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BNL-62484

BIOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION OF THE MAJOR OUTER SURFACE PROTEIN, OSP-A FROM NORTH AMERICAN AND EUROPEAN ISOLATES OF BORRELIA BURGDORFERI.

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CHARACTERIZATION OF THE OUTER SURFACE PROTEINS OF BORRELIA BURGDORFERI

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INTRODUCTION

Lyme borreliosis is the most common vector-borne disease in North America and Western Europe. It is caused by the spirochete *Borrelia burgdorferi*. In humans, the disease is a multisystem illness, with dermatologic, rheumatic, cardiac and neurologic manifestations. The immune response in humans is characterized by an early and persistent response to the flagellin protein, p41, and a significantly delayed response to the major outer surface lipoproteins OspA and OspB. Monoclonal antibodies against OspA as well as immune sera will transfer passive immunity to mice and other rodents. Mice immunized with a recombinant form of OspA are protected from infection from the strain of B. burgdorferi from which the OspA was derived.(Reviewed in Szczepanski and Benach, 1991) The epitopes which are recognized by human sera, as well as the protective epitopes, appear to be conformationally determined and localize to the carboxyl portion of the protein (Schubach et al 1991) As a result of this work, OspA has become a major candidate for a vaccine for Lyme Disease.

Using a panel of monoclonal antibodies against OspA, several unique serotypes of *B. burgdorferi* from North America and Western Europe were identified (Wilske et. al. 1991). These serotypes fall into three distinct phylogenetic lineages, or genotypes, on the basis of their DNA sequences and their reactivities with monoclonal antibodies. The extent of the variability among the groups was somewhat unexpected, as it has been shown that *B. burgdorferi* does not exhibit antigenic variation or switching of its outer surface proteins in the way its close relative *B. hermsii* does (Barbour et. al., 1985). In an effort to understand the basis of the variability of OspA on a structural and molecular level, we have cloned, sequenced and expressed a recombinant form of the protein

from each of the three phylogenetic groups. We are currently testing these proteins for cross-reactivity with several OspA monoclonal antibodies and mapping antigenically important epitopes. We are also using these proteins for fluorescence,circular dichroism,and protein crysallographic trials in an effort to understand the structural basis of OspA antigenicity.

CLONING OF OSPA

Genomic DNA from cultured clinical isolates of each of the variant *B.burgdorferi* strains was used as template for amplification of OspA sequences by polymerase chain reaction (PCR). Oligonucleotides homologous to the 5′ and 3′ ends of OspA from the standard lab strain B31 were used to prime the forward and reverse PCR syntheses. The resulting products were then cloned and sequenced using standard methods.

The OspA DNA sequences were then subjected to computer driven phylogenetic analysis using the PAUP program (Swofford, D.L., 1991). The resulting maximum parsimony tree (Figure 1) shows that the OspAs fall into three distinct lineages, each representing a unique genotype. Group I is comprised of B31 and KA, a clinical isolate which has an OspA identical to that of B31. Group II, the largest group, includes K48, DK29, Tro, and HE. Group III is comprised of PGau, PKO and BO. Preliminary experiments have demonstrated that it is possible to raise monoclonals which will cross-react between Groups I and II, however, none of the monoclonals raised against these two groups will cross-react with Group III. In an effort to simplify our analysis we decided to limit our structural studies to a single representative of each of the genotypes. Groups I, II and III are therefore represented by B31, K48 and PGau respectively.

