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Structure of decorin binding protein B from *Borrelia burgdorferi* and its interactions with glycosaminoglycans

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ABSTRACT

Decorin-binding proteins (DBPs), DBPA and DBPB, are surface lipoproteins on *Borrelia burgdorferi*, the causative agent of Lyme disease. DBPs bind to the connective tissue proteoglycan decorin and facilitate tissue colonization by the bacterium. Although structural and biochemical properties of DBPA are well understood, little is known about DBPB. In current work, we determined the solution structure of DBPB from strain B31 of *B. burgdorferi* and characterized its interactions with glycosaminoglycans (GAGs). Our structure shows that DBPB adopts the same topology as DBPA, but possesses a much shorter terminal helix, resulting in a longer unstructured C-terminal tail, which is also rich in basic amino acids. Characterization of DBPB–GAG interactions reveals that, despite similar GAG affinities of DBPA and DBPB, the primary GAG-binding sites in DBPB are different from DBPA. In particular, our results indicate that lysines in the C-terminus of DBPB are vital to DBPB's ability to bind GAGs whereas C-terminal tail for DBPA from strain B31 only plays a minor role in facilitating GAG bindings. Furthermore, the traditional GAG-binding pocket important to DBPA–GAG interactions is only secondary to DBPB's GAG-binding ability.

1. Introduction

Borrelia burgdorferi is the causative agent of Lyme disease, which is the most prevalent vector-borne disease in North America. As an extracellular bacterium, *B. burgdorferi* relies almost entirely on host cells for nutrients. Because of its parasitic life cycle, *B. burgdorferi* has developed many strategies for adhering to and evading detection by the host. Many of the proteins involved in promoting the adhesion of the bacteria to the host cells have shown to be important to the virulence of the bacteria [1,2]. Understanding the mechanisms of these virulent factors is therefore an important aspect in tackling *B. burgdorferi* infection.

One of the *B. burgdorferi* adhesins identified is decorin binding protein (DBP), a cell surface lipoprotein that is expressed during the mammalian infection stage [3]. Two homologous forms of DBP, termed DBPA and DBPB, exist in the *B. burgdorferi* genome. Both are lipoproteins of approximately 20 kDa in size, and they share ~40% sequence identity. Genetic studies of the two isoforms show that both are important for the bacteria during early stages of infection [4–6]. Although the two isoforms can compensate one another to a limited extent, the absence of either one can produce defects in joint colonization and DBPB overexpression also inhibits proper dissemination of the bacterium [7,8]. Interestingly, DBPA shows high sequence diversity among different strains of *Borrelia* bacteria, while DBPB sequence is well conserved [9–11].

DBPs facilitate bacterial colonization by adhering to proteoglycans in the extracellular matrix (ECM) and on cell surfaces. The ECM proteoglycan decorin is a particular important target for DBPs [3,12], and the glycosaminoglycan (GAG) portion of decorin is a major binding site for the DBPs [13–15]. GAGs are sulfated linear polysaccharides composed of repeating disaccharide units of uronic acids and amino sugars [16]. Because of their high sulfation density and large size, GAGs have strong interactions with a number of extracellular proteins via electrostatic interactions. This enables them to act as receptors for signaling proteins and microbes. Although the GAG chains found in decorin are either chondroitin sulfate (CS) or dermatan sulfate (DS), both of which contain N-acetylgalactosamine (GalNAc), DBPs are also known to interact with other GAG types including heparin and heparan sulfate (HS), both of which contain glucosamine instead of GalNAc. In fact, DBPA's affinity for heparin is significantly higher than its affinity for DS [9,13,14,17]. The core protein of decorin is also suspected to play a role in facilitating the interactions between decorin and DBPs [12,13]. However, there is yet no evidence of direct interactions between the decorin core protein and DBPs.

Although DBPA has been extensively studied functionally and structurally [10,13,18,19], very little information is available on DBPB. The lack of information is curious considering that one reason for the interest in DBPs is their potential as vaccine components. However, the high genetic diversity of DBPA means that a single vaccine may not be sufficient to elicit immunity against all strains of the bacterium. In this respect, DBPB, whose sequence is well conserved among different strains, may be a better candidate for vaccine development. In fact,

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antibody against DBPB has been one of the most common antibodies found in sera of humans infected with the bacterium [20].

