Expression of Blattella germanica allergen 2 gene in E.coli and Production of Its Polyclonal Antibody

Wenhui Xing [a], Ruimin Fu [b]

Department of life science Henan Institute of Education, Zhengzhou 450046, China

[a] xingwenhui429@126.com, [b] angelaminmin@163.com

Abstract

Blattella germanica allergen 2(Bla g 2) gene was amplified by RT-PCR from total RNA of Blattella germanica and inserted into pET-41b vector properly. GST-Bla g 2 was expressed in E.coli via IPTG induction, a relative molecular mass of approximately 74kDa was showed by SDS-PAGE. The expressed fusion protein was purified by Ni+-agarose affinity chromatography, an emulsion was prepared with the purified Bla g 2 and injected into rabbits. The titer of antiserum identified by indirect ELISA reached 1:300000. Moreover, antiserum reacted specifically with purified fusion protein and the extract of Blattella germanica. Preparation of Bla g 2 and its polyclonal antibody offer help to the dialysis and therapy of patients who are allergic to Blattella germanica.

Keywords

Blattella germanica allergen 2, allergy, prokaryotic expression, polyclonal antibody.

1. Introduction

Cockroaches are belonged to *Insecta, Arthropoda, cockroaches mesh*, which is one of the major allergens allergic asthma, their excrement, eggs, carcasses, molt, and other debris through inhalation, contact, ingestion, etc. into the body, causing allergic asthma [1-3] .Currently, the country has risen in the distribution of cockroach, *Blattella germanica* has become one of the dominant species indoors[4-7], the body's main allergenic protein Bla g 2[8-10] is an important change caused by asthma allergens. This study is extracted from the body of the German cockroach total RNA, cDNA obtained by reverse transcription as a template, PCR amplification of gene fragments obtained Bla g 2, the gene fragment was cloned into the prokaryotic expression vector pET-41b[11], and by the transformation, screening, the recombinant vector restriction enzyme digestion and sequencing analysis, transformed into E.coli BL21 (DE3)[12-14], induced by IPTG [15,16]successfully expressed Bla g 2, and thus the expression product to immunize New Zealand white rabbits were prepared anti-Bla g 2 of polyclonal antibodies.

2. Materials and methods

2.1 Materials

German Cockroach provided by the Shanghai Municipal Center for Disease Control and Prevention; New Zealand white rabbits were purchased from Luo Jing feeder animal farms; cloning vector plasmid pET-41b, E.coli BL21 (DE3) provided by the Chinese Academy of Sciences cells. RT-PCR kit was purchased from TOYOBO; A type of small amounts of DNA fragments quick gel extraction kit was purchased from a broad Tektronix; restriction endonuclease BamHI, XHolI, T4 ligase were purchased from TaKaRa; HRP-conjugated goat anti-rabbit IgG were purchased from Sigma.

2.2 Methods

2.2.1 Preparation of the German cockroach extract

German Cockroach fasting 5h, -20 ℃ cryopreservation 0.5h. Ethanol soaked 1h, grinding body. Acetone was added to skim, skim through the upper acetone clarification. With bicarbonate - saline extract extraction 72h, after leaching products sterilized by filtration and stored at 4 ℃.

2.2.2 Cloning of Bla g 2 gene

According to the gene sequence Bla g 2, the design of specific primers (forward primer:5'-gatcggatccgatgattggcctaaagctagt-3'; Downstream primer: 5'- gatcctcgagtta gacgctttctact gaacggc-3'), And the introduction of BamHI restriction sites XHolI two. Using extraction kit German Cockroach RNA, and oligo-dT primer first strand synthesis Bla g 2, Then design specific primers Bla g 2 genes. PCR reaction conditions: 94 ℃ denaturation 50s; 55 ℃ annealing 1min; 72 ℃ extension 2min, 35 cycles. 1% agarose gel electrophoresis of PCR products, and PCR products were recovered.

2.2.3 Building of the recombinant plasmid

Bla g 2 gene amplification and expression vector pET-41b, respectively, and XHolI with BamHI double digestion, digestion products recovered by gel extraction kit. Under the action of T4 ligase overnight at 4 ℃ connection. Take the right amount of ligation product was transformed E.coli BL21 (DE3) competent cells, cultured overnight spread on LB plates containing Kna +.

