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SOMATOTROPIN EFFECTIVE ANTISERA PRODUCTION IN FORTY DAYS

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ABSTRACT

Polyclonal antisera were raised against bovine somatotropin (ST) in rabbits and high titer achieved in less than 40 days after primary immunization. Protocol was developed with slight modification in method of Hu (2002) for antisera production and booster injection given on 28th and 37th day. The serum for antibody titer was collected on 35th and 42nd day. The antisera procured were effective in Western blotting and for purification of ST using affinity column chromatography.

Key words: Affinity column, polyclonal antisera, protein purification, somatotropin

INTRODUCTION

Polyclonal antisera are a useful tool in the characterization of a protein or an antigen and have broad range applications in immuno-serological research and clinical assays. However, immunization is regarded as 'limit step' due to the occasional ineffectiveness of raised sera and time-consuming process (Leenaars and Hendriksen, 2005). Rabbits are considered ideal for raising antibodies, if the final volume of serum required is <100 ml (CCAC, 2002). At least 3 to 4 months are required to get high titer antisera by traditional immunization methods. Many commercial kits are available for getting any recombinant protein and for protein purification but these are expensive. In most of the developing countries, including Pakistan, cheaper products are used instead of commercially available antibodies. Somatotropin has been exploited worldwide to bridge the gap between supply and demand of meat and milk. Therefore, to raise high titer antisera against somatotropin in shorter period shall prove beneficial to researchers involved in somatotropin production worldwide. The present study was aimed to produce effective high titer antisera against somatotropin of 'Nili-Ravi', a reverine type buffalo native to South East Asia, following the method of Hu *et al.* (2002) with slight modifications. The raised antisera were used for the characterization and purification of expressed 'Nili-Ravi' somatotropin (NRST).

MATERIALS AND METHODS

The animals used were procured from the National Institute of Health, Islamabad (Pakistan) and maintained in the Animal House of National Agriculture Research Center, Islamabad, as per the general guidelines. For antisera production, 3 female 'White New Zealand' rabbits of 2.5 kg weight were used and two of them used for the production of polyclonal antibodies and 3rd one maintained as a negative control. The pre-immune sera from all test animals was taken, filtered and preserved at -20°C. Commercially available bovine somatotropin (Boostin 250, LG Life Sciences, South Korea) was used

as antigen and dialyzed somatotropin (ST) used for emulsion preparation for the test animals. The negative control was injected with phosphate buffered saline (PBS) emulsion with Freund's complete adjuvant (FCA). Somatotropin @ 250 µg kg^{-1} animal weight was injected subcutaneously in test animals. Five hundred microlitres of emulsion per animal was injected at four different points along the backbone of animal. Primary immunization with FCA was repeated on 3rd day. The booster injections with Freund's incomplete adjuvant (FIA) were injected on 28th and 37th day and blood collected on 35th and 42nd day. The serum was separated and stored at -20 ºC.

For dot blot analysis, a standard protocol was followed (*www.westernblotting.org*). The sample (2 µl) was placed on nitrocellulose (NC) membrane in each case. The raised serum was diluted 1:100 times in deionized water and dot blot carried out to confirm the presence of ST specific antibodies. Commercially purchased anti-bovine ST antibodies (G8999-06, US Biological) were used as positive control and the serum from pre-immunized animals as negative control. Pre-immunized sera of all test animals and the sera collected at different intervals during immunization process were checked by dot blot. The collected antisera was clarified as per the method of Harkins (2001) and preserved for Western blotting and immuno-affinity column preparation. To obtain protein for column purification, 250 ml bacterial culture was induced with 600 µM IPTG for 4 h and cell pellet washed with 100 ml equilibration buffer (Tris-HCl, 15.76 g; NaCl, 5.85 g L^{-1}; pH, 7.5). Cells were sonicated at 60% amplitude with pulse-on and -off for 30 seconds each. Four cycles of sonication were repeated. After centrifugation the final pellet was reconstituted in 30 ml solubilization buffer (50 mM Tris-HCl, pH 8.0, 8M urea, 1M DTT) and used for purification. For preparation of immuno-affinity column, 'PreACT™ Agarose ALD' kit (Novagen) was used and column packed as per the manufacturer's instructions with 5 ml raised antiserum. The protein bound with resin-antibody slurry was washed with 100 ml equilibrating buffer and then eluted with 8 ml elution buffer (glycine, 7.506 g L^{-1}; pH, 2.5). Fractions (0.5 ml each) were collected in tubes containing 50 µl neutralizing buffer (Tris-HCl, 157.6 g L^{-1}; pH, 8). The column was regenerated with 50 ml elution buffer and then with equilibrating buffer and stored at 4ºC till use.

For protein purification by immuno-affinity chromatography, the presence of protein was first confirmed through SDS-PAGE and dot blot methods. Then the protein was purified by immuno-affinity column as per the protocol provided with the kit (Novagen). The fractions collected were checked by Bradford spot test (Bradford, 1976). The purified protein was lypholized and sample reconstituted in 500 µl distilled deionized water. Reconstituted protein (30 µl) was loaded on SDS-PAGE to check the protein homogeneity (Laemmli, 1970).

