PROTEIN A FROM *STAPHYLOCOCCUS AUREUS**. ITS ISOLATION BY AFFINITY CHROMATOGRAPHY AND ITS USE AS AN IMMUNOSORBENT FOR ISOLATION OF IMMUNOGLOBULINS

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Received 10 July 1972

1. Introduction

Protein A from *Staphylococcus aureus* is covalently linked to the peptidoglycan part of the cell wall [1, 2]. It can be released from the cells by digestion with lysostaphin, and this procedure is the initial step in the isolation of the protein from the bacteria [1]. Further purification of the protein has involved acid and salt precipitation, ion exchange chromatography and gel filtration. The yield of protein A by this technique is approx. 50% of the total amount of the protein in the cell [1].

Since protein A reacts with the Fc-part of IgG [3, 4], non-immune IgG or Fc from IgG coupled to Sepharose can be used as an immunosorbent for isolation of the protein. Conversely, protein A bound to Sepharose can be used as an immunosorbent for isolation of IgG from several species as well as for the isolation of the Fc-fraction.

This article describes the isolation of protein A from *S. aureus* and of IgG from human serum by affinity chromatography. In addition, several uses for protein A bound to a solid support as an immunosorbent will be discussed.

2. Materials and methods

S. aureus, strain Cowan I, was cultured as previously described [1]. Human IgG was kindly supplied by Kabi AB, Stockholm and Sepharose 4B was obtained from Pharmacia Fine Chemicals, Sweden, cyanogen bromide (puriss) from Fluka, AG, Switzerland; all other chemicals were reagent grades.

Coupling of IgG or protein A to Sepharose 4B by CNBr performed essentially as described [5, 6]. To 100 ml washed and packed Sepharose 4B, 10 g CNBr was added. 120 ml of a 2.5% IgG solution was then added to the activated Sepharose. The reaction was allowed to proceed at 4° for 3–4 hr with slow stirring at pH 9.0. The Sepharose was recovered by filtration, suspended in 1.5 l of 0.05 M 2-amino-ethanol – 0.2 M $NaHCO_3$, pH 9.0, and stirred overnight at 4°. The immunosorbent complex was finally washed in 0.1 M $H_2PO_4^-$ – 4 M urea, pH 6.0, and then in 0.1 M $H_2PO_4^-$, pH 7.0, until the A_{280} of the eluate was less than 0.01. The immunosorbent was then thoroughly washed in 0.1 M $H_2PO_4^-$, pH 7.0, on a sintered glass funnel. The gel was stored at 4° with sodium azide added to 0.02%. Approx. 98–99% of added IgG was bound to the Sepharose by this procedure. When protein A was coupled to Sepharose 4B, 4 mg of the protein per ml of packed Sepharose was used. In this case essentially all protein A was bound to the gel.

3. Results

In order to isolate protein A from *S. aureus*, the bacteria were digested with lysostaphin as previously described [1]. The suspension from this digest was centrifuged at 4°, the supernatant was brought to pH

* Part XXIII of a series.

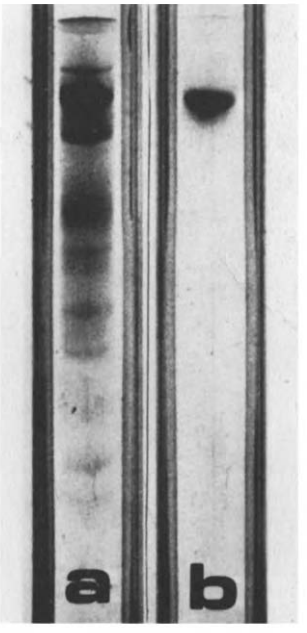

Fig. 1. Disc electrophoresis in 11% polyacrylamide gels, pH 8.9 of a) the neutralized supernatant recovered after acid precipitation before passage through the IgG-Sepharose column and b) protein A released from the immunosorbent with 0.1 M glycine-HCl, pH 3.0. See Results.

