and grey for CFH. b) The hydrophobic interior of the ErpP protein molecule, showing all of the hydrophobic residues. The deletion of either the N- or C-terminus (shown with arrows) may disrupt the shielding of the hydrophobic environment. ErpP is colored in a rainbow color scheme, ranging from blue at the N-terminus to red at the C-terminus.

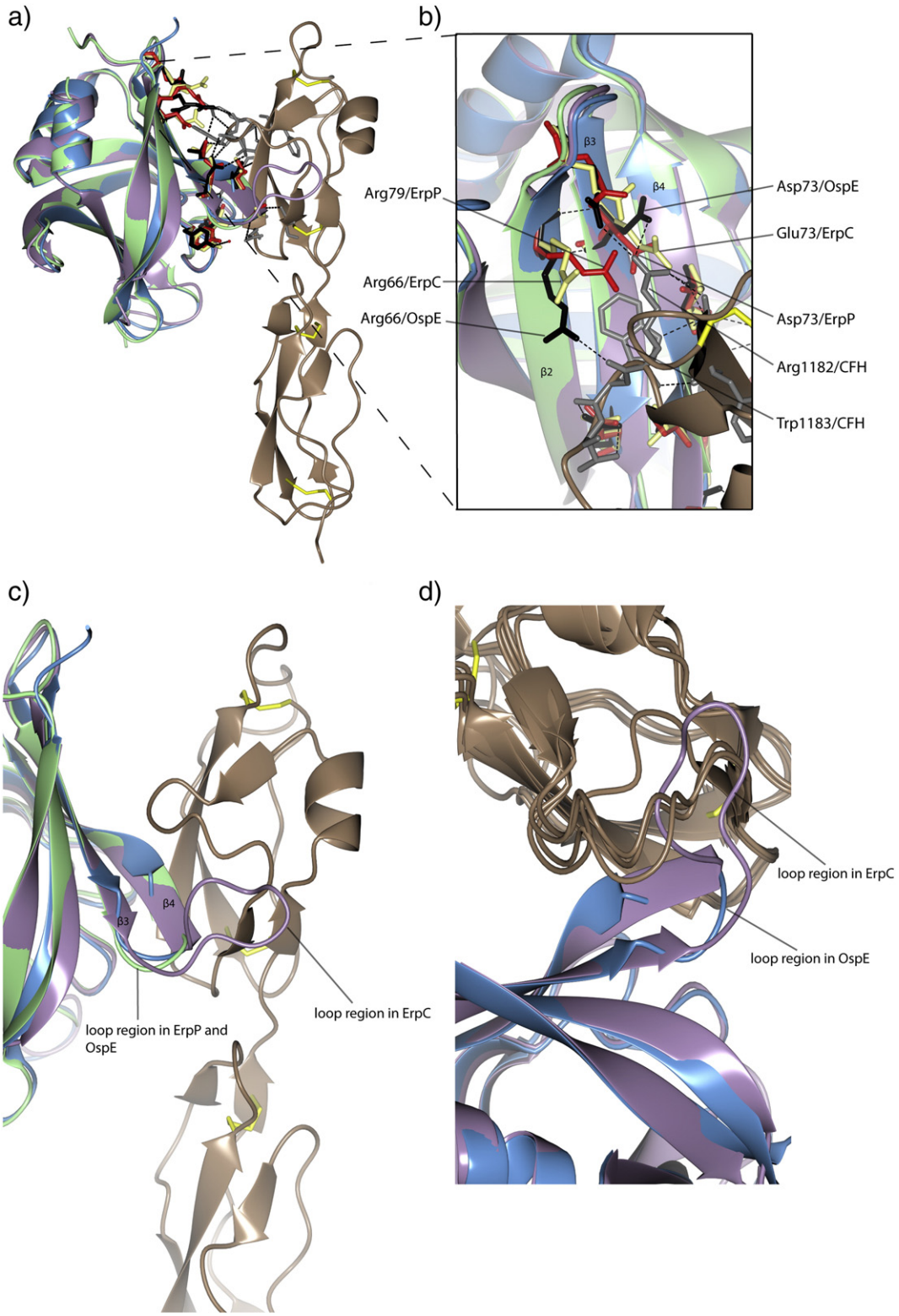

Fig. 3. Binding of CFH SCR20 by OspE with superposed ErpP and ErpC molecules. a) Binding of CFH by OspE with superposed ErpP and ErpC proteins shows the overall binding pattern with the SCR20 domain of CFH [29]. b) Superposed crystal structures of ErpP, ErpC and OspE reveal the residues that have some conformational differences between the homologs. c) The region between β3 and β4 showing the extended loop region in ErpC that likely prevents the binding of CFH; in ErpP and OspE the loop remains short and does not disturb the close interaction with CFH. d) The superposition of CFH crystal structures determined by three different research groups (PDB ID codes: 3RJ3, 3SW0 and 2G7I) with the SCR20 of CFH from the OspE–CFH complex reveals that the extended loop of ErpC will prohibit the binding of CFHR-1. The ErpC is colored in lilac, ErpP is in green and OspE is in blue. The CFH SCR domains 19–20 are colored in pale brown. The side chains are yellow for ErpC, red for ErpP, black for OspE and grey for CFH.

it is able to bind to CFHR1, CFHR2 and, to some extent, to CFHR5, ErpC does not appear to bind CFH; it was not possible to detect any interaction using surface plasmon resonance, Western blotting or native ErpC-producing bacteria exposed to CFH [5,20]. This finding led us to conclude that that there could be some major differences in the binding site between the Erp family proteins.

