IgA IMMUNOGLOBULIN FROM PORCINE SERUM

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Summary

The isolation and immunologic characterisation of a porcine serum immunoglobulin is described. The isolated protein is shown to be distinct from IgG_1, IgG_2 and IgM but to share antigenic determinants with a colostral immunoglobulin, thought to be equivalent to human secretory IgA, which possesses additional determinants. It is suggested that the isolated serum immunoglobulin is equivalent to human serum IgA and that the pig possesses an IgA system analogous to that found in man.

The isolation of an immunoglobulin from porcine colostrum and milk considered to be equivalent to human secretory IgA (for nomenclature see ref.1) has been reported (Bourne[2]). In addition its presence in serum was demonstrated when whole porcine serum was compared with colostral IgA on an Ouchterlony plate using rabbit-raised antisera to colostral IgA absorbed with IgG and IgM. The present communication reports the isolation of this protein from porcine serum and confirms its relationship to colostral IgA. It has been shown to have a β_2 electrophoretic mobility, share some antigenic determinants with IgG_1, IgG_2 and IgM but also possess specific determinants. Antigenic determinants are shared with colostral IgA but 11S secretory IgA molecules possess determinants not present on the isolated protein. It is suggested that the isolated immunoglobulin is equivalent to human serum IgA.

Blood from adult sows was collected at slaughter into sterile containers, incubated for 60 min at 37° and stored overnight at 4° to allow the clot to retract. The serum was decanted and centrifuged at 3000 x g for 15 min at room temperature. Lipoprotein was precipitated by adding to each ml of serum 0.02 ml of 10% (wt.vol.) dextran sulphate 500 (Pharmacia, Uppsala) followed by 0.1 ml 1M $CaCl_2$. The heavy precipitate that formed was removed by centrifugation at 10,000 x g for 10 min at 4°.

100 ml of the supernatant, containing 6g protein, was fractionated by reverse-flow exclusion chromatography on Sephadex G-200 (column size 10 cm x 100 cm) after extensive dialysis against water and then against 0.1M Tris-HCl 1M NaCl buffer, pH 8.0 which was used for elution. Three peaks were found by monitoring at 280 mu. Fractions from the descending limb of the first peak and the trough between the first and second peak (Fractions 69-78 Fig.1) were pooled, dialysed against distilled water and concentrated with carbowax (Gurr Ltd., London).

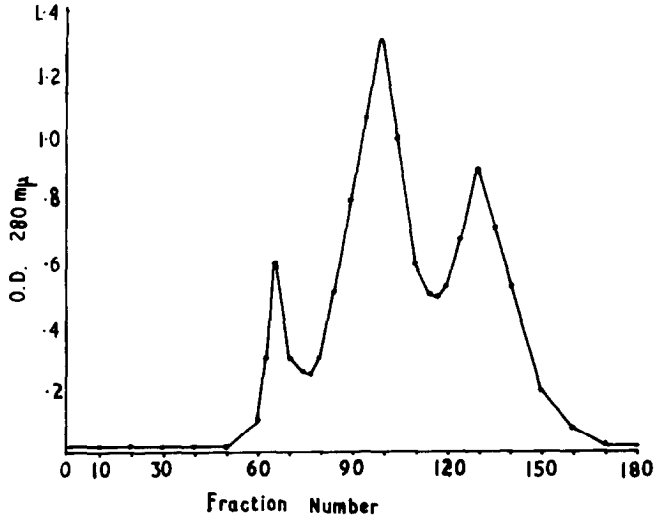

Fig. 1 Gel-filtration of lipoprotein-free porcine serum on Sephadex G-200. A 100 ml portion of serum, containing 6g of protein, was applied to a column 10 cm x 100 cm. Effluent was collected in 30 ml fractions.

