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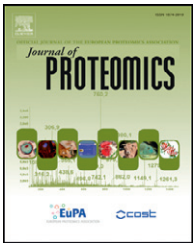

Variable regions in the sushi domains 6–7 and 19–20 of factor H in animals and human lead to change in the affinity to factor H binding protein of *Borrelia*☆

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ABSTRACT

Borrelia binds host's complement regulatory factor H (fH) to evade complement attack. However, binding affinities between fH-binding-proteins (FHBPs) of *Borrelia* and fH from various hosts are disparate. Experiments performed to unfold the underlying molecular basis of this disparity revealed that recombinant BbCRASP-1 (major FHBP of *Borrelia burgdorferi*) neither interacted with sushi 6–7, nor with sushi 19–20 domains of fH in cattle and pig, however, showed binding affinity to both sushi domains of human fH, sushi 6–7 of mouse and sushi 19–20 of sheep. Further, peptide-spot assay revealed three major binding sites (sushi 6:$_{335–346}$, sushi 7:$_{399–410}$ and sushi 20:$_{1205–1227}$) in human fH that can form BbCRASP-1:fH interface, while ^{337}HENMR^{341} residues in sushi 6 are crucial for rigid BbCRASP-1:fH complex formation. Amino acid stretches DTIEFTCRYGYRPRTALHTFRTT in ovine sushi 19–20 and SAYWEKVYVQGQ in mouse sushi 7 were important sites for fH:BbCRASP-1 interaction. Comparative analysis of the amino acid sequences of sushi 6 of cattle, pig and human revealed that bovine and porcine fH lack methionine and arginine in HENMR pocket, that may impede formation of fH:BbCRASP-1 interface. Increasing numbers of FHBPs from animal and human pathogens are being discovered, thus results presented here can be important benchmark for study of other FHBPs:FH interactions.
This article is part of a Special Issue entitled: "Farm animal proteomics".

1. Introduction

Borrelia can infect a wide range of the host species, however, certain host specificity exists for *Borrelia burgdorferi* sensu stricto (*B.b.s.s*). Host specificity of *B.b.s.s* matches with its resistance to complement mediated killing, while complement resistance of *Borrelia* depends on the affinity of *complement factor H binding protein* (FHBP) to factor H (fH) [1,2]. Binding ability of given FHBP to the fH from various host species is not identical [3]. Among the repertoire of FHBPs [4], BbCRASP-1 (complement regulator-acquiring surface protein of *B.b.s.s*) plays a significant role in complement evasion [3–5]. Binding of complement regulatory proteins by bacteria is crucial to avoid the formation of the membrane attack complex and thus cell lysis [6].

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fH is a complement regulatory subunit that serves as a cofactor for the factor I (fI)-mediated cleavage of C3b. By increasing the local concentration of fH at the cell surface, *Borrelia* that binds fH can promote more efficiently the degradation of C3b and thereby decrease the efficiency of complement mediated killing [7–9]. Numerous reports have been published in the past years that show an interaction between CRASPs and human fH. Previously we have reported that *B.b.s.s* readily binds human and mouse fH but not fH of bovine or porcine origin [3], and this differential fH binding correlates with the disparity between evasion of complement attack of animals and human [1]. *B.b.s.s.* can infect human and mouse, infection in cattle and pig is rare (no evidence of experimental infection yet), while *B.b.s.s* infection in sheep is sporadic. These data provide appropriate models to reveal molecular basics of the differential interactions between BbCRASP-1 and fH from animal and human.

The secreted form of human fH is composed of 20 repetitive units known as short consensus repeats (SCRs) or sushi domains [10]. Structural assays showed a central role of sushi 19–20, which encodes heparin, sialic acid and C3b-binding domains, while sushi 7 serves as a binding site for heparin and c-reactive protein [13]. On the other hand, microorganisms can bind fH through various sushi domains, for example: *Streptococcus pyogenes* (M protein) and *Candida albicans* bind to sushi 7, *Streptococcus pneumoniae* (Hic/PspC proteins) interacts with sushi 8–15, *Onchocerca volvulus* (mf protein) uses multiple binding sites between sushi 8 and 20, *Neisseria gonorrhoeae* uses C-terminal sushi domains 16–20, and *B. burgdorferi* FHBPs exploit sushi 7 and sushi 16–20 [11–13]. Most of the fH:pathogen interactions described hitherto are unfolded using human fH. Besides, noteworthy heterogeneity exists between amino acid sequences of fH from human and other hosts. To our knowledge, no study is available that presents comparative binding ability of FHBPs to sushi domains of fH from various host species.

On this background, the work was designed to reveal: 1. comparative binding pattern of BbCRASP-1 to sushi 6–7 and sushi 19–20 (major binding domains for FHBPs from *Borrelia*) of animals and human; 2. potential amino acid residues involved in the BbCRASP-1:fH interactions; and 3. differences or similarities in the BbCRASP-1 binding sites in fH of cattle, sheep, pig, mouse and human.

