PURIFICATION OF PROTEIN A FROM *STAPHYLOCOCCUS AUREUS* BY AFFINITY CHROMATOGRAPHY ON POLYACRYLAMIDE ACTIVATED WITH THIOPHOSGENE

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1. Introduction

Affinity chromatography using modified cellulose and cross-linked dextrans has been used extensively for the purification of a large variety of proteins. The development of simple procedures for the activation of these polymers by cyanogen bromide has greatly expanded their use. The use of polysaccharides [1,2] as the solid matrix in some applications is hampered by their chemical instability and nonspecific adsorption.

The utilization of chemically modified cross-linked polyacrylamide beads as a support medium would greatly enhance chemical stability. Moreover, the polyethylene structure of this material would not be degraded by hydrolytic enzymes and it would not bind proteins by nonspecific adsorption [3,4]. However, the use of polyacrylamide beads in affinity chromatography is limited by the lack of simple and less time consuming procedures for activation and derivatization of the polyacrylamide. A number of specific ligands have been covalently attached to polyacrylamide using lengthy chemical procedures [4]. Proteins have been coupled directly to polyacrylamide after activation of the beads with glutaraldehyde, but only relatively low ratios of ligand to gel were obtained [5].

The present communication describes a very simple procedure for the activation of polyacrylamide. Thiophosgene was found to be a promising activating agent for the direct coupling of proteins to polyacrylamide beads. Thiophosgene reacts with carbamyl groups in polyacrylamide. Two moles of hydrochloric acid are released for each mole of thiophosgene which reacts. Presumably, a reactive isothiocyanate intermediate is formed. Protein is then attached to this reactive intermediate under the same conditions as those used to couple proteins to Sepharose 4B which has been activated with cyanogen bromide [2]. The utility of this procedure was demonstrated by using polyacrylamide beads containing covalently attached porcine IgG for the isolation of protein A from extracts from *Staphylococcus aureus*.

2. Materials and methods

Bio-Gel-P300 was obtained from Bio-Rad labs, Richmond, CA. The gel was suspended in distilled water for 24 h and it was washed twice before use. Thiophosgene was purchased from Aldrich Chemical Co., Milwaukee, WI. This reagent is toxic and all containers should be washed with an organic solvent and ammonium hydroxide after use. Porcine gamma globulin was obtained from Pentex, Kankakee, IL and lysostaphin was purchased from Mann and Swartz.

Protein was estimated by the procedure of Lowry et al. [6]. Polyacrylamide disc-gel electrophoresis was run according to the method of Davis [7] and gels were stained in 3.5% perchloric acid containing 0.04% Coomassie brilliant blue G-250 [8]. *Staphylococcus aureus*, ATCC 12598 was generously provided by Dr Earl Edwards, Naval Health Research Center, San Diego, CA. The cells were grown in CCY broth [9] at 37°C and they were harvested after 24 h. A crude extract of protein A was prepared by digesting the cells with lysostaphin [10].

3. Results

3.1. *Preparation of activated polyacrylamide*

One gram of Bio-Gel P-300 was suspended in 100 ml 80% *N,N*-dimethyl formamide with stirring. Afterwards 1.0 ml of thiophosgene was added and the reaction was allowed to proceed at room temperature for 1 h in a very well ventilated fume hood. The solution became orange initially and gradually turned colorless as the pH decreased. The modified polyacrylamide beads were filtered under vacuum on a Buchner funnel containing a coarse disc. The beads were washed three times with 100 ml absolute ethanol, dried under suction, and quickly transferred to a solution containing protein.

3.2. *Polyacrylamide-porcine IgG*

An immunosorbent column containing covalently bound porcine IgG was prepared using polyacrylamide activated with thiophosgene. Porcine IgG which was purified by chromatography on DEAE cellulose, was dissolved in 0.1 M Na_2CO_3, pH 9.5–0.1 M NaCl. The activated Bio-gel P-300 was added to 15 ml of this solution containing 20 mg/ml porcine IgG and it was incubated at 4°C for 20 h. The gel was then poured into a column and washed extensively with 0.05 M Tris–HCl, pH 8.0 containing 0.1 M ethanolamine. The column was finally equilibrated with 0.1 M sodium phosphate, pH 7.0. The polyacrylamide beads contained 5.5 mg of bound IgG/ml swollen gel. Several other proteins including bovine serum albumin and rabbit IgG were attached to the activated gel by the same procedure.

