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( <http://nslsweb.nsls.bnl.gov> )

### Crystal Clear View of a Lyme Vaccine

In the past few years, our molecular understanding of the mysterious, wavy bacterium that causes Lyme disease has grown tremendously. First identified in 1982, *Borrelia burgdorferi* has been present on Long Island for at least the past century, and pathogenic strains exist worldwide. The organism, a spirochete, exists in the wild and is spread by transmission between a tiny invertebrate arthropod, the *Ixodes* tick, and its natural animal hosts, which include small mammals, birds, and deer. Human infection, with its characteristic bull's-eye rash, fever, and subsequent (if untreated) arthritic, neurological, and/or cardiac symptoms, can result from prolonged attachment of an often unseen infected tick.

Study of the Lyme bacterium has been hampered by poor growth of the organism in culture. Nonetheless, we are beginning to understand how it evades immune attack and is able to transfer between and survive in both tick and animal hosts. The key to the organism's stealth and mobility appears to lie in its ability to change its surface to suit its current environment. When the spirochete migrates between different tissues of the tick or animal, it will alter the composition of its predominant surface-exposed proteins.

Outer surface protein A (OspA) is a 31 kDa lipid-modified protein that was discovered soon after *B. burgdorferi* was isolated. Plentiful in cultured organisms, OspA can also be detected by immunochemical techniques in infected ticks. It was therefore a mystery why antibodies against OspA are usually not found in Lyme disease patients. It was subsequently discovered that a slight temperature increase will cause cultured organisms to stop production of OspA and begin production of a completely different surface protein, OspC. A similar phenomenon was confirmed to occur in the feeding tick: spirochetes migrating toward the warm skin of the mammalian host lose their OspA and begin to express OspC. Since that time, variable expression of a number of other surface proteins has been documented, and sequencing of the entire *B. burgdorferi* genome has revealed the presence of about one-hundred other genes encoding proteins predicted to be targeted to the cell surface. One such gene, called VlsE, can mutate rapidly in a mammalian host so the protein it encodes may play a critical role in evasion of immune attack.

Despite its scarcity on bacteria within the infected animal host, OspA, either isolated from bacteria or produced recombinantly, can act as a protective vaccine. Remarkably, although OspA-generated antibodies cannot stop an established infection, they will kill spirochetes within a feeding tick. The effort to test OspA as a first-generation vaccine against Lyme disease has recently culminated in side-by-side reports in the **New England Journal of Medicine** showing that two OspA-based

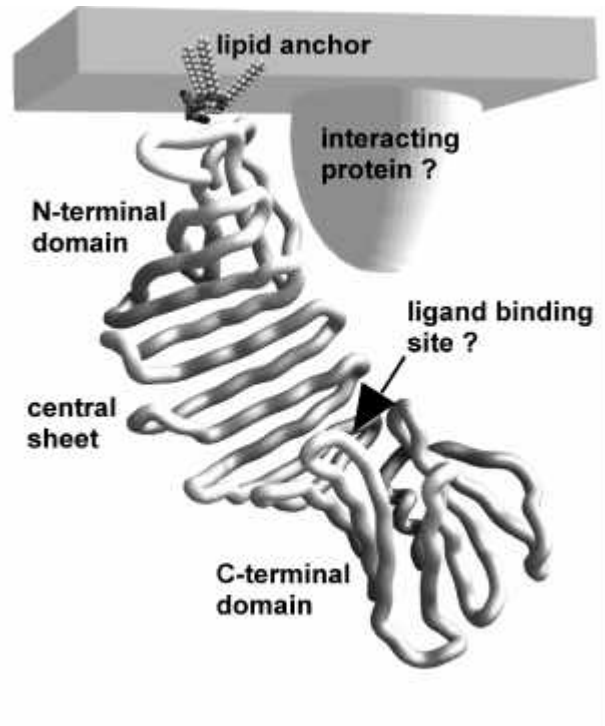


Figure 1. Anatomy of Lyme vaccine candidate OspA.

vaccines are effective in protecting humans.

Before granting approval for general use, the FDA will have to consider several unusual features of these vaccines, which will have both positive and negative impacts on their ultimate utility. Because the gene encoding OspA is not under pressure to evolve within the environment of a stringent immune system, its regional variability is low, and breakthrough mutations are therefore unlikely (though in Europe, several antigenically distinct strains are present, so migration, e.g., through bird hosts, could be a problem). However, because spirochete killing takes place only in the tick, OspA-based vaccines are ineffective after infection is established. Traditional vaccines depend on immune memory (the so-called anamnestic response) to quickly augment low antibody levels. This type of response simply will not occur in an OspA-vaccinated individual, so it is likely that frequent boosters will be necessary to maintain protection.