Table 1 – Primers used in this study for amplification of fragments used to produce recombinant forms of sushi domains and BbCRASP-1.

Sushi domains
Sushi 6–7:
Su6–7 human F— 5′-GCGAAGCTTCCGAGATGTACCTTGAAAC
Su6–7 human R— 5′-GCCTCTAGAGACAAGGATGCACTTGGG
Su6–7 mouse F— 5′-GCGAAGCTTCCAAGATGTACCTTGAAAC
Su6–7 mouse R— 5′-GCCTCTAGAGACACGGATGCATCTGGG
Su6–7 sheep F— 5′-GCGAAGCTTCCAAGATGTGCTTGGAAAC
Su6–7 sheep R— 5′-GCCTCTAGAGACACGGATGCATCTGGG
Su6–7 cattle F— 5′-GCGAAGCTTCCGAGATGTGCCTGGAAAC
Su6–7 cattle R— 5′-GCCTCTAGAGATACGGATGCATTTGGG
Su6–7 pig F— 5′-GCGAAGCTTCCAAGATGTACCTTGAAAC
Su6–7 pig R— 5′-GCCTCTAGAGACACGGATGCATCTGGG
Sushi 19–20:
Su19–20 human F— 5′-CACAAGCTTCCTCAATGCAAAGATTCTACAG
Su19–20 human R— 5′-GTTTCTAGATGCACAAGTTGGATACTCCAG
Su19–20 mouse F— 5′-CACAAGCTTCCAAAGTGCCGAGACTCAACAG
Su19–20 mouse R— 5′-GTTTCTAGATACACAAGTGGGATAATTGAT
Su19–20 sheep F— 5′-CACAAGCTTCCTCAGTGCAAAGACTCTAAAG
Su19–20 sheep R— 5′-GTTTCTAGATCCACATCGAGGATACACCAC
Su19–20 cattle F— 5′-CACAAGCTTCCTCAGTGTAAAGACTCTCAAG
Su19–20 cattle R— 5′-GTTTCTAGATCCACATCGAGGATACACCAC
Su19–20 pig F— 5′-CACAAGCTTCCTCAGTGCAAAGACTCTAAAG
Su19–20 pig R— 5′-GTTTCTAGATCCACACGTAGGATAGGCCAC
BbCRASP-1
BbCRASP1F-5′ TGCGCACCTTTTAGCAAAATC
BbCRASP1R-5′ AAAAGGCAGGTTTTAAGTATC

Primers were constructed based on the sequences in Genbank repository under the accession numbers: Y00716.1 (human), M12660.1 (mouse), GU991526.1 (sheep), BC105258.1 (cattle), AJ278470.2 (pig) and AJ784964.1 (BbCRASP-1). Cycling conditions for all gene fragments were 98 °C for 30 s→35 cycles of [95 °C for 10 s, 72 °C for 1 min]→72 °C for 10 min with Phusion High-Fidelity DNA Polymerase, (Finnzymes). Underlined sequences are sites for restriction enzymes. Primers used to amplify sushi 6–7 and sushi 19–20 produced amplicons ~393 bp and ~399 bp, respectively. Amplicon size of BbCRASP-1 was 678 bp.

2. Materials and methods

2.1. Preparation of r-sushi 6–7 and sushi 19–20 domains

RNA was isolated from cattle, sheep, pig, mouse and human liver biopsy using PureZol kit (Bio-Rad, USA). Regions encoding sushi 6–7 and sushi 19–20 were amplified by using long-range RT-PCR kit (Qiagen, USA). Primers used to amplify the targets are presented in Table 1. All amplicons were sequenced (ABI3100 Avant Applied biosystem, USA), digested with restriction enzymes and then ligated into PYEBME-1 shuttle expression vector (in house modified version of PYES2 vector from Invitrogen; Fig. 1). Ligation mixes were purified with standard phenol–chloroform method and transfected into electrocompetent *Escherichia coli* M15 cells (Qiagen, USA). Transformed *E. coli* were selected based on their ampicillin resistance on LB agar and used for isolation of shuttle plasmid carrying gene of interest. One hundred nanograms of isolated plasmids was transfected into the electrocompetent *Saccharomyces cerevisiae* strain YPH501 (Stratagene, USA). Transformed YPH501 strains were selected on SD agar with uracil auxotrophy (6.7 g yeast nitrogen base, 2% dextrose, 1.3 g amino acid dropout powder without uracil, 2% agar). Selected transformed colonies were cultured further in SD medium for 16 h at 30 °C. Induction of the recombinant proteins in YPH501 was performed in SG medium (dextrose in SD was replaced by 2% galactose) for 4–5 h at 30 °C with constant shaking (300 rpm). Cells were lysed by vigorous shaking on vortex with glass beads (Sigma, Slovakia) in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole) containing 1% of nuclease mix and 1% of a protease inhibitor cocktail (GE Healthcare, Madrid, Spain). Recombinant proteins were eluted using anti-Flag M2 agarose beads (Sigma) under the native conditions as per manufacturer's instructions. The presence of recombinant proteins in elutes was confirmed by western blotting using anti-Flag antibodies (Sigma, Slovakia).

