

Purification of the Mule (*Equus mulus*) IgG by Protein A - Affinity Chromatography

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Abstract

A mule is the off-spring (F1 hybrid) of two equine species: a male donkey and a female horse. Little is known about the immune system of the mule. In this investigation we isolated and characterized molecularly the mule IgG that was purified by Staphylococcal protein A (SpA) affinity chromatography, and then the purified IgG was further study by SDS-PAGE and enzyme-linked immunosorbent assay that concluded that the intact mule IgG has a molecular weight of 150 kDa.

Keywords: Mule; IgG; Purification; ELISA; SDS-PAGE

Introduction

The mule, *Equus mulus*, is the off-spring (F1 hybrid) of two equine species: a male donkey and a female horse. Genetically they differ in the number of chromosomes [1,2]. A mule has been used as a farm animal from ancient times and in China it has been produced by insemination [2]. The immune system of the mule cannot be taken from granted just because we know a bit more about the immune system of its progenitors. In this research we isolated and characterized molecularly the mule IgG that was purified by Staphylococcal protein A (SpA) affinity chromatography [3], separated by SDS-PAGE and a further confirmation of its immunoglobulin nature was assayed by an SpA-enzyme-linked immunosorbent assay (ELISA).

Methodology

Reagents and other materials used in this study were obtained from Sigma-Aldrich, Co, USA. Sera were obtained locally from healthy animals. The phlebotomy was performed by a licensed veterinary doctor that took 10 ml of blood from female mules. IgG purification was performed by SpA-affinity chromatography using a commercially-prepared kit. The instructions of the manufacturer were followed in performing this procedure. Briefly, the serum was added to a SpA-agarose column at pH 8.0 and then elution at a lower pH was followed. The isolated product was dialyzed back against PBS. The fraction eluted were assayed first by A₂₈₀ and then the protein molecular weight was assessed by SDS-PAGE according to Neville, 1971 [4] A SpA-sandwich ELISA was performed as confirmative test.

SpA Sandwich-ELISA

The 96 well polystyrene microplates (U-shaped bottom) were coated with 500 ng of SpA (Sigma-Aldrich) in coating buffer for 4 hr at 37°C. The microplates were washed four times with PBS-Tween-20 and blocked with 3% non-fat milk in PBS, 25 µl/well, 1 hr, at room temperature (RT). The microplates were washed four times again. IgG eluate samples (25 µg/µl) were added and after that were incubated at RT. The microplates were washed four times and 50 µl of peroxidase-labelled protein-A diluted 1:3000 (Sigma-Aldrich) was added to each well. Then the microplates were then incubated for 1 hr at RT and washed four times. Tetramethylbenzidine (TMB) solution (50 µl) was added to the microplates. After a further incubation for 15 min in the dark, the reaction was stopped with 3M H₂SO₄ and read in a microplate reader at 450 nm.

Results and Discussion

Protein A is a surface antigen from the cell wall of *Staphylococcus aureus*. It has a molecular weight of 42 kDa and it has the capacity to bind many IgG from mammalian species and it is considered an immunoglobulin-binding protein [5]. In this research SpA affinity chromatography demonstrated to be efficacious for the purification of mule IgG. This IgG is a precious reagent, pure enough to produce anti-mule secondary antibodies when injected in another animal species such as rabbit, chicken or others for immunodiagnostic purposes. The secondary antibodies can then be labelled to enzymes, colloidal gold, fluorescein and biotin, just to mention some of the molecules to which it can be attached. Since mule is a big animal large amount of the blood can be taken for the preparation of antibodies [6]. On the other hand mules could be immunized with animal and human proteins to produce species-specific antibodies (Figure 1; Table 1).

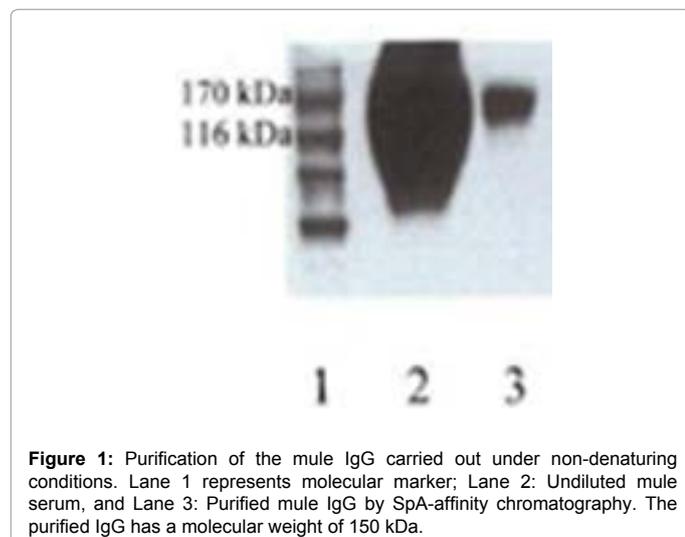
In future work we propose to analyze the IgG by SDS-PAGE under denatured conditions, and we hypothesise that the mule IgG has two heavy chains of approximately 50 kDa and to light chains of approximately 25 kDa as it occurred with other mammalian IgG molecules. In addition a study of the humoral immunity following vaccination and determined by immunodiffusion, and cell mediated-immunity determined by dinitrochlorobenzene skin test can be done later, as previously described in the literature [7]. The ELISA described here confirmed that the mule IgG binds to SpA, and support the result gotten from the purification procedure. It would be important to develop immunodiagnostic kits to monitor mule's diseases [8-10]. The experiment performed in this study demonstrates that is easy to purify mule IgG by affinity chromatography and the intact mule IgG has a molecular weight of 150 kDa. Precisely it is a novel research using a standard separation technique that is Protein-A affinity

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Species IgG	Mean OD at 450 nm	Standard Deviation (SD)
Mule IgG	0.61	0.04
Human IgG (+ control)	0.81	0.02
Chicken IgY (- control)	0.14	0.008
Blank	0.06	0.003

Table 1: Results of the Protein A- Enzyme Linked Immunosorbent Assay (ELISA) for detection of mule IgG. This assay confirmed the previous purification procedure, since the SpA used only binds to IgG molecules.

chromatography. For first time the mule IgG: *Equus mulus* has been purified and there is not report of published papers concerning to the immune system of this equine species.

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