In the past several years we have performed diffraction experiments on crystals of two OspA/antibody fragment complexes at the NSLS that not only have allowed us to determine the overall fold of OspA, but also have permitted definition of non-protective and protective epitopes (the  $\sim 800\text{-}1200 \text{ \AA}^2$  surface of a protein antigen that is recognized by an antibody). The formation of crystals suitable for diffraction resulted from a dedicated collaborative effort between BNL and SUNY Stony Brook, aided by support from BNL, NIH, and DOE. The information gleaned from these crystal structures is now

being employed in the design of improved vaccines that incorporate mainly protective regions, and diagnostics that can measure protective antibody levels directly.

Our first atomic view of this vaccine candidate (Fig. 1) came about when we determined the crystal structure of a soluble unlipidated form of OspA in a complex with a fragment of a nonprotective antibody. Interpretable electron density maps were obtained by combining phases from a molecular replacement solution for the antibody fragment with conventional multiple isomorphous replacement phases using native, platinum, and iodine derivative data collected at X12-C, one of two bending-magnet beamlines operated by the BNL Biology Department for protein crystallography. The structure was subsequently refined against high resolution native data collected on X25, the NSLS-run wiggler beamline that is being used increasingly for crystallographic study of biomolecules.

Like many membrane-anchored proteins, OspA is elongated, stretching 80 Å from tip-to-tip. OspA consists of 21 antiparallel β-strands that fold hairpin-style into four antiparallel β-sheets, plus a single C-terminal α-helix. This unique fold appears to have evolved from 11 repetitions of a basic 25-residue β-hairpin element. Surprisingly, NMR analysis by our University of Rochester collaborators has indicated that the unusual region of "freestanding" β-sheet connecting globular N- and C-terminal domains has stability equivalent to the interior of a globular protein. High stability may derive in part from unusual arrays of alternating charges along the length of the sheet, along with extensive van der Waals contacts between side-chain atoms.

The nonprotective antibody fragment used to crystallize OspA binds to a remarkably well-conserved surface near the N-terminus. The mature N-terminal cysteine residue is the site of triacyl lipid modification that provides a membrane anchor for the otherwise highly soluble protein. The proximity of the conserved surface to the lipid anchor, along with its inaccessibility in the intact spirochete, suggests that it may contact other proteins on the membrane surface.

We have recently determined the structure of a second OspA/antibody fragment complex, this time derived from a monoclonal antibody, called LA-2, that will recognize and kill *B. burgdorferi*. In fact, vaccine effectiveness strongly correlates with the ability of an individual's antibodies to interfere with LA-2 binding. LA-2 recognizes the three loops at the C-terminal end of OspA, opposite the end predicted to be anchored to the membrane. This structure therefore confirms the hypothesis that the C-terminal end of the molecule is accessible on the intact spirochete, and identifies key variable residues that need to be considered when formulating vaccines for world-wide use.

A cellular function for OspA has not yet been identified, and the complex architecture and dynamics of the cellular surfaces on which OspA reside are still not well

understood. Spirochetes are gram-negative bacteria with inner and outer membranes, and they are distinguished by the fact that the rotating flagellar bundles that propel them forward are buried in the intervening periplasmic space. Interestingly, although OspA is readily detected on the outer membrane of cultured organisms, it appears to be even more abundant in the periplasm, presumably attached to the inner membrane. It is not known how OspA gets to the outer membrane, or whether cycling of OspA occurs between inner and outer membranes.

Characteristic of an obligate pathogen, the genome of *B. burgdorferi* encodes an extremely limited repertoire of enzymes for biosynthesis. Its survival therefore depends heavily on transport of premade components into the cell. The relatively flexible C-terminal domain of OspA possesses a highly conserved region that includes a trio of partially buried charged residues and a small hydrophobic cavity within a pronounced cleft. These features strongly suggest that OspA can bind to a small molecular ligand of unknown identity, probably with negative charge. OspA might therefore have a functional role within a nutrient sensing or transport system.

In addition to utilizing the structural information we have gathered for OspA, we are now investigating the structures of *B. burgdorferi* proteins such as OspC and VlsE that are expressed within the tissues of infected mammals. Accurate structural models of these proteins will aid in the design of improved vaccines that will be able to provide protection in an infected individual. We are also likely to uncover structural features that underlie *B. burgdorferi*'s ability to evade destruction in its mammalian hosts.

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