3.5 with 5 M HCl, and the precipitate formed was discarded after centrifugation. The amount of protein A in this precipitate was negligible. The recovered supernatant was neutralized with 5 M NaOH and passed through a Zeiss ultrafilter and then through a column of IgG-Sepharose. The column was washed with 0.1 M $H_2PO_4^-$, pH 7.0, until the absorbancy at 280 nm of the eluate was less than 0.02. Protein A was eluted from the column with 0.1 M glycine-HCl, pH 3.0, and the pH of the column was restored to neutrality by washing with phosphate buffer. The immunosorbent contained 30 mg IgG per ml packed gel, capable of binding approx. 3 mg protein A. The same batch of immunosorbent has been used several times without noticeable loss in capacity to bind protein A.

In a typical isolation experiment 400 g of wet bacteria was used. After digestion with lysostaphin, acid precipitation and filtration through the Zeiss filter, 2 l of solution was recovered, which was divided into 4 equal parts. Each was applied to a 200 ml packed IgG-Sepharose column. The recovery of protein A from 400 g bacteria was approx. 800 mg, which is a 50% higher yield than by the method previously used [1]. In disc electrophoresis the product showed only one band (fig. 1).

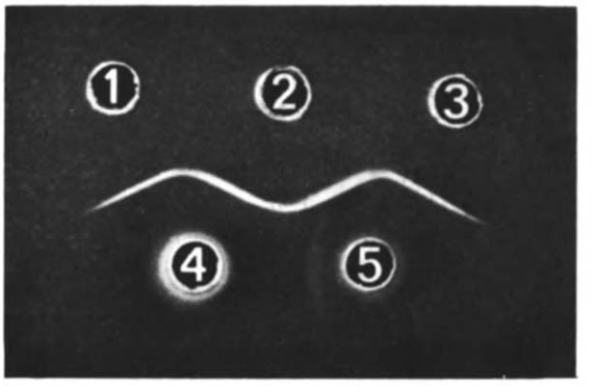

Fig. 2. Immunodiffusion in 1% agarose gel, 0.01 M Tris acetic acid, pH 8.0, of protein A against 4) dog serum and 5) rabbit anti-protein A serum. Numbers 1) and 3) correspond to protein A recovered from the IgG-Sepharose column and 2) protein A prepared according to an earlier method [1].

The amino acid composition and the molecular weight of the isolated product were identical to those of the material previously isolated by a different technique [1]. The products also gave a reaction of identity in gel diffusion against dog serum and rabbit anti-protein A serum (fig. 2).

The second type of immunosorbent, protein A–Sepharose 4B, contained 4 mg protein per ml packed gel and could bind 20 mg of IgG. When normal human serum was passed through the immunosorbent all of the serum IgG was retained. After adding the serum the column was washed with 0.1 M $H_2PO_4^-$ or 0.1 M ammonium acetate until the absorbancy at 280 nm of the eluate was less than 0.02. IgG was then eluted with 0.1 M glycine-HCl, pH 3.0. The recovery of IgG was approx. 95%. Immunoelectrophoresis shows that the fraction eluted with glycine buffer, pH 3.0, appears to be of pure IgG (fig. 3).

4. Discussion

Isolation of protein A has previously been a tedious procedure. By utilizing the known reaction of protein A with the Fc-part of IgG, a method for purifying the protein has been developed based on immunosorption to a complex of IgG-Sepharose 4B.