3.3. *Purification of protein A from* Staphylococcus aureus

A crude extract of *S. aureus* was applied to the Bio-gel P-300-porcine IgG column (2 × 5 cm) and the column was washed with 0.1 M sodium phosphate, pH 7.0, until the absorbancy at 280 nm was less than 0.02. Protein A was eluted from the column with 0.1 M glycine-HCl, pH 3.0 [11]. The protein was eluted in a single symmetrical peak. The active fractions were pooled, concentrated and dialyzed against 0.01 M Tris–HCl, pH 8.0. The preparation was homogeneous as determined by polyacrylamide disc-gel electrophoresis and sucrose density centrifugation. The purified protein A preparation showed

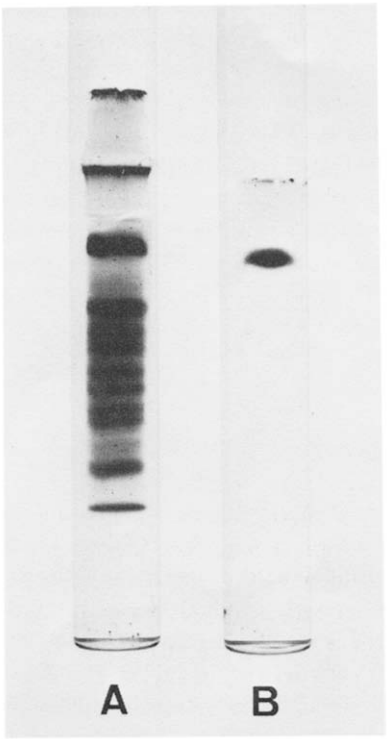

Fig.1. Analytical disc-gel electrophoresis of protein A. Gel A, crude extract of *S. aureus*. Gel B, 50 μg purified protein A.

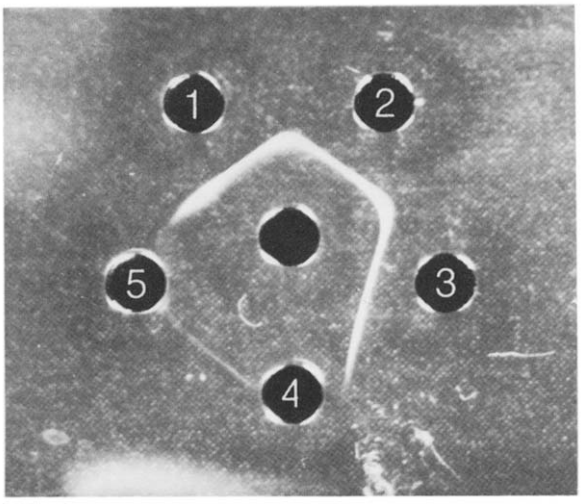

Fig.2. Analysis of purified protein A by immunodiffusion. The reaction was carried out in 1% agarose gel with 0.01 M Tris–HCl, pH 8.0. The center well contained purified protein A. Well 1, Porcine IgG; well 2, Human IgG; well 3, Goat IgG; and wells 4 and 5, Rabbit IgG.

only a single band on electrophoresis at pH 8.0 as seen in fig.1, gel B. Only one band was also observed at pH 7.0 and pH 9.5. None of the other components present in the crude extracts, fig.1, gel A, were detected, even when very large amounts of protein were layered on the top of the gels.

As seen in fig.2, the purified preparation showed only a single line when examined by immunodiffusion against porcine IgG, human IgG and goat IgG. The isolated protein A showed characteristic biological properties in its ability to interact specifically with a number of IgG samples from different species.

4. Discussion

The results obtained in the present study clearly show that thiophosgene can be used to activate polyacrylamide for the preparation of protein derivatives that retain their biological activity. The activation procedure provides an easy method for coupling proteins directly onto polyacrylamide beads under mild reaction conditions. The insolubilized derivative of porcine IgG used to isolate protein A in the present study retained more than 80% of its antigen binding capacity after attachment to polyacrylamide beads [10]. The activation process yielded a derivative with a very high ligand to gel ratio. The amount of porcine IgG bound was consistently greater at 5 mg protein/ml gel.

In the isolation of protein A from extracts of *S. aureus* the column showed little or no nonspecific binding of proteins. A single protein species was removed from the extract by this procedure. The protein A isolated by this method was homogeneous as judged by disc-gel electrophoresis and immunoprecipitation with porcine, bovine and human IgG immunoglobulins. Nonspecific adsorption was not observed even when very large samples of the isolated protein A were examined. The polyacrylamide beads were re-used without any detectable decrease in its antibody binding capacity. The binding of large amounts of protein to the polyacrylamide beads permitted the isolation of very small quantitites of protein A, 5 μg, with quantitative recoveries.

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References

[1] Axen, R., Porath, J. and Ernback, S. (1976) Nature 214, 1302.
[2] Cuatrecasas, P. (1970) J. Biol. Chem. 245, 3059.
[3] Inman, J. K. and Dintzis, H. M. (1969) Biochemistry 8, 4074.
[4] Inman, J. K. (1974) Meth. Enzymol. 34, 30.
[5] Weston, P. D. and Avrameas, S. (1971) Biochem. Biophys. Res. Commun. 45, 1574.
[6] Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265.
[7] Davis, G. J. (1964) Ann. NY Acad. Sci. 121, 404.
[8] Reisner, A. H., Nemes, P. and Bucholtz, C. (1975) Anal. Biochem. 64, 509.
[9] Arvidson, S., Holme, T. and Wadstrom, T. (1971) Acta Path. Microbiol. Scand. Sect. B 79, 399.
[10] Sjoquist, J., Meloun, B. and Hjelm, H. (1972) Eur. J. Biochem. 29, 572.
[11] Hjelm, H., Hjelm, K. and Sjoquist, J. (1972) FEBS Lett. 28, 73.