RESULTS AND DISCUSSION

The sera collected on 0th, 35th and 42nd day were diluted 100 times before checking their antibody titer. The results revealed that the negative control animal, injected with PBS emulsions, did not show any titer against anti-bovine somatotropin antibodies (Fig. 1). However, the positive control depicted titer at different dilutions [1:10, 1:100, 1:1000 and 1:10,000]. The pre-immunized sera dots from the two test animals along with two sample dots also showed high titre by dot blot analysis thereby revealing that high titer antisera can be raised within forty days. Our results are in conformity with the findings of Hu *et al.* (2002). In recent times many antibody manufacturering companies have started to provide, on demand, customized antibodies but they usually take 60 days (Antagene Inc., California, USA) to 112 days (Harlan Laboratories Inc., USA) time. Although many companies claim to raise antibodies in 28 days period (PRF&L, Pennsylvania, USA) but they use some proprietary immune-stimulator for this quick. Therefore the present protocol is advantageous in saving time and money, especially for developing countries.

Before loading protein sample on affinity column for purification, the presence of protein was confirmed through dot blot. In the collected sample fractions blue colour started appearing from fraction 7 upto fraction 26 (Fig. 2).

The brightest blue colour intensity was seen in fraction 13 which showed elution peak in 4th ml of

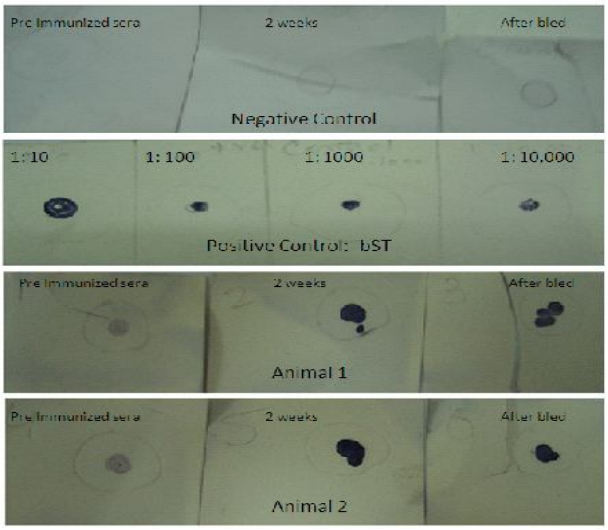

Fig. 1: Dot blot from diffrerent animals

Fig. 2: Bradford spot test of column fractions

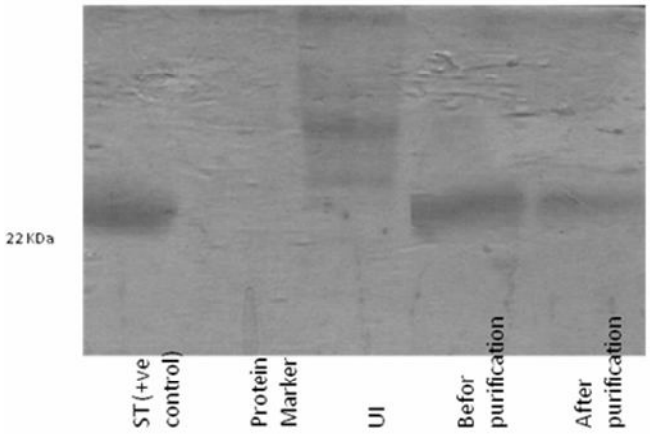

Fig. 3: SDS-PAGE of purified product of immuno-affinity column chromatography

elution buffer. The protein fractions were pooled up, dialyzed and lypholized. After reconstitution the protein purity was tested by SDS-PAGE (Fig. 3). The present study suggested that the antisera raised by following the devised protocol can successfully be used not only for Western blotting and immuno-staining analysis, as reported by Hu *et al.* (2002), but also for column purification on the basis of affinity chromatography.

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REFERENCES

Bradford, M.M. 1976. A dye binding assay for protein. *Analytical Biochemistry*, **72**: 248-254.

CCAC. 2002. *Guidelines on Antibody Production.* Canadian Council on Animal Care, Ottawa, Canada (*http://www.ccac.ca/Documents/Standards/Guidelines/Antibody_production.pdf*).

Harkins, K.R. 2001. Basic methods in antibody purification and characterization. pp.142-168. **In**: *Basic Methods in Antibody Production and Characterization* (eds. G.C. Howard and D.R. Bethell), CRC Press, Florida, USA.

Hu, Y.X., Guo, J.Y., Shen, L., Chen, Y., Zhang, Z.C. and Zhang, Y.L. 2002. Get effective polyclonal antisera in one month. *Cell Research*, **12**: 157-160.

Khalid, S., Mehmood, S.F., Shahzad, M.I., Bashir, Q., Saba, S.Z., Mustansar, Z., Fatima, A. and Khanum, A. 2008. Cloning, expression and modeling studies of somatotropin cDNA of local buffalo breed-Nili Ravi. *Journal of Chemical Society of Pakistan*, **30**: 712-725.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature*, **227**: 680-685.

Leenaars, M. and Hendriksen, F.M. 2005. Critical steps in the production of polyclonal and monoclonal antibodies: Evaluation and recommendations. *Institute of Laboratory Animal Research Journal*, **46**: 269-279.