Thesis Title Chromatography of Horse Antivenom Antibody on Anionic Ion Exchangers

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ABSTRACT

A study was carried out on the fractionation of horse antivenom on two anionic ion-exchangers i.e., DEAE-Sepharose and Q-Sepharose. Dialyzed serum and ammonium sulfate precipitated immunoglobulins of horse anti-cobra (Naja naja siamensis) antivenom were used. An ELISA, specifically detecting the antibody against cobra principal postsynaptic neurotoxin, was employed to follow and to quantitate the antibody during the fractionation. SDS-PAGE was also used to study the serum protein species involved.

Horse antivenom serum was precipitated with ammonium sulfate at various concentrations. It was found that at 30% of the salt, IgG with no antibody activity was preferentially precipitated. At 40-50% ammonium sulfate, the antivenom antibody, the IgG(T), was precipitated, completely at 50% of the salt. The total recovery of the antibody activity was 55-60%.

When ammonium sulfate precipitated horse immunoglobulins were chromatographed on DEAE-cellulose or DEAE-Sepharose, similar profiles were observed. Under the conditions used (20 mM Tris HCl, pH 8.0), IgG was weakly bound to the column while IgG(T) could be eluted, inseparable from albumin, by a sodium chloride gradient. Chromatography at different buffer pH's i.e., 7.5, 8.0, 8.4 and 8.8 did not achieve any better separation of these proteins.

An improved fractionation of IgG(T) from IgG and albumin was observed with Q-Sepharose, even when dialyzed horse serum was studied. Under the conditions, an optimal binding capacity of the Q-Sepharose was about 1.5 ml serum per ml of gel. Using a stepwise gradient elution of 130 mM, 200 mM and 500 mM sodium chloride, it was found that a substantial purification of 1.48 fold could be achieved with 75% antibody activity in the fraction eluted by 200 mM of salt.

These results should be useful to the preparative scale fractionation of horse antibody and its pepsin digested fragment of $F(ab)'_2$.