The OspA DNA sequences were also used to obtain the deduced amino acid

sequences of each of the variant proteins. Figure 2 shows an alignment of the amino acids of B31, K48, and PGau. Both B31 and PGau OspA contain 273 amino acids, whereas K48 has 274. The N-termini of the proteins are remarkably conserved, and all have identical export and lipidation signals as well as the sites required for penicillin binding (Urban, C., et. al., 1990). The carboxyl termini of the proteins have significant variability, however, all have a single conserved tryptophan at amino acid 216. Previous epitope mapping experiments have demonstrated that the carboxyl portion of the protein is important for recognition by human sera, as well as monoclonal antibodies that agglutinate the spirochete (Schubach et. al. 1991)(Sears et. al. 1991). Furthermore, chemical cleavage of OspA at Trp-216 produces two fragments, neither of which react with agglutinating monoclonal antibodies on Western Blots. These results strongly suggest that the region of OspA around Trp-216 is conformationally determined, and is an antigenically important epitope of the OspA protein.

EXPRESSION

Overproduction of the variant OspAs was carried out in *E. coli* using a T7-based expression system (Dunn, et. al. 1990). The expressed OspAs, however, were not full length, but recombinant proteins which lack their export and lipidation signals. This form of the protein is known to be much more soluble than the full-length OspA, and can be accumulated to very high levels in *E. coli* (>150mg/l of culture), without apparent toxicity to the cell.

To obtain the appropriate clones, the OspA sequences were again amplified by PCR using forward primer designed to base-pair OspA DNA 17 codons in from the N-terminal methionine. The resulting PCR products therefore lack sequences encoding the first 17 amino acids of the full length proteins, which include the

sites for export and lipidation. The amplified DNA's were then cloned into a T7 expression vector, and transformed into an *E. coli* expression host as described in Dunn, et. al. 1990. The OspAs were then purified from induced *E. coli* cultures by ion-exchange chromatography and gel filtration. An SDS PAGE of the purified OspAs is shown in Figure 3.

CIRCULAR DICHROISM AND FLUORESCENCE

Circular dichroism and fluorescence studies were first carried out on the recombinant B31 OspA at port U9b at the National Synchrotron Light Source, Brookhaven National Laboratory, as previously described (France, et. al. 1992a and b). The CD results indicate that the B31 protein contains 11% alpha-helix, 34% antiparallel beta-sheet, 12% parallel beta-sheet. Primary sequence analysis predicts that amino acids 204-217 can form one of the few regions of alpha-helix within the protein. This region of OspA includes the agglutinating epitope at Trp-216. The fluorescence emission clearly shows that Trp-216 is buried in a hydrophobic region of the B31 OspA protein. This tryptophan becomes exposed to the solvent when the protein is denatured in 6M guanidine HCl. Taken together, these data support a model whereby the region of OspA between amino acids 204 and 217 forms an oriented alpha-helix. Within this structure, Trp-216 is buried in a hydrophobic pocket, which thereby exposes some of the more polar residues to the hydrophilic solvent.(Figure 4) This leads us to postulate that the antigenic variability within this region of OspA may be due to differences in the solvent exposed amino acids.

To pursue this further we have initiated CD studies on the K48 and PGau OspAs. The data shows that the K48 protein has slightly more alpha-helix (14%) than the B31 OspA, with slightly less antiparallel beta-sheet (28%). PGau,

however, has significantly more alpha-helix (28%) which coincides with a considerable drop in the antiparallel beta-sheet content (22%). Primary sequence analysis predicts that the gain in helix for both K48 and PGau does not occur at the epitope between amino acids 204 and 217, but at a more N-terminal region between residues 143 and 179. This region of OspA is also hypervariable among the genotypes, and may interact with the helical region around Trp-216 to make up the agglutinating epitope.

FUTURE DIRECTIONS

We are currently performing mutagenesis on the B31 OspA between amino acids 204 and 217 to see if replacement of this region with the analogous K48 sequences will result in loss of reactivity with the agglutinating monoclonal against B31, and acquisition of reactivity to a K48 agglutinating monoclonal. We are also continuing the structural studies including the fluorescence emission of the K48 and PGau OspAs to determine whether or not their tryptophans are also in a hydrophobic environment within the helical epitope. Crystallographic trials have also been initiated. Lastly, we are raising monoclonal antibodies to the recombinant OspA variants in order to determine whether the cross-reactivity patterns continue to parallel the division of the genotypes into three distinct groups.

