ISOLATION AND PURIFICATION OF RECOMBINANT OUTER SURFACE PROTEIN C (rOspC) OF *BORRELIA BURGDORFERI* Sensu Lato.

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Received: September 16, 2005; Accepted (with revisions): November 11, 2005

Key words: *OspC/Borrelia burgdorferi/Recombinant protein/Immobilized metal ion affinity chromatography*

The aim of this work was isolation and purification of the major immunodominant protein, Outer surface protein C (OspC) of three members of the species group *Borrelia burgdorferi*, the causative agent of Lyme disease. Our aim was to obtain this protein in a quantity and purity sufficient for immunization of experimental animals. For optimization of protein purification's yield we used immobilized metal ion affinity chromatography (IMAC) under different conditions. The greatest efficiency was achieved by using of HiTrap™ Chelating Column under native conditions.

INTRODUCTION

The *Borrelia burgdorferi* species group is a complex of eleven genomic species of spirochetes, of which three are causative agents of Lyme disease, the most common tick-borne human disease¹. *Borrelia burgdorferi* sensu stricto is the only pathogenic species in USA. *Borrelia garinii* and *Borrelia afzelii* are more common in Europe. The reser-

voir hosts of this pathogens are mostly rodents, deer and rarely birds. The most important vectors are ticks (*Ixodes* spp.)(ref.²).

OspC is a membrane lipoprotein with a native molecular weight of about 23 kDa. The OspC monomer is almost all helical, with four long helices plus one short helix. A biologically active OspC is anchored in the spirochete's outer membrane in the form of dimers³. OspC is probably

Fig. 1. Structure of Outer surface protein C *B. burgdorferi* s., from Kumaran et al³.

important during transmission of spirochetes from ticks to mammals and in the colonization of host tissues⁴. OspC is a good candidate for preparation of vaccine against Lyme disease, due to its strong immunogenicity and availability to neutralizing antibodies⁵.

MATERIAL AND METHODS

The gene sequences coding OspC was amplified from all three species plasmid DNA, and ligated into the expression plasmid pET 28a (Novagen). This construct was then inserted into *Escherichia coli*, strain BL 21. The used plasmid contains Kanamycin resistance cassette and T7 and 6xHis tags for detection and purification of expressed protein. His-tag is a common purification tag used for immobilized metal affinity chromatography (IMAC) one-step purification⁶.

The cells was cultured in LB medium at 37 °C to O.D. = 0.6, then derepressor isopropyl- β -D-thiogalactopyranoside (IPTG) was added and cultivation continued for another 4 h. The cells were harvested by centrifugation (centrifuge Jouan KR 25i, 10.000 RPM, 4 °C, 10 min). From 1 liter of culture about 2 grams of bacterial pellet were harvested. The following methods were used in cell lysis and purification.

- A) Lysis in phosphate lysis buffer (5 ml/g of bacterial pellet) with lysosyme (Sigma, 0,5 mg/ml of buffer) and sonication with ultrasound sonicator (Hielscher UP200S, 10 $\times$ 10 s, amplitude 70 %) followed by purification on Ni-NTA agarose (Qiagen, 1 ml/4ml of lysate). Elution was performed with high concentration of imidazole (250 mM, Fig. 2)
- B) Lysis in denaturant buffer containing 8 M urea (Serva, 5 ml/g of bacterial pellet) and sonication followed by purification on Ni-NTA agarose (Qiagen, 1 ml/4ml of lysate). Elution was performed with decreasing pH (pH 6.3, 5.9 and 4.5, Fig. 3) Elution fractions were dialyzed against phosphate buffer saline (PBS).
- C) Lysis in phosphate lysis buffer with lysozyme and sonication followed by purification on Ni - SepharoseTM High Performance (Amersham Biosciences, 1 ml/4ml of lysate). Elution was performed with high concentration of imidazole (250 mM, Fig. 4)
- D) Lysis in phosphate lysis buffer with lysozyme and sonication followed by purification on Ni²⁺- HiTrapTM Chelating Column (Amersham Biosciences). Elution was carried out by means of:
 - I. high concentration of imidazole (250 mM, Fig. 5) or with
 - II. ethylenediaminetetraacetic acid (EDTA, 50 mM) after washing with gradient of imidazole (Fig. 6).

