P1: Stability of *Camelus dromedarius* IgG_3 heavy chain antibodies

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Introduction:

A breakthrough in immunity has occurred by the discovery of a new type of antibodies which are devoid of the light chains in family *Camelidae*. They were found in *Camelus dromedarius* serum as they comprise approximately 50% of total serum antibodies (Hamers-Casterman et al., 1993). They also were found in milk, their high stability allows them to participate in natural preservation of camel milk in deserts with out using refrigerators (El-Hatmi et al., 2007). In contrast to conventional antibodies, camel heavy chain antibodies and their derived nanobodies showed great stability under different conditions. Dumoulin et al. (2002) studied the effect of different denaturing agents including guanidinium chloride, urea, temperature and pressure on the conformational stability of nanobodies. Omidfar et al. (2007) tested the thermal stability of *Camelus bactrianus* nanobodies, heavy chain and conventional antibodies. They found that IgG_1 lost approximately 40% of its functionality after 50 hours incubation at 37°C, whereas IgG_2 and IgG_3 retained 88% of its functionality after 100 hours incubation at 37°C. Here In our study; camel IgG_3 antibodies showed high resistance to extreme pH values as they retained 90% of their binding activity after 2 h incubation at pH 2. They also showed significant resistance to thermal denaturation at high temperatures (>70°C). Interestingly, these IgG_3 antibodies conserved 60% of their binding activity after one week incubation at room temperature and 55% after 21 days.

Materials & methods:

Animals. Two female camels (*Camelus dromedaries*) from Al-Sanable, Dhulail (Jordan) were used. One as a control and the other has been vaccinated four times subcutaneously with 5 ml of commercial vaccine (Neobactor Syva, Spain). This vaccine contains *Salmonella typhimurium* & *Escherichia coli* (various types). Blood and milk samples were collected by specialist veterinary on weekly intervals immediately before each immunization. Isolated sera & collected milk samples were stored at -20 ºC.

Purification of camel IgG_3. This was done according to Hamers-Casterman method. Then SDS-PAGE & immunoblotting were done to determine the purity of purified IgG_3.

In vitro thermal and pH stability measurement of camel IgG_3. Camel serum, milk, IgG_3 and human serum samples were incubated in water bath at different temperatures (25, 37, 40, 50, 60, 70, 80, 90°C and at boiling temperature 98°C) for 1 hour each. Activity for camel serum, human serum and camel IgG_3 was measured after incubation at 70, 80 and 90°C for 5, 10, 15 and 20 min. For pH stability measurement; all samples were incubated for 2 hours at room temperature in phosphate buffered saline with different pH values (2, 4, 6 and 7). Pepsin digestion of camel IgG_3 was performed by dialysing camel IgG_3 against 0.2 M sodium acetate buffer pH (4.5) and adjusted to 2 mg/ml. Pepsin powder was dissolved in the same buffer (1 mg/ml), then digestion was performed at 1 µg of pepsin: 20 µg of antibody. After 30, 45 and 90 min at 37 °C, digestion reaction was stopped by the addition of 40 µl of 2 M Tris base. For thermal, pH and pepsin digestion ELISA was performed in comparison with control sample.

Results:

Using protein-A as a detection system; camel IgG_3 has optimum activity at 40°C. Pure IgG_3 have higher activity at higher temperatures (above 60°C) when compared with milk and serum activities. Activity of camel serum, human serum and purified camel IgG_3 was detected by protein A after incubation of all samples at 70, 80 and 90°C for 5, 10, 15 and 20 min (Fig1 & 2). There were no differences in activity reduction at 70°C. At 80 and 90°C; human and camel sera lost their activity, while purified camel IgG_3 retained approximately 20% and 15% at 80°C and 90°C respectively. Thermal stability at room temperature for purified camel IgG_3 was measured. It was

found that IgG_3 antibodies retained 62% and 60% of their activity within one week toward *Salmonella* and *E.coli* respectively. An important finding that these antibodies retained 55% of their activity after 21 days incubation at room temperature. *In vitro* pH stability of camel serum antibodies and human serum as well as camel IgG_3 were tested by measuring their activity towards *Salmonella* and *E.coli* antigens after incubation at different pH for 2 hours at room temperature. At pH 2; human serum lost 70% of its activity, while camel serum and IgG_3 retained 80% and 90% of their activity, respectively. Proteolytic stability of camel IgG_3 was measured by measuring their reactivity towards *Salmonella* and *E.coli* antigens after pepsin digestion. Our results showed that camel IgG_3 retained 35% of its binding activity .

References

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