Lab Note

Monoclonal and polyclonal antibodies against the precursor of recombinant human insulin

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The precursor of recombinant human insulin is also called recombinant proinsulin. It is a fusion protein containing protection peptide and insulin. The protection peptide contains 55 amino acids (48 amino acids from human growth hormone, and a connecting peptide containing Arg-residues) and can effectively improve protein stability and expression efficiency [1]. However, in clinical trials the presence of proinsulin in the insulin final products was found to have a negative effect on the heart, leading to an increased incidence of myocardial infarction [2–6]. More importantly, recombinant proinsulin is a heterologous protein. Therefore, the removal of the recombinant proinsulin in the final product of insulin is vital, and the effectiveness of its removal should be analyzed by appropriate methods. In this study, we prepared specific monoclonal and polyclonal antibodies against proinsulin and established enzyme-linked immunosorbent assay (ELISA) for the detection of the residual precursor in the insulin production.

Three adult female BALB/c mice were intraperitoneally injected with 55-peptide as an antigen. Each mouse was injected with 100 µg antigen mixed with complete Freund’s adjuvant (Sigma, St Louis, USA) the first time, and then boosted with 50 µg antigen mixed with incomplete Freund’s adjuvant for three times. The injection interval was 2 weeks. After four immunizations, spleen cells were fused with SP2/0 myeloma cells and the hybridoma cells were selected by growing in HAT (hypoxanthine, aminopterin, and thymidine) medium. Monoclonal cell lines were screened by the limiting dilution method. To obtain a large amount of antibodies, the hybridoma cells were injected into the peritoneal cavity of mice, and antibody-rich ascites was collected. The antibodies were purified by protein-G Sepharose (GE Healthcare, Wisconsin, USA). The titer of anti-55-peptide monoclonal antibody (mAb) was determined by indirect ELISA.

New Zealand white rabbits were immunized with the purified recombinant proinsulin (but not insulin). If the animal is immunized with pure insulin, it would soon become sick and die. Each rabbit was hypodermically injected with 600 µg antigen mixed with complete Freund’s adjuvant the first time, and then boosted three times with 300 µg antigen mixed with incomplete Freund’s adjuvant. The injection interval was 2 weeks. Seven days after the last immunization, the serum of the immunized animals was collected. The anti-insulin polyclonal antibodies (pAbs) in the antiserum were purified by affinity chromatography using recombinant insulin (purchased from Novo Nordisk, Bagsvaerd, Denmark) coupled CNBr-activated Sepharose (GE Healthcare). The titer of the anti-insulin pAbs was assayed by indirect ELISA.

Microtiter plates (Coring, New York, USA) were coated with 20 µg anti-insulin pAbs, and then blocked with 1% bovine serum albumin (BSA) overnight at 4°C. The samples (proinsulin, insulin, and 55-peptide) were added to each well, respectively. The plate was incubated for 90 min at 37°C and washed three times with PBST (10 mM PBS + 0.05% Tween-20). Anti-55-peptide mAbs were added and incubated for 90 min at 37°C. The plate was washed three times with PBST and then incubated with horse radish peroxidase (HRP)-conjugated goat anti-mouse IgG for 90 min at 37°C. After washing, tetramethylbenzidine solution was added into wells and incubated for 10–15 min. Finally, 100 µl of 2 M H2SO4 solution was added to terminate the reaction. The absorbance was read at dual filter 450/620 nm.

The anti-insulin pAbs were efficiently purified with affinity chromatography, and the antibody heavy chains and light chains could be detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [Fig. 1(A)]. The titer for the anti-insulin pAbs was 1 : 32,000 as determined by indirect ELISA [Fig. 1(C)]. The anti-55-peptide mAbs were purified by protein-G Sepharose and both the heavy and light chains of the antibody were detected by SDS-PAGE [Fig. 1(B)]. The titer was 1 : 32,000 [Fig. 1(D)].
Proinsulin could be detected at concentrations ranging from 10 to 640 ng/l using sandwich ELISA. However, insulin and synthetic 55-peptide could not be detected at this range of concentrations (Fig. 2), which indicated that this sandwich ELISA method was effective to specifically detect residual proinsulin in insulin products.

In conclusion, we have successfully prepared anti-insulin pAbs and anti-55 peptide mAbs. These two kinds of antibodies could be used to measure and monitor traces of residual proinsulin in insulin production process.

References