2.2.4 Screening of Positive clones

Picked and grown on LB plates Single colonies were inoculated in LB liquid medium containing the Kna +, 37 ℃ shaking overnight. Alkaline lysis plasmid pumping. Recombinant plasmid with BamHI and XHolI double enzyme digestion; select agarose gel electrophoresis results in colonies send the correct fragment Shanghai Biological Engineering Co., Ltd. sequenced.

2.2.5 Cloning, expression and purification of recombinant E.coli in BL21 (DE3)

When the positive clones were inoculated in LB medium containing the Kna +, 37 ℃ shaking until an OD600 of 0.6-1.0, by adding IPTG (final concentration of 1mmol / L) induced 4h. 0,1,2,3,4h respectively the induction were sampled, centrifuged to collect the cells, cells after treatment, the 12% SDS-PAGE analysis of the expression of the protein, Empty vector transformed E.coli BL21 (DE3) as a negative control, the induction 0,4h sampled. After the collected cells were broken bacteria ultrasound, 10000r / min centrifuge 5min, washing the precipitate 2M urea and dried 8M urea denatured after Ni affinity chromatography purification for the preparation of polyclonal antibodies.

2.2.6 Preparation of Rabbit anti-Bla g 2 polyclonal antibody

After the pre-immune rabbit ear vein blood serum as a negative control, the initial take 100μg purification immune Bla g 2 with an equal volume of Freund's complete adjuvant emulsified subcutaneous multi-point injection. 2 weeks after the first immunization, a second immunization, after taking 100μg of purified Bla g 2 with an equal volume of incomplete Freund's adjuvant emulsion, multi-point injection subcutaneously. Immunized once every two weeks thereafter, the second immunization dose, and the same immunization methods. Fourth 10 days after immunization, ear vein blood, antiserum stored at -20 ℃. ELISA and Western blotting were used to detect its potency and specificity.

2.2.7 Detection of Polyclonal antibodies

The purified Bla g 2 protein was diluted with package solution to 20μg / ml, 4 ℃ overnight coated microtiter plates. After washing three times with PBST, was added to each well 3% BSA, 37 ℃ closed 3h. Washed with PBST 3 times, the control rabbit serum and rabbit antiserum to be detected with a blocking solution of 1: 10000,1: 100000,1: 200000,1: 300000,1: 400000,1: A total of 6 500 000 dilution, 37 ℃ for 1h. After washing four times in PBST, HRP-labeled goat anti-rabbit secondary antibody (1: 4000), 37 ℃ reaction 1h, ABTS chromogenic microplate reader detection A405 value. Preparation of Sample 2 Blattella germanica Bla g were used, and the purified extract, for 15% SDS-PAGE, and then transferred to a nitrocellulose membrane, the membrane was placed in 10% skim milk in TBST at room temperature in a closed 1h, TBST washing 5min. Preparation of rabbit antiserum diluted 5000-fold with TBST, and incubated at room temperature for 2h, and washed 3 times. HRP-labeled goat anti-rabbit IgG (1: 8000), and incubated at room temperature for 1h, and washed 3 times. ECL color development.

3. Results

3.1 The purpose of gene Bla g PCR results and the recombinant plasmid was digested two identification results

German cockroach retroviral gene obtained as a template, with the design of sequence-specific primers for PCR amplification, the resulting product length of about 1059bp, size is consistent with the predicted value. pET41b- Bla g 2 double digested by BamHI and XholI, there are four clones at about 1059bp purposeful bands sequenced correctly.

3.2 Bla g 2 gene expression in Escherichia coli

The positive identification of the strains after the culture was added IPTG induced to express the time gradient, by SDS-PAGE analysis to determine the amount of time to achieve the desired optimal expression of 3h. Compared with the control, the opposite occurs at approximately 74kDa apparent molecular weight of the strip to enhance expression, for GST- Bla g 2 fusion protein, consistent with the expected molecular size, and then cultured on a large, to obtain the desired recombinant protein.