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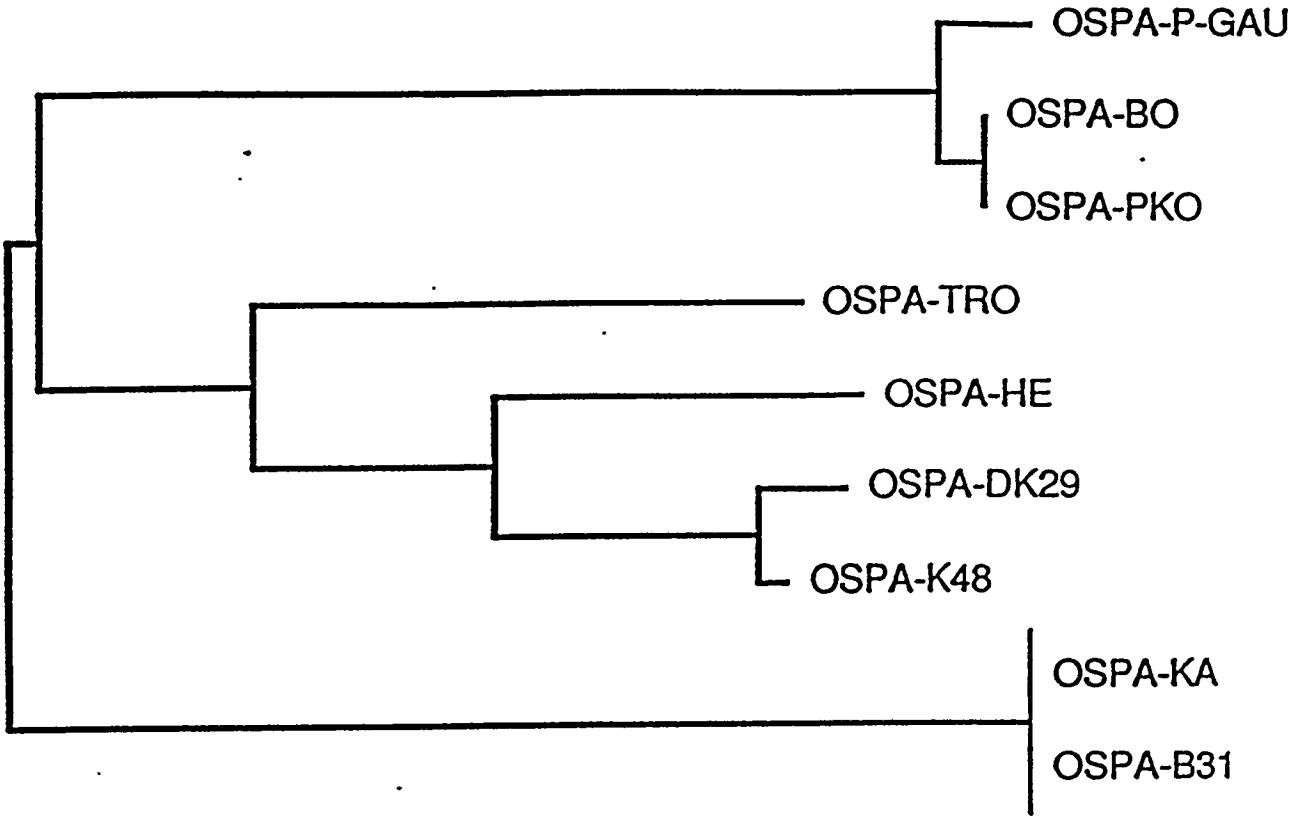
FIGURE LEGENDS

Figure 1. Phylogenetic tree of the OspA protein of *B. burgdorferi*. The branch lengths are proportional to the number of inferred character changes The tree is rooted by midpoint rooting.

Figure 2. Amino acid alignment of OspAs B31, K48 and PGau.

