Generation of high affinity anti-peptide polyclonal antibodies recognizing goat αs1-casein

ABSTRACT

The chemical, technological and allergy properties of goat's milk are significantly affected by the level of αs1-casein. Detection and quantification of αs1-casein requires high-specificity methods to overcome high-sequence similarity between this protein and others in the casein family. Unavailability of antibodies with high affinity and specificity towards goat αs1-casein hinders the development of immuno-based analytical methods such as enzyme-linked immunosorbent assay (ELISA) and biosensors. Here, we report the generation of polyclonal antibodies (or immunoglobulins, IgGs) raised towards goat αs1-casein N- (Nter) and C-terminal (Cter) peptide sequences. The Nter and Cter peptides of goat αs1-casein were immunized in rabbits for the generation of antisera, which were purified using protein G affinity chromatography. The binding affinity of the antisera and purified IgGs were tested and compared using indirect ELISA, where peptide-BSA conjugates and goat αs1-casein were used as the coating antigens. The Nter antiserum displayed higher titer than Cter antiserum, at 1/64,000 and 1/32,000 dilutions, respectively. The purification step further yielded 0.5 mg/mL of purified IgGs from 3 mL of antisera. The purified Nter IgG showed a significantly ($p < 0.05$) higher binding affinity towards peptide-BSA and goat αs1-casein, with lower Kd value at $5.063 \times 10{-}3$ μM compared to $9.046 \times 10{-}3$ μM for the Cter IgG. A cross-reactivity test showed that there was no binding in neither Nter nor Cter IgGs towards protein extracts from the milk of cow, buffalo, horse and camel. High-quality antibodies generated will allow further development of immuno-based analytical methods and future in vitro studies to be conducted on goat αs1-casein.

Keyword: IgG purification; Goat's milk allergy; αs1-casein; Anti-peptide polyclonal antibody; Immunogenic peptides