3.3 Identification of rabbit polyclonal antibody anti Blag 2

Elisa method for detecting antibodies, the antibody titre of 1: 300,000. Western blotting analysis confirmed GST- Bla g 2 purified fusion protein at about 74KDa Department has specific bands appear, and the German cockroach extract bands appear at 36kDa place, with the reported molecular weight natural Bla g 2 consistent.

4. Discussion

Asthma is a common chronic disease in recent years, the incidence and mortality of asthma have risen. Indoor allergens increased prevalence of asthma is one of the important reasons for the increasing [2], The first time since Kang et morbidity related to cockroaches and bronchial asthma since many studies have shown that cockroach allergy is one of the original cause many allergic diseases mainly indoors, close ties with asthma. Periplaneta americana, Blattella germanica, Periplaneta fuliginosa are common allergens and other varieties of this study is to select German Cockroach study.

The complexity of the pathogenesis of asthma, is not yet fully understood, according to the World Allergy Organization reported that asthma patients 30 years or older, about 50 percent of people also suffer from allergic diseases, asthma patients 30 years of age the proportion of concurrent allergic diseases more high.

Studies have shown that the occurrence of some allergenic proteins closely related activity and asthma, as has cysteine protease activity of house dust mite allergen Der P1, and have chymotrypsin or serine protease activity of dust mite allergen Der f6 and house dust mite allergen Der P2, Der P9, has phospholipase A2 activity of bee venom [3]. Bla g 2 molecular weight of about 36k Da, and its amino acid sequence homology and aspartic proteases. In the presence of high concentrations of Blattella germanica vivo digestive organs Bla g 2, which may be a presumed digestive enzymes, but does not itself play activity, need some auxiliary molecule that has the activity.

Because cockroaches indoors widespread, simply by avoiding contact with allergens original relieve symptoms is not realistic, and the extensive use of various hormones, antihistamines and other drugs cause the body burden of adjustment mechanism heavier patients, seriously interfere with the patient had a precise endocrine system, so that the patient enters a vicious circle.Specific immunotherapy is considered to be the only method for the treatment of asthma due, and can change the way asthma, can block worsening of symptoms, prevention of new allergens allergic reaction occurs, the dual role of both prevention and treatment, are widely used in the treatment of allergic diseases.

But the cockroach allergen used in clinical extracts most of its body, with the strains of raw materials, gathering places, such as the difference in the extraction process will increase the quality of raw material change, causing the clinical products used in protein components, allergenic protein content in the different aspects of the activity, affect the accuracy of the diagnosis of the efficacy and safety

of. With the application of molecular biology techniques, many allergenic proteins have been cloned and expressed. In this study, the expression in E. coli in the main body of the German cockroach allergens protein Bla g 2, while preparing a rabbit anti-Bla g 2 serum and better quality. In addition, with systemic extracts Blattella germanica Bla g 2 with rabbit anti sera positive band at 36kDa appears, its size is consistent with the literature reports on the molecular weight of Bla g 2, illustrating antibody capable of recognizing native Bla g 2 protein. Furthermore, westen blots at 46 kDa is detected positive bands, the size of the other allergenic proteins in the body of the German cockroach Bla g 1 molecular size consistent, suggesting Bla g 1 and Bla g 2 have the same epitope portion, it is possible be prepared rabbit anti-Bla g 2 sera. In addition, the German cockroach extract at about 30kDa have combined, has yet to see reports on the German Cockroach similar molecular weight proteins in the body, its nature remains to be further studied.

Acknowledgement

This work is supported by Key research project of Henan Province (15B180002), Basic and advanced technology research in Henan Province (152300410092).The corresponding author is Wenhui Xing, Henan Institue of Education,Email:xingwenhui429@126.com

References

[1] Vliagoftis, H.; Asaduzzaman, M.; Davidson, C. Functional inhibition of par2 prevents inflammation and tissue remodelling in a long-term model of cockroach-mediated allergic airway inflammation. *Journal of Allergy and Clinical Immunology* 2015, 135, p.62-64.

[2] Dai, C.; Yao, X.; Nugent, G.; Keeran, K.; Yu, Z.; Aponte, A.; Gucek, M.; Levine, S.; Mushaben, E. The macrophage scavenger receptor cd163 binds the german cockroach protein, vitellogenin, and attenuates allergic sensitization and airway hyperreactivity in a murine model of experimental cockroach-induced asthma. *Am J Respir Crit Care Med* 2015, 191, p.5661-5663.