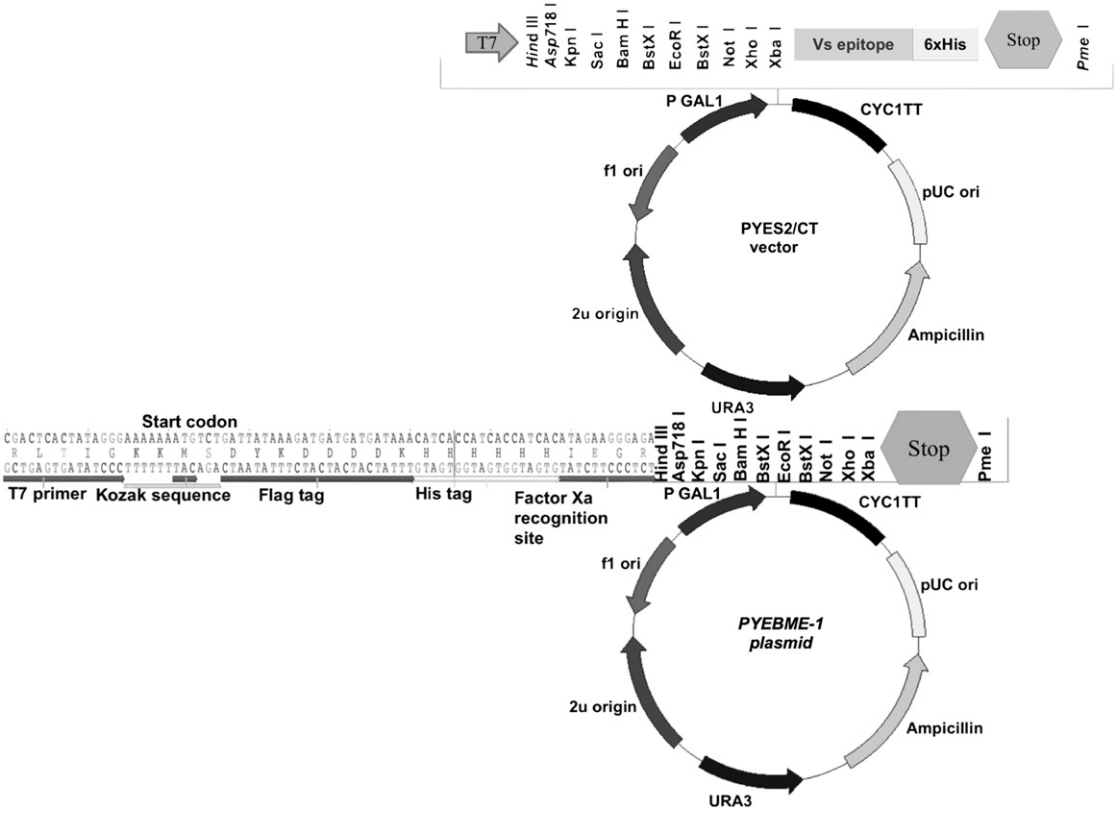

Fig. 1 – PYEBME-1 plasmid. In-house modified version of PYES2/CT plasmid. PYEBME-1 plasmid contains N-terminal double tags (Flag and hexa-histidine), Kozak consensus sequence around start codon to ensure consistent translation, factor Xa cleavage site to cleave tags from rest of the protein, if necessary.
Sequence of PYES2/CT can be found at Invitrogen: http://tools.invitrogen.com/content/sfs/vectors/pyes2ct_seq.txt, while other features of this plasmid are here: http://tools.invitrogen.com/content/sfs/vectors/pyes2ct_map.pdf.

2.2. *Preparation of r-BbCRASP-1*

DNA was isolated from *B.b.s.s* (strain SKT-2, GenBank accession no. GQ344484.1). Whole protein coding region of BbCRASP-1 was amplified by using long-range high fidelity PCR kit (Qiagen, USA) with primers depicted in Table 1. Amplicons were cloned into pQE-UA expression vector (His-tag at N-terminal) using QIAexpress cloning kit (Qiagen). Recombinant constructs were electrotransfected into *E. coli* M15. Transformants were selected on LB agar (1% tryptone, 0.5% yeast extract, 1% NaCl and 1% agar) containing ampicillin (100 μg/ml) and kanamycin (25 μg/ml). Expression of recombinant protein was induced with 1 mM IPTG. After induction cells were pelleted and sonicated in the lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0) containing 1 mg/ml lysozyme, 1% of nuclease mix and 1% of a protease inhibitor cocktail (GE Healthcare, Madrid, Spain). Recombinant His-tagged protein was purified using Ni-NTA agarose (Qiagen) under the native conditions.