Figure 3. SDS PAGE of purified recombinant OspAs from variant strains of *B. burgdorferi*.

Figure 4. Helical wheel projection for residues 204-217. Capitals indicate hydrophobic residues; lowercase letters indicate hydrophilic residues; +/- indicate positively/negatively charged residues. dashed line indicates division of the alpha-helix into a hydrophobic arc (above the line) and a polar arc (below the line). (France et. al. 1992b)


OSPA-P-GAU
OSPA-BO
OSPA-PKO
OSPA-TRO
OSPA-HE
OSPA-DK29
OSPA-K48
OSPA-KA
OSPA-B31

Sequence Range: 1 to 274

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                5   10    15   20    25   30    35   40    45   50    55   60    65   70    75   80    85   90    95  100
                *   *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
A-B31       MKKYLLGIGL ILALIACKQN VSSLDEKNSV SVDLPGEMKV LVSKEKNKDG KYDLIATVDK LELKGTSDKN NGSGVLEGVK ADKSKVKLTI SDDLGQTTLE

P-K48              10         20         30         40         50         60         70         80         90         100
[  998 ]    .......... .......... .......... ......g.t. ......d... ..s.e..... .......... ....t...e. t......... a...s..kf.>

P-PGAU             10         20         30         40         50         60         70         80         90         100
[  981 ]    .......... .......... .........a .......... ......d... ..s.k..... i........d ........t. d....a.... a...sk..f.>

              105  110   115  120   125  130   135  140   145  150   155  160   165  170   175  180   185  190   195  200
                *   *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
A-B31       VFKEDGKTLV SKKVTSKDKS STEEKFNEKG EVSEKIITRA DGTRLEYTGI KSDGSGKAKE VLKGYVLEGT LTAEKTTLVV KEGTVTLSKN ISKSGEVSVE

                                                                                                    g
                                                                                                    |
P-K48              110        120        130        140        150        160        170        | 180        190        200
[  998 ]    i....a.... .....l.... .......... .t...t.v.. n.......d. .......... ...dft.... .a.d....k. t....v.... .l....it.a>

P-PGAU             110        120        130        140        150        160        170        180        190        200
[  981 ]    ll........ .r..s.r..t ..d.m..... .l.a.tm..e n..k....em ....t..... ...kft...k vand.v..e. .........e .a.....t.a>

              205  210   215  220   225  230   235  240   245  250   255  260   265  270
                *   *     *    *     *    *     *    *     *    *     *    *     *    *
A-B31       LNDTDSSAAT KKTAAWNSGT STLTITVNSK KTKDLVFTKE NTITVQQYDS NGTKLEGSAV EITKLDEIKN ALK*

P-K48            210        220        230        240        250        260        270
[  998 ]    .d.s.ttq.. ...gk.d.k. .....s...q ...n...... d.....k... a..n...k.. ...t.k.l.. ...>