Composition of all buffer used was in accordance with QIAexpressionist manual (Qiagen) with addition of protease inhibitors (PMSF, leupeptin, aprotinin) and β - mercaptoethanol (Fluka, 5 mM) in native buffers. Lysis buffer with higher concentration of imidazol (30 mM) was used for washing. Volume of wash fractions was the same as the lysate volume.

Fig. 2. SDS - PAGE analysis of lysis in phosphate buffer followed by purification on Ni - NTA agarose (method A). lane 1 - standard, lane 2 - lysis pellet, lane 3 - flow through fraction, lane 4 - wash 1 fraction, lane 5 - wash 2 fraction, lane 6-10 - elution fractions.

Fig. 3. SDS - PAGE analysis of lysis in denaturant buffer followed by purification on Ni - NTA agarose (method B, standard same as on fig. 2). lane 1 - standard, lane 2 - flow through fraction, 3 - lysis pellet, lane 4-5 - wash fractions with pH 6.5 buffer, lane 6-7 wash fractions with pH 5.9 buffer, lane 8-11 - elution fractions with pH 4.5 buffer.

Separated fractions were analyzed using SDS - PAGE and Western blotting. Electrophoresis gels were stained in Coomassie Blue, membranes with blotted proteins were analyzed with antibody anti-T7 HRP conjugate (Novagen, data not shown). All these methods were performed as described in the literature⁷. Protein concentration was measured by densitometric comparison with standard.

The Results of the method used on cells with plasmid containing rOspC *B. garinii* sequence are shown in Figures 2-6.

Fig. 4. SDS - PAGE analysis of lysis in phosphate buffer followed by purification on Ni - Sepharose (method C) lane 1 - flow through fraction, lane 2 - wash 1 fraction, lane 3 - wash 2 fraction, lane 4-8 - elution fractions.

Fig. 5. SDS - PAGE analysis of lysis in phosphate lysis buffer followed by purification on Ni²⁺ - HiTrapTM Chelating Column, elution with imidazole (method D I, standard same as on fig. 2): lane 1 - flow through fraction, lane 2 - standard, lane 3 - wash 1 fraction, lane 4 - wash 2 fraction, lane 5-8 - elution fractions.

RESULTS AND DISCUSSION

Several methods of cell lysis and IMAC purification of rOspC *B. garinii* were performed. Using method A, only a low yield of protein was obtained (15 µg/ml) and the purity was inadequate. We had better results with method B, but it wasn't any forcible tool for verification of the native structure of obtained recombinant protein. Using method C we obtained a protein of extremely low purity (< 20% of total protein) and low concentration. We had the best results with method D. With imidazole elution we obtained protein of sufficient purity and relative high concentration (400 µg/ml). With elution with EDTA, the concentration was somewhat lower (about 200 µg/ml), but the purity was very high (min. 95%). Results with rOspC *B. burgdorferi* and *B. afzelii* were very similar (data not shown), so HiTrapTM Chelating Column was chosen as an optimal tool for purification of rOspC of *Borrelia burgdorferi* sensu lato.

ACKNOWLEDGEMENT

We are grateful to Doc. RNDr. J. Kopecký, CSc of the Institute of Parasitology, the Czech Academy of Sciences, České Budějovice and RNDr. J. Turánek, CSc from Veterinary Research Institute, Brno and their co-workers for unselfish help. This work was supported by grant of Ministry of Industry and Business FD -K3/100.

Fig. 6. SDS - PAGE analysis of lysis in phosphate lysis buffer followed by purification on Ni²⁺ - HiTrapTM Chelating Column, elution with EDTA (method D II, standard same as on fig. 2): lane 1 - second elution fraction, lane 2 - standard.

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