2.3. *Affinity ligand binding assay (ALBI assay)*

Purified BbCRASP-1 was fractionated by SDS-PAGE (20 cm long well) and electrotransferred onto nitrocellulose membranes. Nitrocellulose membrane was cut into 5 mm vertical strips (protein concentration per strip ~ 100 ng). To confirm the presence of BbCRASP-1 on nitrocellulose membrane (input control), one strip was blocked in TBSM (10 mM Tris/HCl (pH 8.3), 150 mM NaCl, 1% skim milk) for 1 h and then incubated with nickel-HRP conjugate (1:5000 diluted in TBSM). After five times washing with TBS containing 0.05% tween-20 (TTBS), strips were incubated in enhanced chemiluminescence substrate (Pierce) for 5 min and signal was documented on x-ray film (Fujifilm).

For ALBI assay, after overnight blocking in TBSM, strips were incubated for 2 h either with recombinant sushi 6–7 or sushi 19–20 of different animals and human, or in TBSM (negative control). Unbound proteins were washed two times with TTBS. Sushi domains bound to BbCRASP-1 were detected with mouse anti-Flag antibody diluted at 1:1500 (in TBSM) and goat anti-mouse peroxidase conjugated antibody (Pierce, USA) diluted at 1:10,000 (in TBSM). Enhanced chemiluminescence and x-ray films were used to document signals. Assay was repeated three times.

2.4. *Co-precipitation assay*

Interactions between BbCRASP-1 and sushi domains were further confirmed with co-precipitation in which recombinant Flag-tagged sushi domains were immobilized on anti-Flag agarose beads (protein load ~ 0.3 mg) as per manufacturer's instructions. Agarose beads were washed with TTBS and hybridized overnight at 4 °C with purified his-tagged BbCRASP-1 (protein load ~ 0.3 mg). After stringent washings with TTBS for four times, beads were

boiled in SDS sample buffer, directly load on the SDS-PAGE and proteins were transferred on the nitrocellulose membrane. Membrane was cut horizontally into two strips (approximately at 20 kDa position). Lower strip containing sushi domains (~15–16 kDa) was subjected for protein detection (input control), with anti-Flag antibody and goat anti-mouse HRP conjugate as described above, while BbCRASP-1 (~30 kDa) on upper strip was detected with nickel-HRP conjugate. Enhanced chemiluminescence and x-ray films were used to document signals in both cases. Assay was performed in three replicates to confirm the results.

2.5. *In silico analysis*

Nucleotide sequences of sushi 6–7 and sushi 19–20 of cattle, sheep, pig, mouse and human obtained in our study were in silico translated and amino-acid sequences were aligned with clustal W (Geneious, www.biomatters.com). Amino acid sequences were also compared with the sequences available in Uniprot repository under the entry numbers: human — P08603, mouse — P06909, sheep — D6PZY4, cattle — Q28085, and pig — Q8MI72.

Putative ligand binding sites in human fH were first mapped with data mining [5,11,14,15]. Furthermore, residues in ligand–fH interactions were predicted by *protein interaction calculator server* (http://pic.mbu.iisc.ernet.in/job.html) and *PDBePISA* (Protein Interfaces, Surfaces and Assemblies) for human factor H. Residues in the binding pockets of sushi domains, which possess potential to form hydrogen bonds and potential C–H…π interactions were predicted with CHpredict server (http://bioinformatics.uams.edu/mirror/chpredict/).

2.6. *Peptide-spot assay*

A library of peptides of different sizes (10–23 amino acid stretch; Sigma-Aldrich) covering sushi 6–7 and sushi 19–20 was used. In case of sushi 6–7 only human and mouse fH were included in this assay, as sushi 6–7 from other species showed no interaction with BbCRASP-1, similarly sushi 19–20 of only human and sheep fH were targeted for peptide-spot assay. Most of the peptides carried putative sites/residues having potential to form hydrogen bond or salt bridges or formation of beta strand. Peptides were spotted on nitrocellulose membrane and non-specific binding sites were blocked with TBSM. Membranes were then incubated with His-tagged BbCRASP-1, and binding was detected with nickel-HRP His-tag protein identification system coupled with chemiluminescence (Pierce, Slovakia) as per manufacturer's instructions. Experiment was repeated three times. No non-specific binding between peptides and nickel-HRP conjugate was observed (data not shown).

3. Results

3.1. *Interactions between of BbCRASP-1 and sushi 6–7 of different host species*

In ALBI assay we found interactions between BbCRASP-1 and sushi 6–7 of human and mouse, however, sushi 6–7 domains from cattle, pig and sheep were unable to form complex with BbCRASP-1 (Fig. 2a). It is important to note that in ALBI assay protein candidates on nitrocellulose membrane are in the denaturated state that may cause hindrance in protein–protein interactions. Thus, protein interactions were also performed in their native state with co-precipitation assay; nevertheless, results obtained were consistent to those obtained previously (Fig. 2b).

