IDENTIFICATION OF SERUM PROTEINS FOLLOWING DIFFERENT STAGES OF SODIUM SULFATE PRECIPITATION OF CHICKEN SERUM*

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Abstract—1. Immunoglobulin G and IgM can be separated from serum by the addition of different concentrations of sodium sulfate.

2. Acrylamide gel electrophoresis of the resulting supernatants and precipitates revealed that some IgG and IgM are lost during the separation procedure.

3. Bands 4–9 on an acrylamide gel have not been identified and have been difficult to separate from major transferrin.

4. Acrylamide gel electrophoresis has revealed the presence of bands 4–9 and not major transferrin in the precipitate resulting from the addition of 9% sodium sulfate.

INTRODUCTION

BENEDI~ (1967) proposed the use of sodium sulfate for the fractionation of immunoglobulins in chicken serum. This procedure is extensively used for the isolation, and purification of IgG, IgM and alpha 2 macroglobulin (Leslie & Clem, 1969, 1972; Leslie & Benedict, 1970). In the present investigation, disc electrophoresis was employed to study each step of Benedict's procedure for the presence of immunoglobulins and other serum proteins.

MATERIALS AND METHODS

Serum (60 ml) was collected from 5-week-old New Hampshire birds and the salting out procedure of Benedict (1967) was performed. The fractionation of serum proteins was begun by adding 18 g% of sodium sulfate to the serum. The precipitate was collected following centrifugation at 1500 rev/min for 1 hr. The supernatant was separated and the precipitate dissolved in one-half of the original serum volume in phosphate buffered saline (PBS), pH 7.5. Solid sodium sulfate was added at the level of 14 per cent of the volume in ml. The supernatant and precipitate were collected as before, the precipitate was dissolved in one-quarter of the original serum volume in PBS and solid sodium sulfate added at the level of 14 per cent of the volume in ml. The resulting precipitate was separated from the supernatant, and dissolved in one-quarter of the original serum volume in PBS and dialyzed at 4°C for 16 hr against PBS. The dialysate was precipitated by adding 9% sodium sulfate.

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and then centrifuged. The 9% precipitate was dissolved in PBS and dialyzed against several changes of distilled water at 4°C.

The samples of supernatant and precipitate from each step of the procedure were electrophoresed on a 7% acrylamide gel (Davis, 1964). Relative mobility (RM) values for each band were obtained by dividing their distance from the origin by the distance of major transferrin from the origin. Reference to a standard electropherogram based on RM values identified each band by a number and in some cases named the band (Glick, 1968, 1973).

RESULTS AND DISCUSSION

The 18 per cent precipitate contained IgM, IgG, acetylcholinesterase (band 14), band 12, minor transferrin (band 11), major transferrin (band 10), bands 4–9 and albumin (Fig. 1). IgG appeared to be more concentrated in the precipitate than in the 18 per cent supernatant while IgM concentration in the precipitate was similar to the 18 per cent supernatant.

The second 14 per cent supernatant showed distinct IgM, IgG, albumin, major and minor transferrin, band 12 and 4–9 bands (Fig. 2).

The second 14 per cent precipitate contained IgG, a faint IgM and albumin (Fig. 2). The concentration of IgG was similar to the 14 per cent supernatant.

The 9 per cent supernatant contained IgG, IgM and a faint albumin (Fig. 3). Concentrating this fraction by one-half intensified IgG and revealed the presence of band 14 (acetylcholinesterase) and bands 4–9.

The 9 per cent precipitate contained IgG. A band appeared close to the position of IgM, but we were unable to identify it as IgM by immunoelectrophoresis. This band may have been alpha 2 macroglobulin. Albumin appeared as a faint band.

Disc electrophoresis of the samples of supernatant and precipitate revealed that variable amounts of IgM and IgG are lost during the sodium sulfate procedure. Further precipitation of these samples should improve the collection of Ig.

A major goal of this laboratory has been to identify the various serum proteins of the chicken. Application of serum on a G-100 Sephadex column has been successful in separating IgG from bands 4–9 but not in separating bands 4–9 from transferrin (Kulkarni, 1972). Our success in effecting the latter separation with sodium sulfate should help us in our program to purify and characterize bands 4–9.

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Fig. 1. Disc electrophoretic pattern of the precipitate (left) and supernatant (right) following the addition of 18 g % of sodium sulfate to chicken serum: A, albumin; P1, prealbumin; and T. tracer.

Fig. 2. Disc electrophoretic pattern of precipitate (left) and supernatant resulting from the second addition of 14 g % of sodium sulfate: A, albumin.

Fig. 3. Disc electrophoretic pattern for the concentrated (left) and unconcentrated (right) supernatant resulting from the addition of 9% sodium sulfate: A, albumin.
REFERENCES


Key Word Index—Serum proteins; Sodium sulphate precipitation; IgM; IgG.