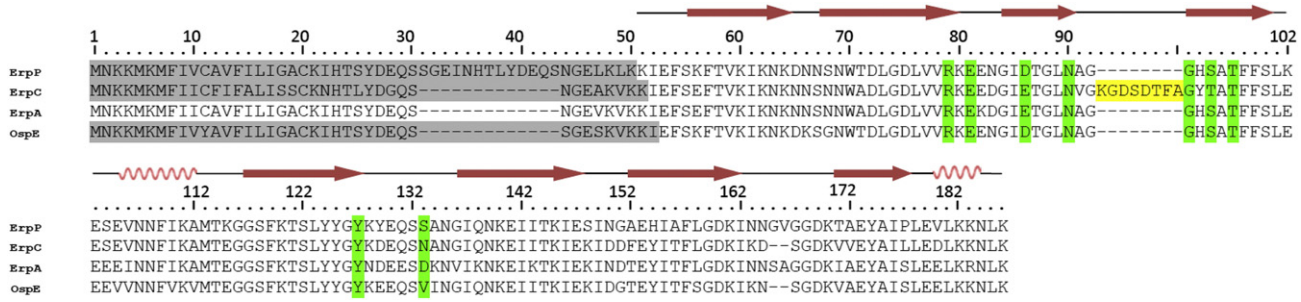

Fig. 4. Sequence alignment of the homologous proteins ErpP, ErpC, ErpA (all from *B. burgdorferi* B31) and OspE (from *B. burgdorferi* N40). The residues in OspE protein that are involved in the formation of the OspE–CFH complex [43] are shown for all members on a green background. For ErpP, ErpC and OspE proteins that already have known crystal structures, the amino acid sequence corresponding to the signal peptide and residues at the N-terminus that are not modeled in the crystal structures are shown on a grey background. The eight additional residues, found only in ErpC, that form an extended loop region and potentially inhibit the binding of CFH are highlighted in yellow. α-Helices are indicated as coils, and β-strands are arrows above the alignment. The numbering and the secondary structure elements are given for ErpP.

The sequence alignment of ErpP, ErpC, ErpA and OspE revealed that the residues involved in complex formation between OspE and CFH are fully or partially conserved among the members, except for the residue Ser133 in ErpP, which corresponds to Asn128 in ErpC, Asp120 in ErpA and Val120 in OspE (Fig. 4). However, Val120 in the OspE–CFH complex makes a hydrogen bond with Arg1215 in CFH by using just the backbone oxygen atom of the residue. Because the residues involved in the binding of CFH are essentially the same in all member proteins, there should be some other divergence explaining the different affinities for CFH.

We first examined the positions and conformations of the residues involved in complex formation between the proteins. This comparison revealed some slight differences, particularly with respect to the residues Arg66, Glu68 and Asp73 in OspE (Fig. 3b). However, it is still possible that under some conditions, the residues in the other homologous proteins adopt the same conformation, as observed in the OspE–CFH complex. However, the residues in the crystal structures are the same among the homologous proteins, so the different conformations of the residues can explain only minor differences in the affinities for CFH.

In comparison with other homologs, ErpC contains an additional eight residues between β-strands β3 and β4 that are located near the residues involved in the potential binding site. The crystal structure of ErpC protein has been determined [31] and revealed that these eight additional residues are indeed located near the CFH binding site. However, due to the poor electron density, the loop region was not included in the model. In our crystal structure of ErpC, the same loop region between β-strands β3 and β4 is clearly visible. Using the superposed crystal structures of ErpC, ErpP and the OspE–CFH complex, it is clear that the extended loop region in ErpC precludes CFH binding, whereas in other homologous proteins, the loop region remains short, thus permitting the binding of CFH (Fig. 3c).

This finding agrees with the previous studies showing that of the Erp proteins, only ErpP and ErpA are able to bind both CFH and CFHRs (CFHR-1, CFHR-2 and CFHR-5), whereas ErpC can bind only CFHR-1, CFHR-2 and CFHR-5 [5,20]. Questions remain about how Erp proteins are able to distinguish CFHR from CFH, given that the SCR domains 4 and 5 from CFHR-1 display 100 and 97% identity to SCR19–20 from CFH and that the SCR domain 20 from CFH is involved in Erp protein binding. One explanation could be that unlike for CFH, the extended loop region observed in ErpC does not interfere with the binding as a result of some conformational change in CFHR proteins and that CFHRs can therefore bind to ErpC. Another hypothesis is that CFHR proteins bind at some other site on the surface of ErpC, likely as dimers forming a unique binding surface [14].

There is no CFHR-1 crystal structure deposited in the PDB to explain the structural differences between CFH and CFHR-1. But there are several CFH SCR domain 19–20 structures showing 97 to 99% identity with the primary sequence of SCR domain 5 from CFHR-1 (PDB ID codes: 3RJ3, 3SW0 and 2G7I). The superposition of the respective CFH crystal structures with the SCR domain 20 of CFH from the OspE–CFH complex confirms that the extended loop of ErpC would prohibit the binding of CFHR-1 (Fig. 3d). Therefore, the assumption that CFHR proteins bind to some other site in ErpC is the more likely explanation and would explain the observation that ErpA can bind both to CFH and CFHR-1 simultaneously [20].

Transparency document

The Transparency document associated with this article can be found, in the online version.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbapap.2014.12.025.

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