3.2. *Interactions between BbCRASP-1 and sushi 19–20 domains of animals and human*

Interestingly, sushi 19–20 of the sheep showed interaction with BbCRASP-1 in both ALBI and co-precipitation assays (Fig. 2). Binding ability of the sushi 19–20 of human fH to

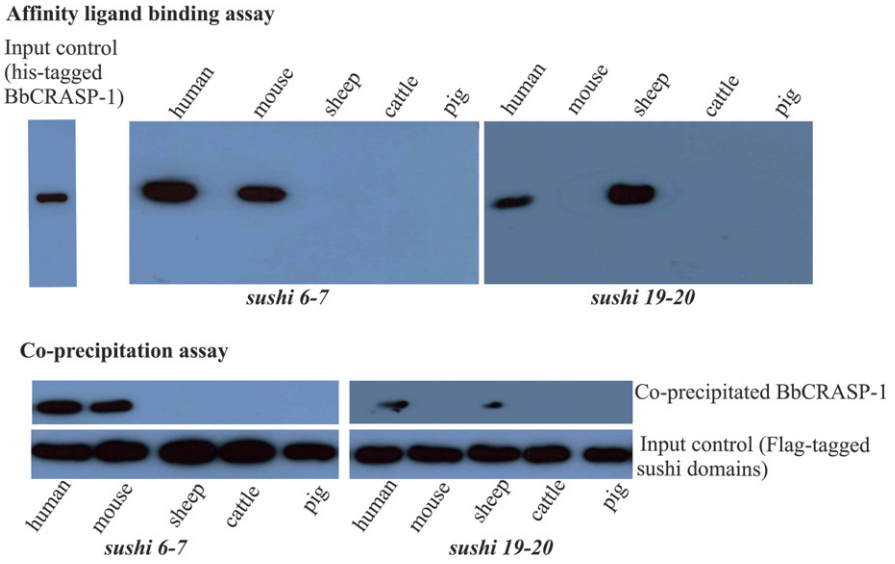

Fig. 2 – Binding of BbCRASP-1 to sushi domains of mouse, sheep, cattle, pig and human. a. ALBI assay; b. co-precipitation assay.

BbCRASP-1 was also observed; however this interaction might be weaker than BbCRASP-1:sushi 6–7. Interaction between sushi domains 19–20 of cattle, pig and mouse failed to show any interaction with BaCRASP-1.

3.3. *Prediction of residues involved in the formation of hydrogen bonds and salt bridges in BbCRASP-1:sushi 6–7 and BbCRASP-1:sushi 19–20 interfaces*

Residues having potential to form hydrogen bond and salt bridges were either predicted in-silico or data from previous studies [14,15] were retrieved. All together, in sushi 6–7 of human fH, data revealed that amino acid residues HENMR in sushi 6:$_{335–346}$, KYYSYYCDE in sushi 6:$_{349–361}$, SGSYWDH in sushi 6:$_{363–374}$ and HGRK in sushi 7:$_{399–410}$ possess potential to form hydrogen bonds (bold letters). Three amino acid residues (underlined) involved in salt bridge formation were predicted in sushi 6: arginin, lysin and histidin (Fig. 3). In sushi 19–20 of human fH, tyrosine in sushi 19:$_{1120–1138}$, tyrosine and alanine in sushi 20:$_{1174–1191}$, five residues (in bold letters, ASSVEYQCQNLYQLE) in sushi 19:$_{1141–1155}$, tyrosine and serine in sushi 20:$_{1205–1227}$ (GYRLSSR), and tyrosine and alanine in sushi 20:$_{1221–1241}$ (EYPTCA) showed potential to form hydrogen bond (Fig. 3). Hydrogen bonds and salt bridges play a central role in protein–protein interaction, as they are the major contributors in the electrostatic interactions between proteins.

To probe further the underlying principles of disparity in the interactions between BbCRASP-1 and sushi domains from different host species, amino acid sequences of sushi 6–7 and sushi 19–20 from human and animals were aligned (Fig. 4). Alignment of sushi 6:$_{335–346}$ revealed the absence of amino acid residues at positions 340 and 341 in sheep, cattle and pig. In our in silico analysis methionine at 340 was found as an important residue for the formation of hydrogen bond, while arginine at 341 was critical for salt bridge formation between ligand–fH interface. Such indel polymorphisms were not found in mouse sushi 6:$_{335–346}$, however methionine$_{340}$ was changed to serine and arginine$_{341}$ was replaced by leucine. Apart from these two changes, polymorphism in other hydrogen bond forming residues was observed in sushi 6:$_{335–346}$ (histidine to tyrosine and asparagine to glutamic acid), sushi 6:$_{349–361}$ (tyrosine to lysine and glutamic acid to asparagine), sushi 6:$_{363–374}$ (serine to tyrosine and tyrosine to serine) and sushi 7:$_{399–410}$ (histidine to tryptophan, glycine to glutamic acid and lysine to valine). Two salt bridge formation sites, lysine in sushi:$_{349–361}$ and histidine in sushi 6:$_{363–374}$, also showed polymorphism in murine sushi 6 (Figs. 3 and 4).