[3] Oseroff, C.; Sidney, J.; Tripple, V.; Grey, H.; Wood, R.; Broide, D.H.; Greenbaum, J.; Kolla, R.; Peters, B.; Pomés, A. Analysis of t cell responses to the major allergens from german cockroach: Epitope specificity and relationship to ige production. *The Journal of Immunology* 2012, 189, p.679-688.

[4] Martynova, E.; Kapelinskaya, T.; Schal, C.; Mukha, D. Intracellular localization of regulatory proteins of the german cockroach blattella germanica densovirus. *Molecular Biology* 2014, 48, p.301-304.

[5] Varadínová, Z.; Frynta, D.; Aulický, R.; Stejskal, V. Detection of cockroach faeces: Consumption of fluorescent bait and production of uv - light - detectable faeces from german cockroach, blattella germanica. *Entomologia Experimentalis et Applicata* 2015, 155, p.167-175.

[6] Liu, J.-L.; Sakuma, M. Olfactory conditioning with single chemicals in the german cockroach, blattella germanica (dictyoptera: Blattellidae). *Applied entomology and zoology* 2013, 48, p.387-396.

[7] Xiao, B.; Chen, A.-H.; Zhang, Y.-Y.; Jiang, G.-F.; Hu, C.-C.; Zhu, C.-D. Complete mitochondrial genomes of two cockroaches, blattella germanica and periplaneta americana, and the phylogenetic position of termites. *Current genetics* 2012, 58, p.65-77.

[8] Li, M.; Gustchina, A.; Glesner, J.; Wünschmann, S.; Vailes, L.D.; Chapman, M.D.; Pomés, A.; Wlodawer, A. Carbohydrates contribute to the interactions between cockroach allergen bla g 2 and a monoclonal antibody. *The Journal of Immunology* 2011, 186, p.333-340.

[9] Bassirpour, G.; Zoratti, E. Cockroach allergy and allergen-specific immunotherapy in asthma: Potential and pitfalls. *Current opinion in allergy and clinical immunology* 2014, 14, p.535-541.

[10] Dietrich, D.E.; Martin, A.D.; Brogden, K.A. Human β-defensin hbd3 binds to immobilized bla g2 from the german cockroach (blattella germanica). *Peptides* 2014, 53, p.265-269.

[11] Geng, A.; Zou, G.; Yan, X.; Wang, Q.; Zhang, J.; Liu, F.; Zhu, B.; Zhou, Z. Expression and characterization of a novel metagenome-derived cellulase exo2b and its application to improve

cellulase activity in trichoderma reesei. *Applied microbiology and biotechnology* 2012, 96, p.951-962.

[12] Robichon, C.; Luo, J.; Causey, T.B.; Benner, J.S.; Samuelson, J.C. Engineering escherichia coli bl21 (de3) derivative strains to minimize e. Coli protein contamination after purification by immobilized metal affinity chromatography. *Applied and environmental microbiology* 2011, 77, p.4634-4646.

[13] Xu, H.; Han, D.; Xu, Z. Erratum to: Overexpression of a lethal methylase, m. Tnedi, in e. Coli bl21 (de3). *Biotechnology letters* 2014, 36, p.1861-1861.

[14] Waegeman, H.; Maertens, J.; Beauprez, J.; De Mey, M.; Soetaert, W. Effect of iclr and arca deletions on physiology and metabolic fluxes in escherichia coli bl21 (de3). *Biotechnology letters* 2012, 34, p.329-337.

[15] Arita, Y. Reprint of "paradoxical decrease of an adipose-specific protein, adiponectin, in obesity". *Biochemical and biophysical research communications* 2012, 425, p.560-564.

[16] Li, F.-q.; Ma, C.-f.; Shi, L.-n.; Lu, J.-f.; Wang, Y.; Huang, M.; Kong, Q.-q. Diagnostic value of immunoglobulin g antibodies against candida enolase and fructose-bisphosphate aldolase for candidemia. *BMC infectious diseases* 2013, 13, p.253-254.