Journal of Chromatography, 376 (1986) 269—272
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2962

GLYCIDYL METHACRYLATE COPOLYMERS AS SUITABLE SUPPORTS FOR IMMOBILIZING ANTIGENS AND ANTIBODIES

JAN KÂŠ*, MIROSLAV MAREK and JITKA BERANOVÂ

Department of Biochemistry and Microbiology, Prague Institute of Chemical Technology, Suchbátarova 3, 166 28 Prague 6 (Czechoslovakia)

and

FRANTIŠEK ŠVEC

Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Place of Heyrovský 2, 162 06 Prague 6 (Czechoslovakia)

SUMMARY

Glycidyl methacrylate copolymers modified with 1,6-diaminohexane were activated with glutaraldehyde. Such activated supports were directly bound to the chosen antisera. Prepared immunosorbents contained ca. 40 mg of bound protein and were able to purify 2–5 mg of antigen (alkaline proteinases Esperase and Maxatase) per 1 g of solid support. Chaotropic agent (2.5 *M* ammonium thiocyanate in phosphate or Tris buffer) enabled antigen to be released quickly, the properties of the immunosorbent remaining unchanged.

INTRODUCTION

Antigen or antibody purification by affinity chromatography is a very effective procedure, but only when a suitable insoluble affinant (with sufficient stability, capacity, etc.) and a gentle technique of ligand desorption (retaining its original properties) are available. Glycidyl methacrylate copolymers, chemically modified, may be used as suitable supports for enzyme immobilization [1–4]. The aim of this study was to use them for the preparation of immunosorbents.

EXPERIMENTAL

Materials

Macroporous glycidyl methacrylate support (pore volume 0.96 cm^3/g, most

0378-4347/86/$03.50

frequent pore radius 25 nm, specific surface area 60.2 m^2/g, bead fraction 150—250 μm) was prepared in the Institute of Macromolecular Chemistry (Czechoslovak Academy of Sciences, Prague, Czechoslovakia). This support was modified with 1,6-diaminohexane by employing the method described by Švec et al. [2]. Microbial proteinases were commercial technical preparations (Maxatase from Gist Brocades, The Netherlands; Esperase from Novo Industry, Denmark), ovoalbumin was from Difco (U.S.A.) and other chemicals were from Lachema (Brno, Czechoslovakia). All antisera used were prepared by Dr. Paluska from the Research Institute of Haematology and Blood Transfusion (Prague, Czechoslovakia).

Procedure

Modified glycidyl methacrylate support was activated by glutaraldehyde treatment [4]. The activated support was coupled with the corresponding protein (antibody) in 0.05 *M* Tris- HCl buffer (pH 8.5) at 4° C for 16 h. The immobilized antiserum or antigen was then washed with the same buffer to remove all unbound protein. The immunosorbent obtained was used for affinity chromatography of the corresponding antigen or antibody. The adsorbed ligand was eluted with the chaotropic agent (2.5 *M* ammonium thiocyanate in phosphate or Tris buffer, pH 7.0 or 8.5) [5], in batch operation or continuously. The eluted protein was assayed by immunochemical methods (radial immunodiffusion [6]; rocket immunoelectrophoresis [7]), by spectrophotometry and, in the case of proteinases, by determination of proteolytic activity with casein substrate (Delft's method [8]).

RESULTS

Purification of microbial proteinases

Alkaline proteinase Esperase (EC 3.4.21.14) was purified on the immunosorbent in both batch and continuous operations. Figs. 1 and 2 show that the ligand (Esperase) in continuous operation is eluted in a smaller volume and

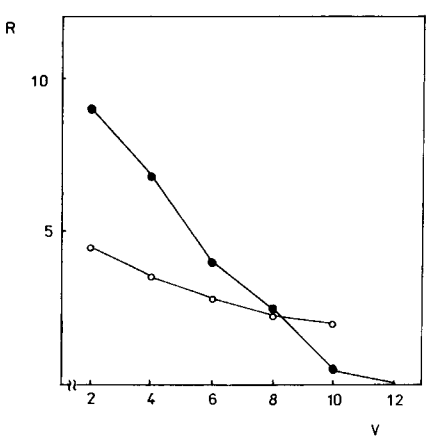

Fig. 1. Elution of Esperase from immunosorbent in continuous and batch operation. R = Rocket height (mm); V = elution volume (ml). (○) Continuous operation; (●) batch operation.