Changes in the amino acids, crucial in the formation of hydrogen bond, observed in ovine sushi 19–20 were: alanine$_{1141}$ to glycine and glutamic acid$_{1155}$ to glutamine in sushi 19, while tyrosine$_{1187}$ to histidine, alanine$_{1190}$ to glutamine, serine$_{1218}$ to arginine and alanine$_{1241}$ to glycine in sushi 20.

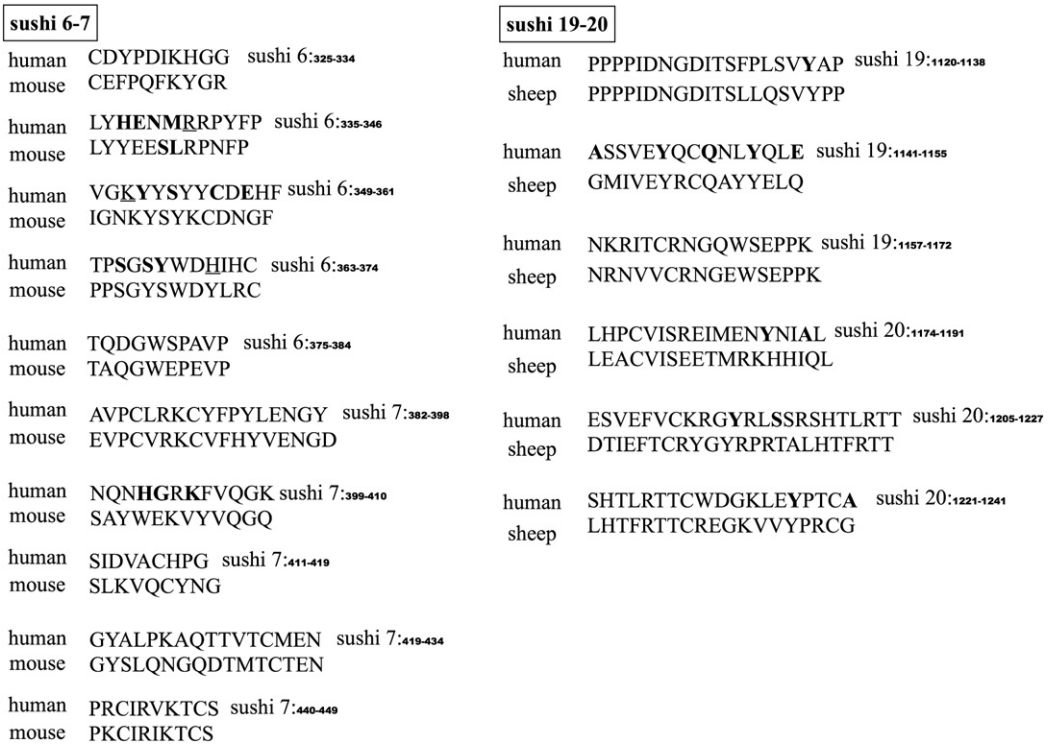

Fig. 3 – Peptide used in the peptide-spot assay. Peptides were designated based on their location in sushi domain and amino-acid stretch (e.g. sushi 6:$_{325–334}$). Residues in bold letter are with potential to form hydrogen bond in protein–protein interaction. Residues involved in formation of salt bridges are underlined (both prediction were based on data mining and bioinformatics tools).

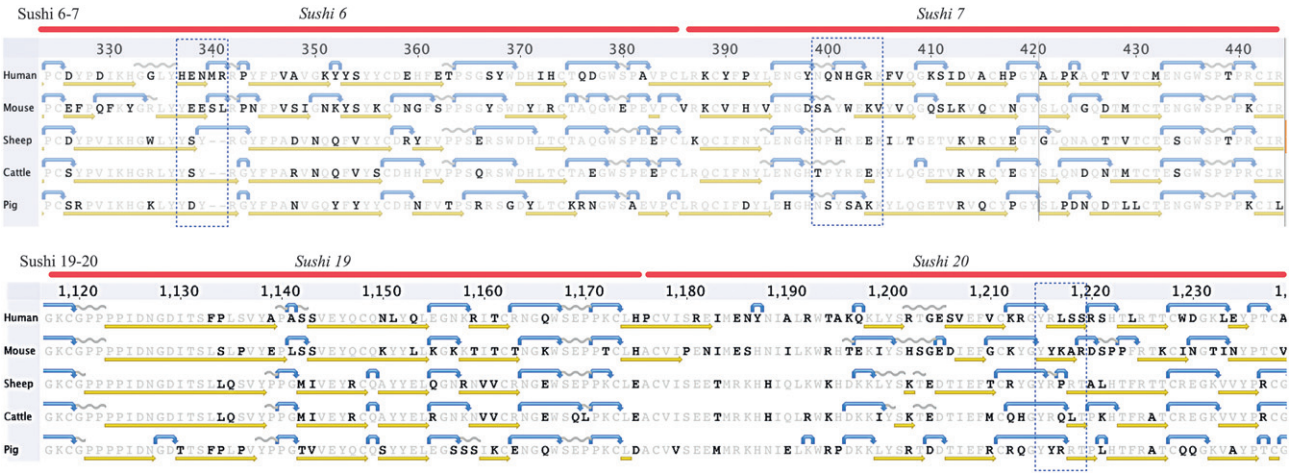

Fig. 4 – Amino acid sequence alignment of sushi domains of fH from animals and human. Squares with dashed line indicate important BbCRASP-1 binding sites. Secondary structure was predicted (Garnier server, http://emboss.sourceforge.net/) as a part of comparative in silico analysis: beta strands — yellow arrows; turns — blue arrows, coils — gray coiled lines.