We have determined the solution structure of DBPB from strain B31 of *B. burgdorferi* using solution NMR and characterized its interactions with GAGs. The structure of DBPB is homologous to the known DBPA structures. In particular, it is composed of five helices with an unstructured linker between helices one and two as well as a flexible C-terminal tail. However, the C-terminal helix of DBPB is considerably shorter than the helix in DBPA, resulting in a longer unstructured C-terminal tail that is enriched in basic amino acids. Characterizations of DBPB–GAG interactions showed that DBPB has similar GAG affinities as DBPA, but possesses different binding sites than DBPA. In particular, although some of the lysine residues deemed important to DBPA's affinity for GAGs are also conserved in DBPB, the most important GAG-binding site in DBPB is its lysine-rich C-terminus, the elimination of which reduced the GAG affinity of DBPB significantly. These results indicate that DBPB may be as important in facilitating bacterial adhesion as the well-studied DBPA.

2. Material and methods

2.1. Expression and purification of B31 DBPB

The open reading frame (ORF) of the wild type (WT) B31 DBPB (residues 21–187) was synthesized by GenScript Inc. (Piscataway, NJ) and cloned into the pHUE vector with ORF of His-tagged ubiquitin at the 5′ end [21]. Residue C21, which acts as the lipid anchor *in vivo*, was mutated to serine to prevent dimerization [3]. To construct DBPB mutants, the following forward primers were designed: K65S/K69S, 5′-GCGTTCACCGGCCTGAGCACGGGTAGCAGCGTTACCTCTGG-3′; R78S/K81S, 5′-GGCGGTCTGGCCCTGAGCGAAGCAAGCGTGCAGGCGATTG-3′; K81S, 5′-GGCCCTGCGCGAAGCAAGCGTGCAGGCGATTGTG-3′; K169S, 5′-GAAAGTGGTTAAAGAAAGCCAGAACATCGAAAACGG-3′; ^{184}SSSS^{187}, 5′-GGGCTCCGCGGTGGATCGAGC-3′; and DBPB$_{21–183}$, 5′-GAAAAACAACAAAAGCTAAAAGAAAAAATGAAAG-3′. The reverse primers were designed: K65S/K69S, 5′-CCAGAGGTAACGCTGCTACCCGTGCTCAGGCCGGTGAACGC-3′; R78S/K81S, 5′-CAATCGCCTGCACGCTTGCTTCGCTCAGGGCCAGACCGCC-3′; K81S, 5′-CAACAATCGCCTGCACGCTTGCTTCGCGCAGGGCC-3′; K169S, 5′-CCGTTTTCGATGTTCTGGCTTTCTTTAACCACTTTC-3′; ^{184}SSSS^{187} 5′-GGGAAGCTTTCAGCTGCTGCTGCTGCTTTTGTTGTTTTT-3′; and DBPB$_{21–183}$, 5′-CTTTCATTTTTTCTTTTAGCTTTTGTTGTTTTTC-3′. The mutagenesis was done with the Agilent Quickchange site-directed mutagenesis kit according to the manufacturer's protocol, and confirmed by sequencing.

2.2. Escherichia coli

BL21 (DE3) cells transformed with the expression vectors were grown in an M9 medium at 37 °C to an OD_{600} of 0.8. The cells were then induced with 0.5 mM IPTG before overnight incubation at 30 °C. $^{15}NH_4Cl$ and/or ^{13}C glucose were added into an M9 medium for desired isotopic labeling. After cell harvesting by centrifugation, the resuspended cells were treated with 1 mg/mL lysozyme for 20 min and lysed via sonication. After centrifugation, the supernatant was subjected to Ni-affinity chromatography with a 5 mL HisTrap column (GE Life Sciences). The bound DBPB was eluted from the column by applying an imidazole gradient of 35 to 500 mM at a flow rate of 3 mL/min. After exchanging the pooled protein into 25 mM Tris and 100 mM NaCl buffer (pH 8.0), the fusion protein was cleaved with 1/20 molar equivalent of USP2 (deubiquitinase) overnight at room temperature [21]. Another Ni-affinity chromatography was applied to separate cleaved DBPB from His-tagged ubiquitin and His-tagged USP2.