P-PGAU           210        220        230        240        250        260        270
[  981 ]    ....nttq.. ...g..d.k. .....s.... ..tq.....q y....k.... a..n...t.. ..kt...l.. ...>
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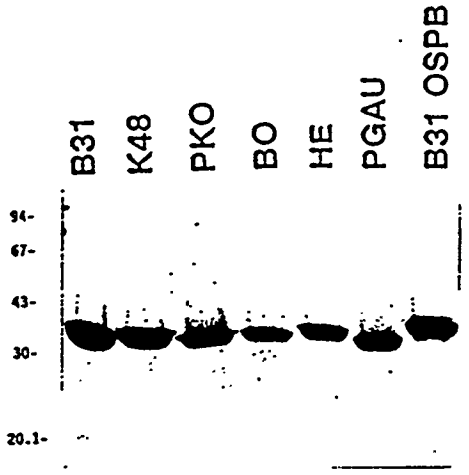
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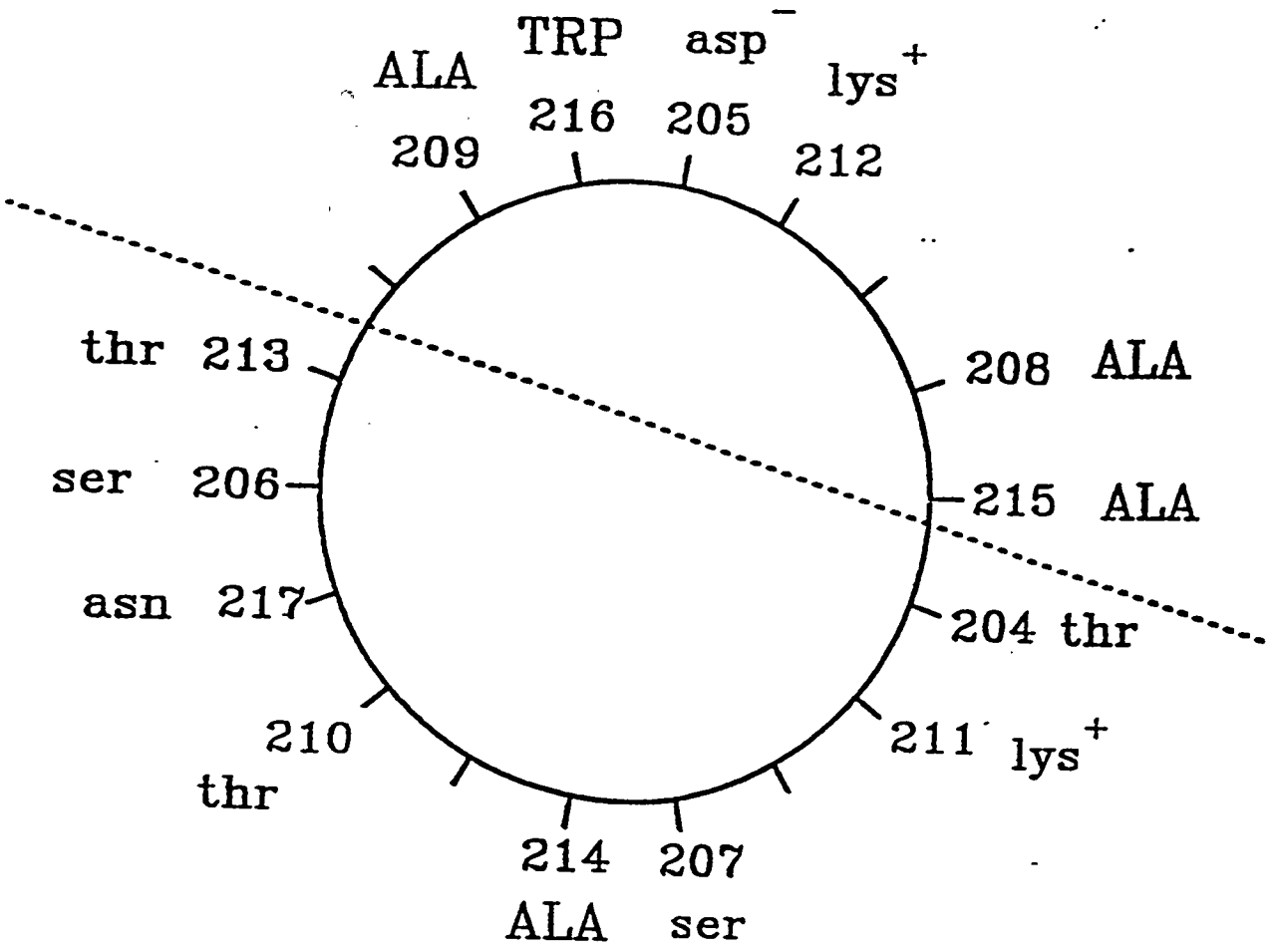
K48
PKO
BO
HE
PGAU
B31 OSPB
94-
67-
43-
30-
20.1-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