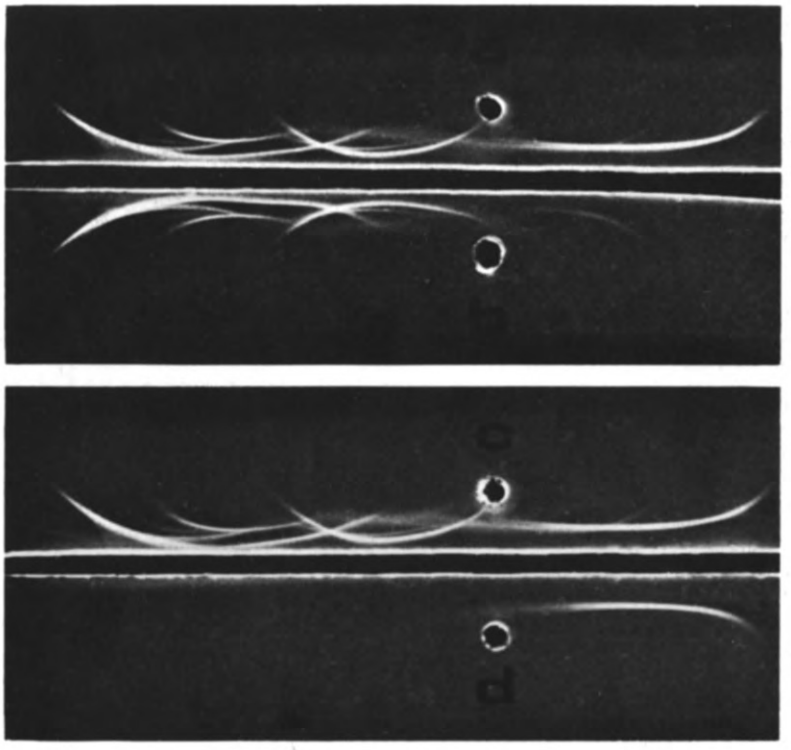

Fig. 3 Immunoelectrophoresis in 1% agarose gel, 0.01 M Tris-acetic acid, pH 8.0, against rabbit anti-human serum of a) and c) normal human serum, b) human serum after passage through the protein A-Sepharose column and d) the IgG recovered from the column after elution with glycine buffer, pH 3.0.

This step is applied directly after the release of the protein from the bacteria by lysis with lysostaphin. It is rapid and convenient and gives a pure product in high yield. The immunosorbent has also been applied to the isolation of extracellular protein A as well as reactive fragments obtained after chemical and enzymatic cleavage of the intact protein. IgG-Sepharose or Fc-Sepharose can be used in screening for other substances which react with the immunoglobulin in a way similar to protein A.

Conversely, protein A coupled to Sepharose 4B can be conveniently used to remove and isolate IgG from serum of different species. The immunosorbent will also remove IgG from preparations of other immunoglobulin classes and thus facilitates the preparation of IgA, IgM, IgD and IgE, which do not react with protein A. As it has been shown that human IgG3 does not react with protein A [7], the immunosorbent can be used to separate this subclass from the others. The immunosorbent of course binds Fc and is therefore suitable for isolation of Fc from Fab and for isolation of fragments of Fc with retained activity against protein A. Using the device described by Edelman et al. [8], protein A bound to nylon fibers could be used as a tool for isolation of lymphoid cells with IgG receptor molecules on their surfaces.

Protein A coupled to Sepharose is superior to anti-IgG as an immunosorbent in that adsorbed immunoglobulins are easily removed and the same adsorbent can be used for isolation of immunoglobulins from different species.

Acknowledgements

This investigation was supported by grants from the Swedish Medical Research Council and from the Medical Faculty, University of Uppsala, Uppsala, Sweden.

References

[1] J. Sjöquist, B. Meloun and H. Hjelm, European J. Biochem., in press.
[2] J. Sjöquist, J. Movitz, I.-B. Johansson and H. Hjelm, European J. Biochem., in press.
[3] A. Forsgren and J. Sjöquist, J. Immunol. 97 (1966) 822.
[4] J. Sjöquist, A. Forsgren, G.T. Gustafson and G. Stålenheim, Cold Spring Harbor Symp. Quant. Biol. 32 (1967) 577.
[5] R. Axén, J. Porath and S. Ernback, Nature 214 (1967) 1302.
[6] P. Cuatrecasas and C.B. Anfinsen, Ann. Rev. Biochem. 40 (1971) 259.
[7] G. Kronvall and R.C. Williams, Jr., J. Immunol. 103 (1969) 828.
[8] G.M. Edelman, U. Rutishauser and C.S. Milette, Proc. Natl. Acad. Sci. U.S. 68 (1971) 2153,