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Research paper

Preparation of an immunoadsorbent coupled with a recombinant antigen to remove anti-acetylcholine receptor antibodies in abnormal serum

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Abstract

An immunoadsorbent that removes anti-acetylcholine receptor antibodies (AChRAb) in abnormal serum of myasthenia gravis (MG) patient was efficiently prepared by an expression product, the functional fragment of $AChR_{\alpha 205}$ fused with maltose binding protein (MBP). The ligand can then covalently bind to amylose resin through MBP fusion protein. It was shown from the result of this study with anti-AChR mice sera that the removal rate of AChRAb on this immunoadsorbent reached $87 \pm 10\%$ (mean value of 10 mice) and the maximally binding capacity of AChRAb was approximately 260 μg/g immunoadsorbent (wet weight). Moreover, the immunoadsorption test of sera in two MG patients indicated that about 90% and 96% of abnormal AChRAb could be eliminated, while other serum components such as albumin, IgG, IgM and IgA only dropped 18%, 35%, 22%, 15% and 24%, 27%, 15%, 12%, respectively, for two MG patient sera. It is anticipated from this study that the immunoadsorbent reported here could, with further development, find its clinical application for removal of AChRAb from patient serum.
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Keywords: Immunoadsorbent; AChRAb; $AChR_{\alpha 205}$; Recombinant antigen

1. Introduction

The nicotinic acetylcholine receptor (AChR) is a ligand-gated ion channel receptor, formed by four kinds of subunits: α_2, β, γ (ε) and δ, of which α-subunit is the main immunogen involved in the autoimmune disease myasthenia gravis (MG) (Takamori, 1994; Lindstrom, 2000). Anti-AChR antibodies (AChRAb) in the serum of MG patient can accelerate the degradation of AChR, cause complement-mediated destruction of postsynaptic membranes and block ACh-binding to AChR, resulting in myasthenic weak-

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ness (Richman and Agius, 2003). AChRAb are usually classified into two subclasses. The first subclass is "blocking antibody", which acts on the segment of the α-subunit 183–200 residues to prevent the binding of ACh to AChR (Takamori et al., 1988). The second subclass is "binding antibody", which acts on the segments of α-subunit 67–76 and 125–147 residues to accelerate AChR degradation and to disrupt the postsynaptic membrane (Tzartos et al., 1988; Lennon et al., 1985).

Plasma exchange and double filtration plasmapheresis have been used to remove the abnormal AChRAb in MG patient serum. However, these methods may remove some normal plasma components simultaneously, indicating that replacement fluid such as fresh frozen plasma or albumin preparation is required (Chiu et al., 2000; Yeh and Chiu, 2000). Therefore, immunoadsorption based on specific antigen–antibody reaction seems to be an efficient method for eliminating AChRAb in MG patients. It was reported that adsorbents coupled with ligands such as sulfathiazole, tryptophan, protein A or synthetic peptide $AChR_{\alpha 183-200}$ by chemical method could selectively or semi-selectively remove AChRAb, but these adsorbents could be low specificities or only eliminate blocking antibody (Yamamoto et al., 1990; Splendianid et al., 1992; Flachenecker et al., 1998; Takamori and Maruta, 2001). Therefore, it is necessary to find novel adsorbents to completely and specifically remove AChRAb in MG patient serum. In this article, a novel immunoadsorbent was produced by using amylose resin as the support conjugated with functional region of acetylcholine receptor α-subunit ($AChR_{\alpha 205}$) via maltose binding protein (MBP). The experimental results demonstrate that this novel immunoadsorbent can efficiently remove AChRAb from anti-AChR serum of mice or MG patients.

2. Materials and methods

2.1. Plasmids, antibodies and sera

The host strain *E. coli* BL21(DE3), plasmid pUC-$AChR_{\alpha 205}$ and vector pMAL-c2 were stored in this laboratory at − 70 °C. All enzymes used and amylose beads were purchased from New England Biolabs (NEB) (Beverly, MA, USA). Anti-AChR monoclonal antibody (mAb198, mAb35) and AChR α-subunit from *Torpedo* (T-AChR) were generous gifts from Prof. W.E. Trommer (Department of Chemistry, University of Kaiserslautern, Germany). Secondary antibodies, rabbit anti-rat, goat anti-mouse or goat anti-human immunoglobulin G conjugated with horseradish peroxidase were purchased from Sigma (Missouri, USA). Anti-AChR mice Sera were prepared by immunizing the C_{57}BL/6 mice with native AChR α-subunit from *Torpedo* according to the established procedure (Yin et al., 2000). Two MG patient sera were obtained from the First Hospital attached to Shanxi Medical University and Xiehe Hospital attached to Huazhong Science and Technology University, respectively.

2.2. Cloning and fusion expression of recombinant $AChR_{\alpha 205}$ gene

The DNA fragment encoding $AChR_{\alpha 205}$ was amplified by PCR with plasmid pUC-$AChR_{\alpha 205}$ as the template, and the PCR product was digested with *Bam*HI/*Hin*dIII and ligated into vector pMAL-c2 to generate a recombinant plasmid pMAL-$AChR_{\alpha 205}$ (Fig. 1). The plasmid generated was then transformed into *E. coli* BL21(DE3) by $CaCl_2$ method (Sambrook and Russell, 2001). The resulting engineered strain *E. coli* BL21(DE3)/pMAL-$AChR_{\alpha 205}$ was grown in LB medium supplemented with 100 μg/ml ampicillin at 37 °C until the optical density (OD) at 600 nm reached

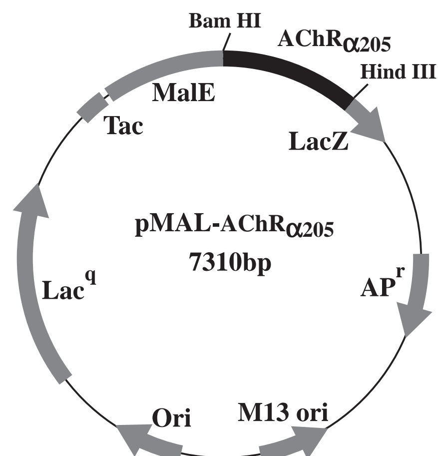

Fig. 1. Schematic diagram of the expression construct pMAL-$AChR_{\alpha 205}$.

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