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EXPRESSION AND PURIFICATION OF RECOMBINANT STAPHYLOCOCCAL PROTEIN A FUSED TO A MALTOSE - BINDING PROTEIN

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ABSTRACT

Recombinant protein A is widely used in biotechnology for the purification of immunoglobulin G antibodies. In this study, truncated protein A (protein A (5), encoding only five IgG binding domains (E, D, A, B, and C) and lacking signal sequence S and cell-wall anchoring region X M, was cloned into pMBP his parallel 2 and expressed in BL21 (DE3) cells. The gene encoding protein A was inserted downstream of the maltose binding protein (MBP) encoding *malE* gene. MBP is often fused with other proteins to improve their solubility, enhance stability, and increase the final yield. Proteins expressed using this system can be found in the soluble fraction, which is likely due to the solubilizing properties of MBP. A strain producing recombinant protein A (5) fused with MBP was obtained. The recombinant protein was purified and concentrated by gradient affinity chromatography. The weight of the protein fused with MBP was approximately 77.3 kDa, and without MBP was approximately 37 kDa. The IgG binding specificity of the recombinant protein was assessed using the agar gel immunodiffusion test with the target protein, bull, mouse, rat, and rabbit IgGs. The antigen antibody interaction showed a pattern not character to that described previously.

Keywords: protein A, cloning, expression, purification, *spa*, SpA.

INTRODUCTION

Bacterial proteins that capable to bind immunoglobulins of different mammalian species are widely used in immunology, medical biotechnology and in the fundamental researchers as secondary antibodies and as ligands for antibody purification [1]. These proteins are mainly found on the surface of gram-positive and gram-negative bacteria. However, it can also be found in the growth media and in the capsule [2]. The most well studied immunoglobulin binding proteins (IBP) of bacteria are protein A from *Staphylococci* (SpA) [3], protein G from *Streptococci* (SpG) [4], protein L from *Peptostreptococci* (PpL) [5, 6] and M proteins. In this work, we are focusing mainly on SpA and SpG proteins. Each group of proteins is different in binding specificity to immunoglobulins of various species and to various subclasses of immunoglobulins within the species [2]. It has been identified that there is a high affinity of protein A with human and rabbit IgGs. However, it also has been shown that SpA binds weakly with goat, sheep and rat IgGs [7]. The interaction of SpA with human Ig1, Ig2 and Ig4 subclasses is strong, although the interaction with Ig3 has not been identified [8].

Moreover, the mechanism of interaction is also varied. The interaction mainly occurs through Fc region of Ig molecule, although some of them might be via Fab region. The previous study [9] has shown that the interaction of protein A with human IgA and IgM occurs through Fab region and it is relatively weak [1]. It's important to point out that the interaction for L protein is different. This protein binds to k type IgGs through the light chains, suggesting that the binding ability of L protein is broad [6].

Regarding domain structures, both proteins of gram-positive bacteria, protein A from *Staphylococcus aureus* and protein G from *Streptococcus* GX7809 and G148 strains have three main regions [10]. In detail, they are signal sequence so called region S that is processed during secretion; IgG binding domains and XM region responsible for cell-wall and membrane bindings [11] as cited in [1]. Protein A has five homologous IgG binding domains E, D, A, B and C, whereas protein G from streptococcal GX7809 strain has only two [4, 10]. Protein M [12] and L [2], like G and A proteins, have

highly homologous repetitive domain structure. In the structure of M protein, like G and A proteins, there are also C terminal membrane anchor region (M), signal sequence and cell wall binding part [13]. The structure of L protein is similar to G protein in that both of them have repeating domains with albumin binding functions [2].

Both proteins SpA and SpG have previously been produced in recombinant form [10, 14, 15]. In recombinant form of production, S, X and M regions are often removed from the proteins in order to avoid using proteolytic enzymes for release of Protein A or G from cell wall that might causes degradation of protein. The truncated form of protein A lacking X region has weight of about 31 kDa [16].

Although, Proteins A and G have similar binding characteristics, they are far not identical in terms of sequence as well as in applications. SpA is more suitable to use on a large scale as this protein is more stable rather than SpG. At the same time SpG have ability to bind broader range of IgG subclasses. As in case with, human IgGs of different subclasses. That allows separating total fraction of human IgGs more efficiently. However, if there is a requirement to separate specific subclasses of IgGs, for example, mouse IgG1, IgG2a or IgG2b, it is better to use SpA [1].

The aim of the present study was to remove non –IgG binding regions from protein A, clone it into vector with MBP and produce recombinant protein A (5) from *Staphylococcus aureus* in *E.coli* cells.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, Reagents and Media

The genomic DNA was isolated from *Staphylococcus aureus* by using Promega Purification Kit.