2.3. Production of GAG fragments and TEMPO-labeled GAG fragments

Heparin and DS from Sigma-Aldrich were partially depolymerized using heparinase I (IBEX Inc.) and chondroitinase ABC (Sigma-Aldrich), respectively [22,23]. Digested fragments were separated based on size with a 2.5 cm × 175 cm size exclusion chromatography column (Bio-Rad Biogel P10) at a flow rate of 0.2 mL/min. Fractions containing fragments of the same size were pooled, desalted, and lyophilized. For paramagnetic relaxation enhancement (PRE) studies, DS dodecasaccharide, or dp12 (degree of polymerization 12) fragments were paramagnetically labeled by modifying the reducing end with the nitroxide radical, 4-amino-TEMPO, through reductive amination [19]. Specifically, 300 μM TEMPO was mixed with 1 mg of GAG fragments and 25 mM $NaCNBH_3$, and incubated at 65 °C for three days. After desalting, labeled fragments were further purified using SAX-HPLC.

2.4. Acquisition and analysis of NMR data for DBPB structure and backbone dynamics

NMR experiments were conducted on Bruker Ultra-Shield 600 MHz and Varian Inova 800 MHz spectrometers. Most of the pulse sequences were provided by the manufacturer. For backbone assignment, HNCACB, HNCOCACB, HNCO, and HNCOCA spectra were acquired for ^{13}C- and ^{15}N-labeled DBPB. To determine DBPB structure, ^{15}N- and ^{13}C-edited NOESY-HSQC spectra were obtained for ^{13}C- and ^{15}N-labeled DBPB. Methyl group assignments were made with the methyl HCCH-TOCSY experiments [24] while side chain proton assignments were made using a combination of HCCH-TOCSY, HCCONH and ^{13}C-edited NOESY-HSQC. HN and NC residual dipolar couplings (RDCs) were measured with DBPB aligned in a 7% neutral polyacrylamide gel using J-modulated pulse sequences [25]. NMR samples contain 100–600 μM of ^{13}C- and/or ^{15}N-labeled DBPB in 50 mM NaH_2PO_4 and 150 mM NaCl buffer (pH 6.5). All NMR data were processed with NMRPipe [26] and analyzed using NMRView [27].

For PRE studies, 400 uL of 150 μM WT ^{15}N-labeled DBPB was mixed with 8 molar equivalents of TEMPO-labeled DS dp12. PRE effect arising from the TEMPO-labeled fragments was estimated by collecting a $^{1}H–^{15}N$ HSQC spectrum before and after the radical was reduced by adding 3 μL of 1 M ascorbic acid [28].

To investigate the effects of GAG-binding on backbone mobility, backbone nitrogen T_1, T_2, and steady state heteronuclear nuclear Overhauser effect (NOE) were measured for WT ^{15}N-labeled DBPB with or without 10 molar equivalents of heparin dp10. Relaxation delays for longitudinal relaxation (T_1) and transverse relaxation (T_2) experiments were 0.1, 0.25, 0.4, 0.6, 0.8, 1.0 s and 10, 20, 40, 60, 80, 100 ms, respectively. Steady state heteronuclear NOE was extracted by calculating peak intensity ratios of spectra collected with or without proton saturation of 3 s. The order parameter S^2 was calculated with the program relax [29] using the isotropic global rotational diffusion model. The global rotational correlation time, τ_m, was approximated as the average rotational correlation times of all structured residues. The residue-specific correlation times (τ_c) were determined according to the method of Kay et al.[30]. Specifically, τ_c is estimated using the equation: $\tau_c = 1/(4\pi\nu_N) \times [6(T_1/T_2) - 7]^{1/2}$, in which ν_N is the resonance frequency of ^{15}N in Hz. DS-induced millisecond time scale conformational exchange was measured on a sample containing 300 μM ^{15}N-labeled DBPB and 3 mM DS dp10 using the CPMG-based relaxation dispersion experiment designed by Tollinger et al.[31]. The R_2 values were extracted by conducting two-point transverse relaxation measurements at relaxation delays of 5 and 50 ms. The field strength was varied from 10 to 210 Hz. The exchange component of the relaxation was estimated as the difference in R_2 values at field strengths of 10 and 210 Hz.

2.5. Structure calculation

Backbone dihedral angles of well-ordered residues were determined with the online server TALOS + [32]. ^{13}C- and ^{15}N-edited NOESY-HSQC spectra were analyzed manually to find unambiguous long-range contacts. The partially assigned peak lists were then used as input for CYANA's automatic structure determination procedure [33]. The