The concentrated fraction was then exhaustively dialysed against 0.02M Tris-HCl buffer, pH 7.4 and applied to a column (1.8 cm x 90 cm) of DEAE-cellulose (DE52 Whatman Ltd., London) which was then eluted by the following stepwise changes of molarity at the same pH: 0.02, 0.05, 0.075, 0.10, 0.125 and 0.15.

The protein that was identified as serum IgA was eluted at 0.075M and 0.1M buffer. These fractions were pooled, concentrated and after dialysis against the Tris-HCl-NaCl buffer, pH 8.0 applied to Sephadex G-200 (column size 2.5 cm x 100 cm) and eluted with the same buffer using reverse-flow. A double peak was obtained and the second was collected (Fractions 80-92 Fig.2), concentrated and recycled on Sephadex G-200 under the same conditions. A single peak was obtained and fractions from the apex were pooled, dialysed and concentrated. The concentrated material was exhaustively dialysed

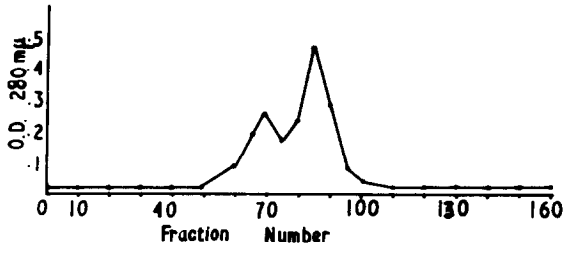

Fig. 2 Gel-filtration of partially purified serum IgA immunoglobulin on Sephadex G-200. A 5 ml sample, containing 30-40 mg of protein, was applied to a column 2.5 cm x 90 cm. Effluent was collected in 3 ml fractions.

against 0.05M barbital buffer pH 8.6 and fractionated electrophoretically, using the same buffer, in 1% agar (Oxoid 1.D. Agar) on glass plates (10 cm x 20 cm). A central trough was cut in the agar to take 0.5 ml of the concentrated material (about 5 mg protein) and electrophoresis was carried out for 2 hours at 4° with a constant voltage of 30 V/cm. A strip of

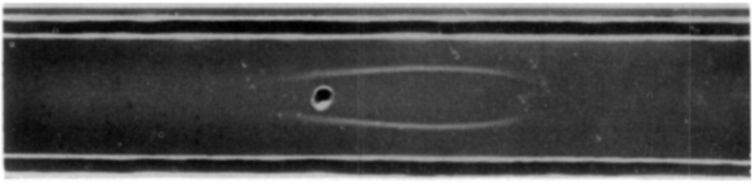

Fig. 3 Immunoelectrophoresis of porcine serum IgA. The troughs contain rabbit antiserum against porcine whole serum.

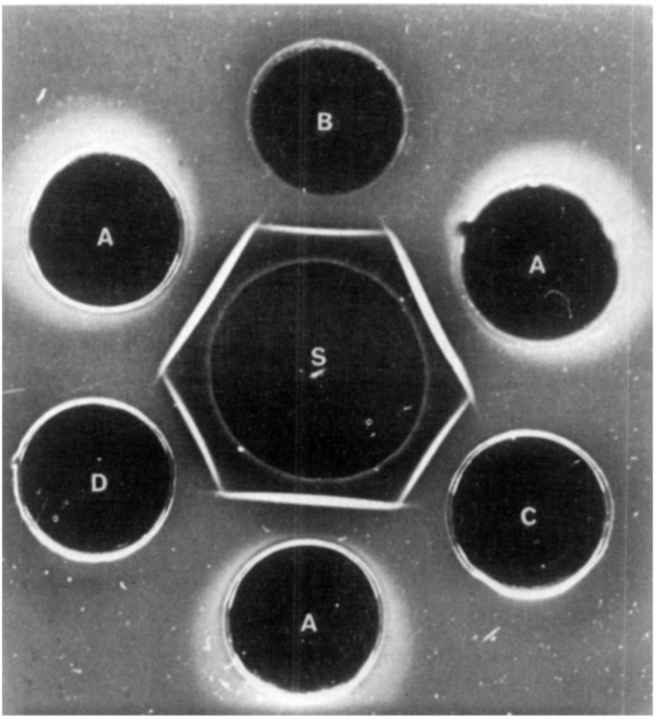

Fig. 4 Immunodiffusion of porcine serum IgA, IgG_1, IgG_2 and IgM with antiserum raised against porcine serum IgA.