In this work following strains were used: DH5α for the production of expression vectors and selection of ligated plasmids; BL 21DE3 for the recombinant protein expression. For protein purification, 1 ml HiTrap Chelating HP (GE Healthcare, USA) columns charged with Ni^{2+} ions were used. To construct expression vector, *pMBP his parallel 2* was used. The plasmid was the generous gift of Dr. D. Utepbergenov (Dept. of Chemistry, Nazarbaev University) [17].

Bacterial cells were grown in LB media with the addition of ampicillin (150 μg/ml). After transformation cells were cultivated in SOC.

All reagents used in this work are from Sigma-Aldrich, AppliChem, Promega, Amresco for molecular biology.

DNA preparation and Cloning

Genomic DNA encoded SpA from *Staphylococcus aureus* was extracted according to Purification Kit (Promega). Then the gene *spa*, lacking non-IgG binding regions was amplified by using specific primers with inserted *Nco* (5'-CAT GCC ATG GCA CAC GAT GAA GCT CAA CAA AAT GCT TTT-3') and *Not* (5'-TTT TCC TTT TGC GGC CGC TTA AGC ATC GTT TA G CTT TTT AGC TTC TGC TAA AAT-3') restriction sites. Thermo Scientific Phusion High-Fidelity DNA Polymerase was used for PCR. Cloning was done according to previous described protocols [18, 19]. Amplified *spa* gene was cloned into expression vector *pMBP his parallel 2* restricted by *Nco*/*Not* restriction sites. Chemically competent cells DH5α were transformed by heat shock method. Electro competent cells BL21 (DE3) were transformed by electroporation using MicroPulser (Bio-Rad, USA) as described in previous paper [20].

Isolation of Plasmid DNA

Plasmid DNA was isolated by using MiniPrep (Invitrogen) according to the supplier's recommendations. Some changes were introduced. We used 5 ml of dense culture obtained after cultivation for 15-18 hours. The time of incubation with elution buffer was prolonged from 1 to 5 minutes.

PCR Screening of Colonies

Screening of colonies to check the presence of target vector with gene was conducted by PCR. The universal M13 primer was used.

Purification by metal-affine chromatography

Recombinant protein was purified from 50 ml of induced cultivated culture. The culture was centrifugated at 4600g, at 4°C for 7 minutes. Lysis of cells was conducted as described earlier [21]. The protein lysate was run through 1 ml HiTrap Chelating HP (GE Healthcare, USA) column charged with

Ni^{2+} ions. The washing steps were included different concentration of imidazole starting from the concentration 20 mM to 250 mM. It also was contained of 500 mM NaCl and 20 mM Hepes (pH7, 5).

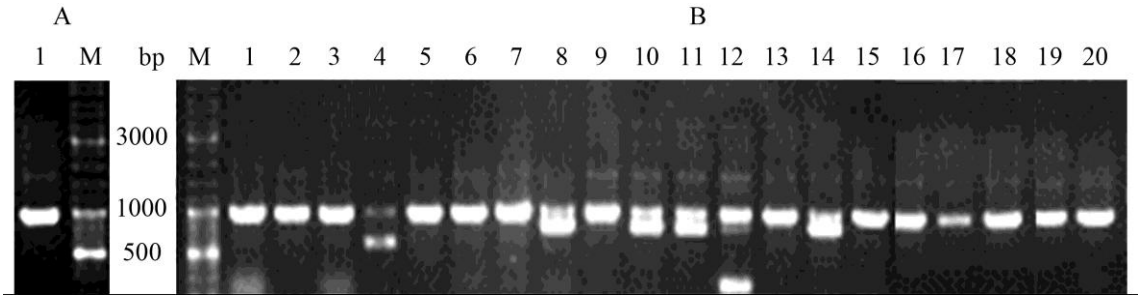
AGID test

This analysis was done according to previously described method [22] in 1.2 % agar.

RESULTS AND DISCUSSION

Cloning of truncated *spa* encoded 5 IgG binding domains *(spa (5))* into *pMBP-his parallel 2*

Gene *spa* encoded five domains were successfully amplified from *Staphylococcus aureus* template. The detection of PCR product was in 1% agarose gel. The results show the presence of band at the position of 1000 base pairs (Figure1A). Purification of DNA from a PCR reaction was conducted by chloroform method. Gene *spa (5)* was further cloned into *pMBP-his parallel 2* vector that had been restricted by *Nco* and *Not* restriction sites in buffer Tango 2x. After chloroform purification, DNA fragments were ligated by using T4 ligase and then transformed into DH5a competent cells. Screening reaction was conducted to check the presence of the vector with target gene. Using M13 universal primers, PCR screening analysis was conducted, the results of PCR screening are shown in (Figure1B).