3.4. *Experimental mapping of the residues involved in BbCRASP-1 and sushi interfaces*

To map the residual stretch involved in the ligand–receptor interaction, peptide-spot assay was performed with the peptides depicted Fig. 3. Only sushi domains of species, which interacted with BbCRASP-1, were tested by peptide-spot assays: human and mouse sushi 6–7 and human and sheep for sushi 19–20.

Results of peptide-spot assay clearly showed that both sushi 6 and sushi 7 possess BbCRASP-1 binding pockets, while amino acid residue stretch 335 to 346 (sushi 6:$_{335–346}$) is an important ligand binding site (interpretation is based on strongest binding affinity observed to BbCRASP-1, Fig. 5). Sushi 6:$_{335–346}$ of mouse was also interacted with BbCRASP-1. Another binding pocket in human sushi 6–7 was sushi 7:$_{399–410}$, however binding affinity of this pocket was weaker than sushi 6:$_{335–346}$. Murine sushi 7:$_{399–410}$ showed lack of BbCRASP-1 binding affinity. Change in amino acid residues that possess potential to form hydrogen bond (histidine to tryptophan, glycine to glutamic acid and lysine to valine) might be the probable cause of differential affinity of human and murine sushi 7:$_{399–410}$ to BbCRASP-1 (Figs. 3 and 5).

In case of sushi 19–20, only sushi 20:$_{1205–1227}$ of human and sheep showed affinity to BbCRASP-1. Moreover, binding affinity between BbCRASP-1 and Sushi 20:$_{1205–1227}$ was weaker than in BbCRASP-1:sushi 6:$_{335–346}$ complex (Fig. 5), which may suggests that sushi 19–20 might not be the primary binding site for BbCRASP-1. In contrast, since sushi 6–7 of sheep showed no binding with BbCRASP-1, Sushi 20:$_{1205–1227}$ might be the only site involved in ovine fH:BbCRASP-1 interface formation.

4. Discussion

To date, majority of the studies are focused on the interaction between human factor H and FHBPs of *Borrelia* [5,16–18]. BbCRASP-1 protein, a major candidate in the FHBP family, has been linked with various factors like virulence [19], resistance of *B.b.s.s.* against complement attack [5,19–22], host specificity of *B.b.s.s.* [3] etc. We and others have reported previously, that BbCRASP-1 binds strongly with full length fH protein from human, mouse, sheep, but not with fH from cattle and pig [3,5,21,23]. Reports show that cattle and pig are not suitable hosts for Lyme disease related borreliae like *B.b.s.s* [1,3,42,43]. Thus binding of BbCRASP-1 to fH correlates with ability of *B.b.s.s* to infect human, mouse and sheep, but not cattle and pig.

Hitherto, binding site for human fH on BbCRASP-1 molecule has been described [23,24], however, scanty reports are available, if at all, which show binding pockets for FHBPs present on fH protein from various hosts. Study of comparative binding ability of FHBPs to various domains of fH (like sushi 6–7 and sushi 19–20), depending on the host species, might be the promising way to understand fundaments of FHBPs:fH interaction. Moreover, comparative analysis of binding of BbCRASP-1 to sushi 6–7 and/or sushi 19–20 from various hosts (susceptible to borrelial infection vs. resistant) can serve as a model for study of FHBP:fH interactions for other pathogens.

Previously it was found that the sushi 6–7:FHBP (from *Neisseria*) complex is held together by extensive interactions between both ß-barrels of FHBP and sushi 6, and with more minor strength with sushi 7 [14]. Peptide-spot assay in our study also showed that binding affinity in sushi 6:$_{335–346}$:BbCRASP-1 complex was stronger than in sushi 7:$_{399–410}$:BbCRASP-1. In another study [5] binding pockets in BbCRASP-1 and human fH were experimentally assessed in which authors found interaction of BbCRASP-1 with peptides YNQNHGRKFVQGKSID (identical to our core residues of sushi 7:$_{399–410}$) and KRGYRLSSRSHTLRTT (identical to our core residues of sushi 20:$_{1205–1227}$). Authors further reported that binding affinity of former peptide was evidently stronger than peptide from sushi 20, while mere sushi 6 was not assessed in their work [5]. We found similar differences among affinity of peptides from sushi 7 and sushi 20 to BbCRASP-1. Strong interaction between sushi 6:$_{335–346}$:BbCRASP-1, observed in our study, suggests that

