SHORT COMMUNICATION

Amino Acid Sequences of Proteins from *Leptospira* Serovar *pomona*

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This report describes a partial amino acid sequences from three putative outer envelope proteins from Leptospira serovar pomona*. In order to obtain internal fragments for protein sequencing, enzymatic and chemical digestion was performed. The enzyme clostripain was used to digest the proteins 32 and 45 kDa. In situ digestion of 40 kDa molecular weight protein was accomplished using cyanogen bromide. The 32 kDa protein generated two fragments, one of 21 kDa and another of 10 kDa that yielded five residues. A fragment of 24 kDa that yielded nineteen residues of amino acids was obtained from 45 kDa protein. A fragment with a molecular weight of 20 kDa, yielding a twenty amino acids sequence from the 40 kDa protein.*

Key words: *Leptospira* - proteins - amino acid - sequence

Leptospirosis is an economically important zoonosis occurring worldwide (Ellis 1986). Host immune defense mechanisms in leptospirosis are mainly humoral (Adler et al. 1980) with production of specific antibodies primarily to the outer envelope antigens. Whole cell (Bey 1982) and outer envelope antigens (Auran et al. 1972, Zuerner et al. 1991) have been extracted from leptospiras by different methods and proven to be immunogenic. Investigators isolated and characterized the outer envelope proteins in the range of 22 to 66 kDa from *Leptospira interrogans* using different detergent and extraction methods (Nunes et al. 1985, Brown et al. 1991, Haake et al. 1991, Zuerner et al. 1991). Haake et al. (1991) and Zuerner et al. (1991) used Triton X-114 extraction to isolate and characterize outer envelope antigens of serovar *grippotyphosa* and *pomona*, respectively. They concluded that these detergent phase proteins were related to integral membrane proteins. Alves (1993) used this method to isolate detergent-phase proteins from six common serovars. Three major (32, 40, 45 kDa) and one minor (22 kDa) protein were common to several serovars. These proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting and demonstrated to be immunologically conserved in all serovars tested. The objective of this investigation was to partially characterize the 32, 40 and 45 kDa antigens of *Leptospira* serovar *pomona* through internal amino acid sequencing. One serovar *pomona* was propagated in Bacto Leptospira Medium Base EMJH supplemented with 10% Bacto Letospira Enrichment EMJH and incubated at 30°C for 7 to 10 days. Extraction and phase partitioning of outer envelope leptospiral proteins were performed with Triton X-114 as previous described (Bordier 1981). The detergent-phase proteins were resolved on 12% acrylamide gels (Laemmli 1970) and transferred to Immobilon polyvinylidene difluoride (PVDF) membranes using a semidry blotting system. The membranes were washed (3 times in Milli-Q ddH_2O), stained with 0.1% Ponceau S and the desired proteins excised for sequencing. To obtain internal sequence information the 32 kDa and 45 kDa were cleaved with clostripain (Arg-C). Nonspecific protein binding was prevented incubating the membranes (room temperature for 3-5 min) with 1% polyvinylpyrollidone in MeOH (500 µl). Briefly, the membranes were washed in sterile Milli-Q ddH_2O and imersed in 50 µl of 20 mM Tris pH 7.6 containing 1 mM $CaCl_2$, 5 mM DTT. After heating (10 min at 80°C) and cooling, the digestion (12 h at 37°C) took place by adding 1µg of the enzyme. The peptides were eluted with 70% isopropanol/1% TFA solution (100 µl, 30 min at

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room temperature) and dried using a concentrator evaporator.

In situ digestion (room temperature, 24 h in the dark) of the 40 kDa protein was accomplished adding 1.4 mg of CNBr dissolved in 70% formic acid. The peptides were eluted (two times) with 50 mM Tris, pH 9.2, containing 2% SDS and 1% Triton X-100.

For N-terminal amino acid sequence, both pooled eluates were subjected to SDS-PAGE (15% acrylamide, Schagger & Jagon 1987) and transferred to PVDF or Pro-blot membranes as described before (Matsudaira 1987). The bands were detected with 0.1% (w/v) Coomassie Brilliant Blue R-250 in 50% HPLC methanol grade and the peptides sequenced on a pulser-liquid automated sequencer (model ABI 477; Applied Biosystems, Foster City, CA).

The enzymatic cleavage of the 32 kDa protein with clostripain (Arg-C) generated a10 kDa fragment with the following sequence I K I P N (P) and one addition with 21 kDa not sequenced. A 24 kDa fragment could be obtained from the 45 kDa protein using the same enzyme. In this case, the polypeptide presented the sequence A A A Q N T E G G T G L Q Y N (S) G A N D. The chemical cleavage of the 40 kDa protein with CNBr generated a major fragment with 20 kDa (L I P L D A T L I K V E T G E (S) K K A I V).

Data bank comparison (NBRF Protein and Swiss Protein) revealed a highly similarities of the 32, 40 and 45 kDa of *L. pomona* (95.2 and 90.5%) with *L. interans* (accession number U31426-LppL1) and *L. kruschnersi* (accession number L46794), respectively. The impossibilty of the 32 kDa to be sequenced (data not shown) suggests that this protein may contains the N-terminus acylated, a characteristic of lipoproteins, as that of *Borrelia burgdorferi* (Brandt et al. 1990). Thus, assuming that the 32, 40 and 45 kDa proteins are also acylated proteins, the strategy used in this study to identify the *L. pomona* antigens sequence seems to be fundamented to characterize the proteins. The obtention of amino acid sequence from these proteins would be also useful to design synthetic oligonucleotides that may allow the identification and cloning of the respective genes.

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