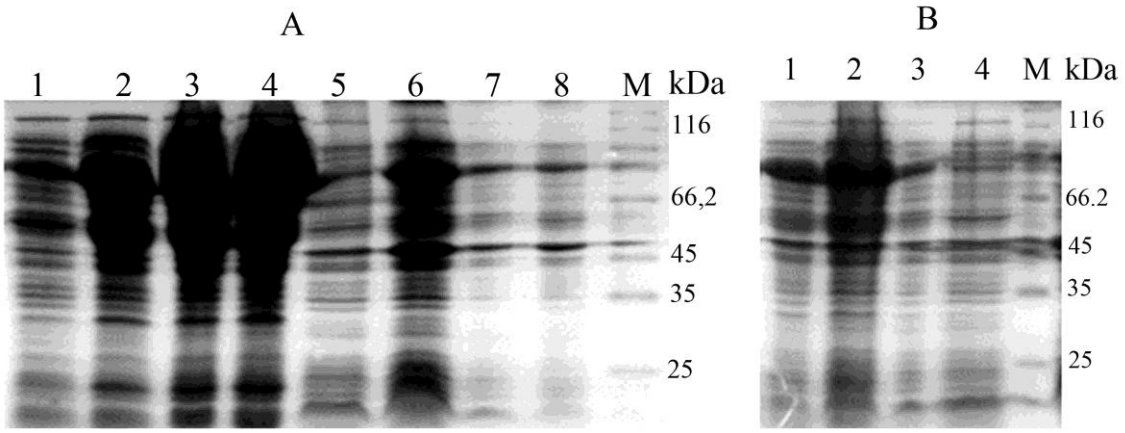


A. M- O'Gene Ruler Mix marker, 1-PCR amplicon; B. M- O'Gene Ruler Mix marker, 1-20 clones

Fig. 1. The result of PCR amplification of *spa (5)* from *Staphylococcus aureus* (A) and PCR screening of clones with insertions (B)

This analysis shows the protein bands at the position of 1000 base pairs suggesting that a cassette was integrated into host cells (Figure1B).

In order to check the expression of protein, construction was transformed into BL21 (DE3) cells. The culture is grown until it reaches OD_{600}=0.6, adding IPTG with final concentration of 0.5 mM and growing on a continuous culture for 2, 4 and 16 additional hours (are shown in Figure 2).

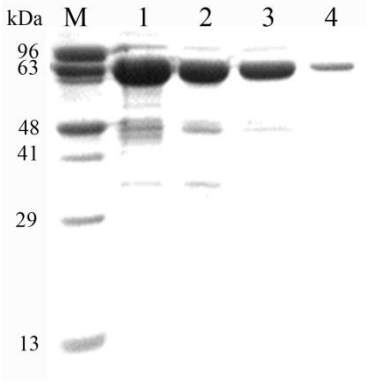


A.1-Sample of supernatant collected from culture before induction (NI); 2-4 Samples of supernatants collected from culture induced after 2 ,4 and 16 hours; 5-Sample of pellet collected from culture before induction (NI); 6-8 Samples of pellets collected from induced culture after 2,4 and 16 hours; M-protein molecular weight marker.

B. Lane 1-3,Samples of totals collected from induced culture after 16,4 and 2 hours; 4-Sample of total collected from non- induced culture(NI); M- protein molecular weight marker.

Fig. 2 A and B. SDS –PAGE analysis showing the effects of induction time on the expression of SpA by *Echerichia coli*

These data show that the fusion of recombinant protein with MBP does not interfere with the expression of former and even increases solubility. There is a band at the expected position of around 70 kDa. Regarding time of cultivation, the most appropriate time is 16 hours (Figure 2A). Therefore, BL 21(DE3) *pMBP his parallel 2 spa (5)* was cultivated following optimized conditions. Purification of the protein was done using gradient affine-chromatography (Figure 3).



M-protein molecular weight marker; Lanes 1 and 2, elution of SpA from Ni-affinity column by 50 mM Imidazole; Lanes 3 and 4, elution of SpA from Ni-affinity column by 100 mM Imidazole.

Fig. 3. SDS-PAGE analysis of recombinant protein A (5) fused with MBP and purified by gradient affine-chromatography.

According to this data, protein started to elute by washing with 50 mM Imidazole. By the elution with 100 mM Imidazole, protein had become most pure, and was collected. The final concentration of protein was 520 μg/ml collected from 600 ml.