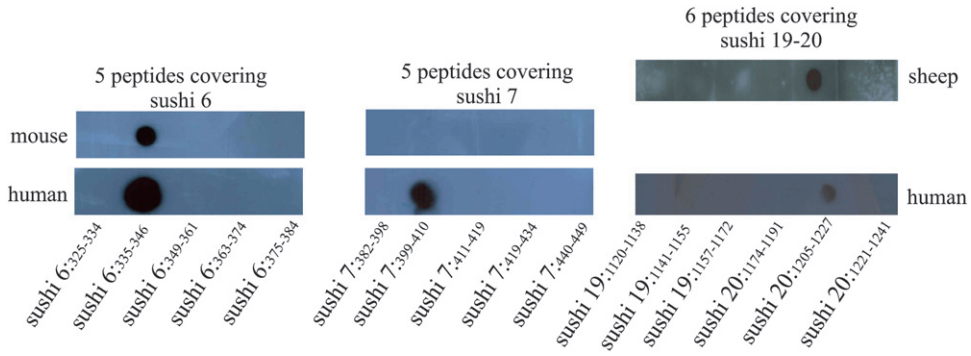

Fig. 5 – Mapping of BbCRASP-1:fH interface with peptide-spot assay.

residues in sushi 6:$_{335–346}$ are crucial for rigid protein complex formation. Alignment of the amino acid residues in this pocket from human, mouse, sheep, cattle and pig fH exposed that sushi 6:$_{335–346}$ of cattle, pig and sheep lack two major residues: methionine at 340 (important for the formation of hydrogen bond) and arginine at 341 (critical for salt bridge formation). Absence of these residues seems to be the most probable reason why sushi 6–7 of cattle, sheep and pig were unable to bind BbCRASP-1. In case of mouse sushi 6:$_{335–346}$ these two amino acids were replaced by serine and leucin, which although not abolished interaction with BbCRASP-1, but reduced strength of complex formation (Fig. 5). Lack of the interaction between mouse sushi 7:$_{399–410}$ and BbCRASP-1 suggests that complex formation between mouse fH and BbCRASP-1 is mediated only through sushi 6. In case of sheep fH, sushi 20:$_{1205–1227}$ was the only peptide, which showed affinity to BbCRASP-1. When sushi 20:$_{1205–1227}$ from human and sheep was compared for differences between amino acid residues, we found that hydrophilic residue serine$_{1218}$ (hydropathy index −0.8. It is also involved in the formation of hydrogen bond Fig. 4) in human was replaced by strong hydrophilic arginine (hydropathy index −4.5) in sheep. Strong hydrophilic pocket in sheep sushi 20:$_{1205–1227}$ might be the cause of higher affinity between this peptide and BbCRASP-1 (Fig. 5). The hydrophilic effect, total charge in the protein–protein interface, hydrogen bonds and salt bridges all play crucial roles in stability of the protein complex. Hydrogen bonds and salt bridges are principally essential in determining binding specificity [25,26]. A hydrogen bond and/or salt bridge provide favorable free energy to the binding [27–29]. Hydrogen bonds, C–H…π with π-acceptor establishes another considerable fraction. C–H…π interactions have been described in the formation of proteins–ligands complexes [30,31]. Previous work [32] has described hydrogen bonds in proteins involving aromatic acceptors, while elsewhere [33] occurrence of interactions involving all possible C–H groups (C^{α}, $C_{aliphatic}$–H and $C_{aromatic}$–H) as donors and all possible side chain π systems as acceptors are described.

Many domestic animals are susceptible to *B. burgdorferi* infection, often exhibiting symptoms similar to those of Lyme disease in humans [34–37]. In agricultural animals, symptoms of Lyme disease, such as weight loss and decreased milk production, can have significant financial impact. Previous studies have reported that *Borrelia* can infect sheep [36–41]. Serum complement sensitivity assay in our previous study [1] revealed significant resistance of *B.b.s.s.* to sheep complement, which correlates with ability of *B.b.s.s.* to bind fH (mediated through sushi 20 as observed in this study) and cause infection in sheep.

5. Conclusion

Numbers of human and animal pathogens are found to express FHBPs such as pneumococcal PspC and Hic proteins [44–47], streptococcal M and Fba [48–50]; neisserial Por1A protein [51] and ~58 kDa protein of *B. coriaceae* (causes bovine abortion) [3]. Comparative studies, like the presented here, are essential to unfold interactions between FHBPs from various pathogens and fH different animals and human. Comparative amino acid sequence and structural analysis of fH from wide range of animal species will not only help to understand basic principles of pathogen:fH interactions, but also shade a light on the fH related diseases like glomerulonephritis in human and pig, atypical hemolytic uremic syndrome, uncontrolled activation of the alternative pathway in animals etc. In summary, natural diversities found in the amino acid sequences and protein structures among different animals and human can be used as effective tool to probe novel ligand–receptor interactions.

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