A - serum IgA
B - IgG_1
C - IgM
D - IgG_2
S - rabbit-raised porcine serum IgA antiserum

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agar 1.5 cm wide was cut from the cathodal side of the trough and the protein eluted in saline by freezing at -20^{o}. On thawing the

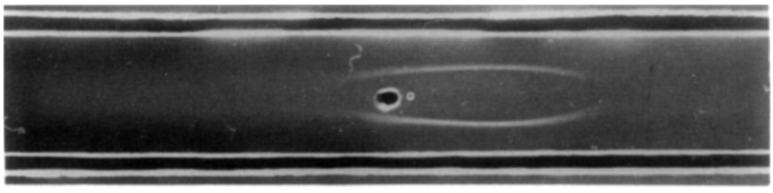

Fig. 5 Immunoelectrophoresis of porcine serum. The troughs contain rabbit antiserum against porcine IgA made specific by absorption with IgG_1, IgG_2 and IgM

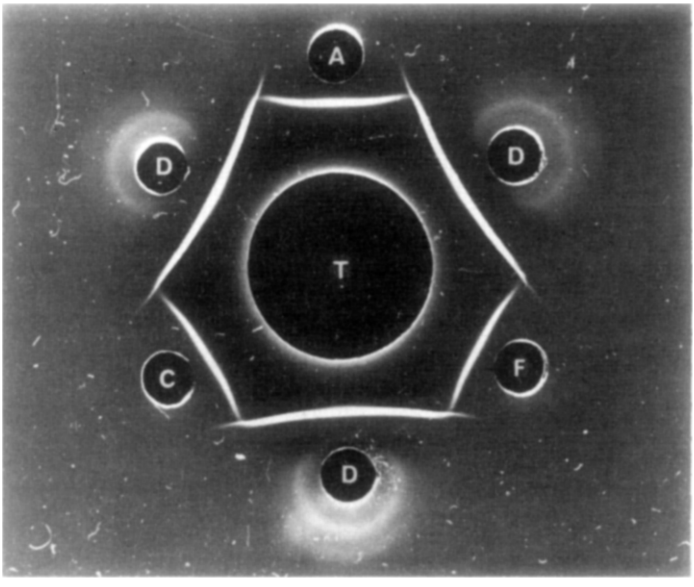

Fig. 6 Immunodiffusion of porcine IgG_2, IgM, serum IgA and 11S colostral IgA with antiserum raised against IgG_2

A - serum IgA
D - IgG_2
C - IgM
F - 11S colostral IgA
T - rabbit-raised IgG_2 antisera

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mixture was centrifuged at 10,000 x g for 10 min at room temperature and the supernatant dialysed and concentrated. Immunoelectrophoresis of this fraction against a rabbit-raised antiserum to whole porcine serum showed a single arc in the β_2 position (Fig.3).

The antisera raised against the isolated protein in rabbits showed a reaction with IgG and IgM immunoglobulins on immunoelectrophoresis of porcine serum. The antigenic relationship to the immunoglobulins thus shown was confirmed on a Ouchterlony plate comparing IgG_1, IgG_2 and IgM (Bourne[3]) with the isolated IgA using the technique described by Fahey & McLaughlin[4], which also showed that the isolated protein possessed specific antigenic determinants (Fig.4). Absorption of the antisera with IgG_1, IgG_2 and IgM rendered it specific for the serum isolate and immunoelectrophoresis of whole serum against this antisera showed a single arc in the β_2 position (Fig.5). A comparison of the isolated immunoglobulin and of IgM with IgG_2 using rabbit-raised antisera to porcine IgG_2 on an Ouchterlony plate further confirmed their relationship (Fig. 6).