AGID analysis to test the reactivity between recombinant SpA protein and IgGs

Checking obtained recombinant protein SpA with ability to bind IgGs of different organisms, an immunodiffusion test was carried out with serum from rabbit, bull, mouse and rat. The antibody binding test was conducted according to method described in Materials and Methods section. This analysis is based on principal that 'antigen and antibody can diffuse through a semisolid medium (usually agar) and form stable immune complexes at optimal concentrations, which then can be analysed' [23]. As a result, truncated form of SpA cloned and expressed in BL21 (DE3) reacted with only serum of rat (Figure 4). There is no precipitation line was observed between the protein SpA and IgG of rabbit, bull and mouse. These findings are different from those described earlier [1, 24, 25] and suggest that due to genetic manipulations the binding characteristics for protein A might change.

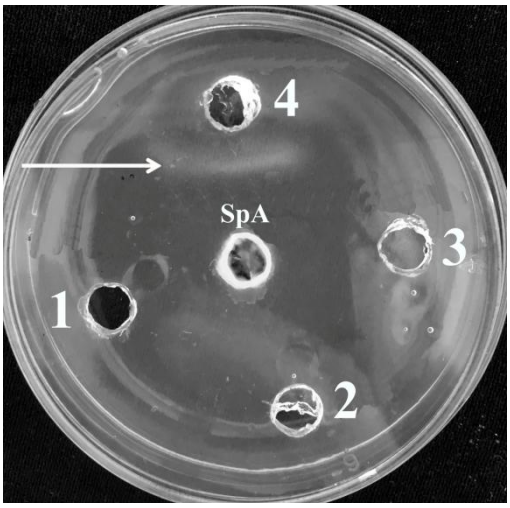


Fig. 4. Diffusion in agar gel against recombinant protein SpA (5) expressed in BL21 (DE3) of: serums of rabbit (1), bull (2), mouse (3) and rat (4).

CONCLUSION

The first strategy was to clone recombinant protein A encoded five IgG binding domains into pET-28c (+). However, the results did not show any level of expression (Data not shown). The second strategy was to clone the same protein into *pMBP his parallel 2* vector and express in BL 21(DE3) cells. The protein was successfully expressed and accumulated in soluble fraction. Then, the protein was purified by gradient metal-affinity chromatography. The final yield of protein was 520 μg/ml collected from 600 ml.

The immunodiffusion analysis for recombinant protein A was shown to give precipitation lines against only rat serum. Unlike literature data, protein did not react with rabbit, bull and mouse Igs imply a novel pattern of interaction.

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MBP-н ҚҰЙЫЛЫСТЫРЫЛҒАН РЕКОМБИНАНТТЫ *STAPHYLOCOCC*-қ А АҚУЫЗЫН ЭКСПРЕССИЯЛАУ ЖӘНЕ ТАЗАЛАУ

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ТҮЙІН

Рекомбинантты А ақуызы иммуноглобулин G антиденелерін тазалау үшін биотехнологияда кеңінен қолданылады. Берілген зерттеуде IgG байланысатын тек E, D, A, B және C домендерін кодтайтын, сигналдық S тізбегі жоқ, және жасуша қабырғасына бекінетін X M аймағы бар А ақуызының өзгертілген түрі *pMBP* his parallel *2* векторына клондалып, BL 21 (DE3) жасушаларында экспрессияланды. MBP (мальтоза байланыстырушы ақуыз) ақуыздардың үлкен отбасына жатады, берілген ақуыз жиі басқа ақуыздармен, ерігіштігін жоғарлату үшін, тұрақтандыру үшін және ақырғы шығысын арттыру үшін, құйылыстырылады. А ақуызын кодтайтын ген MBP ақуызының кодталуына жауапты *maLe* генінен кейін клондалды. Белгілі болғандай, ақуыз экспрессиясы суда еритін фракцияда жүреді, бұл MBP-ң ерігіштікті жоғарлату қасиетімен тікелей байланысты. MBP-н құйылыстырылған рекомбинантты А ақуызын өндіретін штамм алынды. Рекомбинантты ақуыз градиентті аффинді хроматография әдісімен тазаланып, концентрацияланды. MBP-н құйылыстырылған рекомбинантты ақуыздың салмағы шамамен 77,3 кДа, MBP есептемегенде шамамен 37 кДа. Рекомбинантты ақуыздың IgG байланыстыру ерекшелігі нысана ақуыз, бұғы, тышқан, егеуқұйрық және қоян IgGs мен агар геліндегі иммунодиффузия тесті (AGID) арқылы тексерілді. Антиген-антидене әрекеттесуі бұрын соңды сипатталмаған үлгінің пайда болуына әкелді.

Кілтті сөздер: А ақуызы, клондау, экспрессия, тазалау, *spa*, SpA.