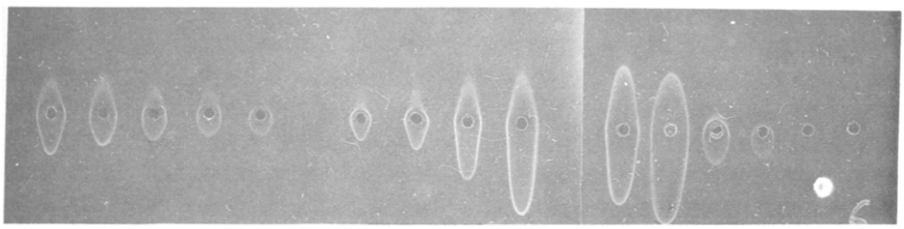

Fig. 2. Evalution of Esperase elution from immunosorbent by rocket immunoelectrophoresis. From left to right: batch operation; calibration; continuous operation.

TABLE I

EVALUATION OF IMMUNOSORBENT PREPARATION AND ITS APPLICATION IN ANTIGEN (ESPERASE) PURIFICATION

Analysed subject	Protein per 1 g of dry support (mg)	
	Batch operation	Continuous operation
Antiserum before immobilization	70	70
Bound protein	44	44
Bound IG fraction (calculated)	7	7
Protein added	7.5	7.5
Enzyme bound	4.1	6.3
Purified enzyme	2.8	4.9

faster than in batch operation. Desorption is gradual and complete. Subsequent washing with buffer in order to remove the chaotropic reagent prepared the column for repeated experiments. No decrease in immunosorbent capacity was observed. The experiments proved that the modified glycidyl methacrylate copolymers may serve for the preparation of suitable immunosorbents, preferably used in continuous operation (Table I).

Another technical proteinase, Maxatase (EC 3.4.21.14), was purified on a

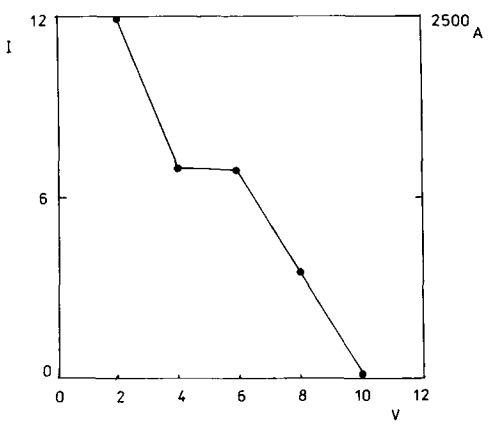

Fig. 3. Maxatase elution from immunosorbent in continuous operation. I = Evaluation by radial immunodiffusion (D^2 squares of zone diameters); A = proteolytic activity (Delft units/ml); V = elution volume (ml).

corresponding immunosorbent in continuous operation. Similar results were achieved under conditions close to those used for Esperase purification. In the latter case, elution of proteinase was assayed by radial immunodiffusion and by measurement of proteolytic activity (Fig. 3).

Other experiments

Similar experimental conditions were used for immunoaffinity chromatography of ovalbumin. Phosphate buffer (pH 7.0) and the same concentration of chaotropic reagent were applied for its elution.

REFERENCES

1 F. Švec, J. Hradil, J. Čoupek and J. Kálal, Angew. Makromol. Chem., 48 (1975) 135.
2 F. Švec, H. Hrudková, D. Horák and J. Kálal, Angew. Makromol. Chem., 63 (1977) 23.
3 J. Drobník, V. Saudek, F. Švec, J. Kálal, V. Vojtíšek and M. Bárta, Biotechnol. Bioeng., 21 (1979) 1317.
4 J. Káš, P. Rauch and L. Fukal, Biotechnol. Lett., 5 (1983) 219.
5 J.W. Eveleigh, in T.C.J. Gribnau, J. Visser and R.J.F. Nivard (Editors), Affinity Chromatography and Related Techniques, Elsevier, Amsterdam, 1982, p. 293.
6 O. Ouchterlony, Prog. Allergy, 6 (1962) 30.
7 C.B. Laurell, Anal. Biochem., 15 (1966) 45.
8 Booklet of Royal Netherlands Fermentation Industries Ltd., Delft, 1967.