When rabbit-raised antisera to the isolated serum immunoglobulin, made specific for this protein by absorption with IgG_1, IgG_2 and IgM, was used on an Ouchterlony plate to compare

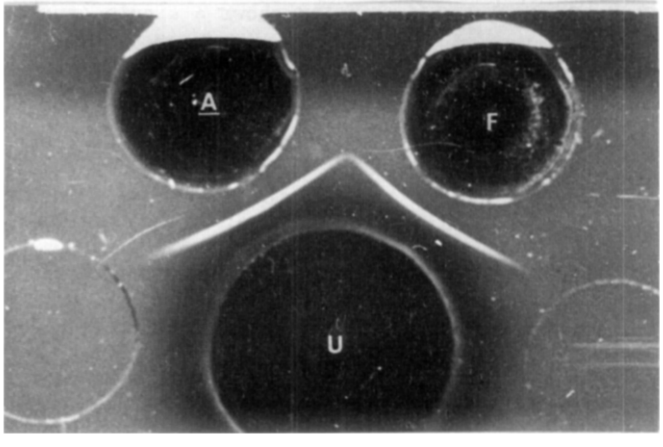

Fig. 7 Immunodiffusion of porcine serum IgA and 11S colostral IgA showing a reaction of identity.

A - serum IgA
F - colostral 11S IgA
U - antisera to serum IgA made specific by absorption with IgG_1, IgG_2 and IgM.

the serum immunoglobulin with colostral IgA, they were shown to share antigenic determinants (Fig. 7). When this examination was repeated using antisera specific to colostral 11S IgA a single spur of secretory IgA formed over the isolated serum immunoglobulin, showing that colostral 11S IgA possessed antigenic components not present on the serum protein (Fig. 8).

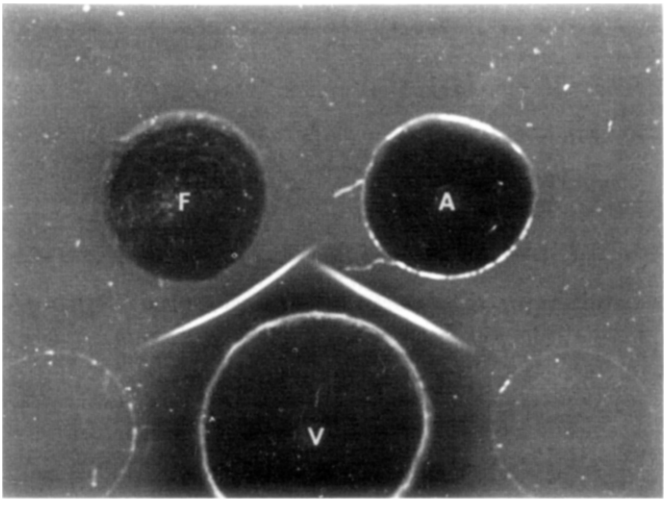

Fig. 8 Immunodiffusion of porcine serum IgA and 11S colostral IgA. The colostral immunoglobulin shows a single spur over serum IgA.

A - serum IgA
F - 11S colostral IgA
V - antisera to 11S colostral IgA made specific by absorption with IgG_1, IgG_2 and IgM.

The presence in porcine serum of an immunoglobulin distinct from IgG_1, IgG_2 and IgM whose antigenic determinants are shared by a colostral immunoglobulin which possesses additional determinants suggests that the isolated serum immunoglobulin is equivalent to human serum IgA (Tomasi et al.[5]). Thus it appears that the pig possesses an IgA system analogous to that found in man. Further work is being carried out on the characterisation of porcine IgA and its comparison with human IgA which will be reported in greater detail later.

Acknowledgements

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