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AFFINITY CHROMATOGRAPHY OF YEAST ANTIBODIES ON MODIFIED MANNAN

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SUMMARY

Mannan, which forms the major antigen of yeast cell walls, was cross-linked with epichlorohydrin under alkaline conditions and used as an immunoadsorbent for the corresponding antibodies.

Cross-linked mannans of *Saccharomyces cerevisiae* and *Candida albicans*, representing two different chemotypes, were found to be efficient, stable and specific immunoadsorbents, which, packed in a column, can be used for repeated separations of yeast antibodies.

INTRODUCTION

Recent immunological studies of the yeasts have demonstrated that mannans occurring on the surface of the cell wall form the principal antigen of the yeasts. These highly branched homopolysaccharides consist of an $\alpha(1-6)$-linked backbone and numerous side-chains containing $\alpha(1-2)$ and $\alpha(1-3)$ linkages. Mannans isolated from various yeast species show different immunochemical properties, which depend mainly on the length of the side-chains. It was found that mannotetraose is the immunodominant group in *Saccharomyces cerevisiae* mannan, and mannohexaose in *Candida albicans* mannan[1,2]. Yeast mannan is soluble in water and cannot be used directly as an immunoadsorbent for the isolation of antibodies. This paper describes a method in which mannan is insolubilized by cross-linking with epichlorohydrin under alkaline conditions to afford a sorbent that is suitable for the separation of specific antibodies from yeast antisera.

EXPERIMENTAL

Material and methods

Mannans of *Saccharomyces cerevisiae* CCY 21-4-13 and *Candida albicans* CCY 29-3-109 were extracted from cell paste with 0.2 *M* sodium chloride solution[3] and purified via the insoluble copper complex formed with Fehling's solution.

Cross-linking of mannan with epichlorohydrin was carried out in a homogeneous phase according to the following procedure. Mannan (10 g) was dissolved in 8 ml of water and 3.5 ml of 40% sodium hydroxide solution and 1.5 ml of epichloro-

hydrin were added. The reaction mixture was mixed thoroughly in order to obtain a homogeneous gel, and then the reaction vessel was closed. The cross-linking reaction was carried out for 1 h at 20° then for 1 h at 50°. The reaction product was cooled and dispersed in water in a high-speed mixer or blender to a suitable particle size, neutralized with acetic acid and purified by washing on a glass filter. The product was then washed with methanol and acetone and finally dried overnight at room temperature.

The bed volume of the cross-linked mannan sample was 7.8 ml/g. The number of monoether glycerol mannan bonds, which shows the extent of the non-cross-linking etherification reaction between mannan and epichlorohydrin, was determined by periodate oxidation of dry cross-linked mannan and by quantitative determination of formaldehyde, a product of the oxidation in the filtrate after washing the oxidized cross-linked mannan[4]. The cross-linked mannan used in this work contained 8 cross-links per 100 anhydromannose units and 1.7 monoether glycerol mannan bonds per 100 anhydromannose units.

The rabbit antisera employed were prepared by immunization of whole yeast cells as described by Šandula and Vojtková-Lepšíková[5].

The concentration of proteins was determined on an MOM 201 spectrophotometer at 280 nm and by the method of Lowry *et al.*[6]. The amount of antibody in whole antisera or in the eluates from the immunoadsorbent was determined by quantitative precipitation[7] and by immunodiffusion in agar gel.

Separation of specific antibodies on cross-linked mannan. Cross-linked mannan (3 g) was swollen in buffered saline (pH 7.4) and packed in a 20 × 2 cm chromatographic column. A 5-ml volume of antisera was applied and allowed to enter the bed slowly. After 2 h of adsorption, the non-specific serum proteins were washed with saline at a flow-rate of 12 ml/h, and 2-ml samples were collected on a fraction collector and the protein content was measured with the spectrophotometer.

The adsorbed antibodies were then eluted either with 2 *M* magnesium chloride solution or with 0.2 *M* hydrochloric acid–glycine buffer of pH 2.4. Antibodies obtained with the hydrochloric acid–glycine buffer were immediately neutralized with 1 *M* dipotassium hydrogen orthophosphate solution. Fractions containing specific antibodies were collected and dialyzed against cooled saline. All of the above operations were carried out at 4°.

RESULTS AND DISCUSSION

The separation of anti-*S. cerevisiae* serum antibodies on cross-linked homologous mannan is shown in Fig. 1. The first fraction obtained by elution of the column with saline contains non-specific serum proteins, which do not give any precipitin reactions with homologous mannan. The second fraction obtained after desorption of the column with 2 *M* magnesium chloride solution contains antibodies that react in immunoprecipitation with *S. cerevisiae* mannan. Similar results were achieved when the column was eluted with hydrochloric acid–glycine buffer of pH 2.4. Fig. 2 shows the application of *C. albicans* antiserum on cross-linked *C. albicans* mannan.

The specificity of the immunoadsorbent used was revealed by the application of *C. albicans* antiserum on cross-linked *S. cerevisiae* mannan; no immunologically

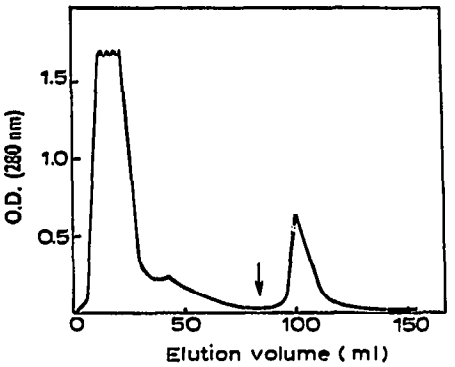

Fig. 1. Separation of anti-*Saccharomyces cerevisiae* serum antibodies on cross-linked homologous mannan with 2 *M* magnesium chloride solution.

active proteins were separated, which could be due to the structural differences of their mannans and the corresponding antibodies.

On the other hand, cross-linked *S. cerevisiae* mannan can be used successfully for other Saccharomyces antisera[5] and also an immunoadsorbent based on *C. albicans* mannan is suitable for sera that contain antibodies against other pathogenic yeast species, such as *C. tropicalis*, *C. stellatoidea* and *C. guilliermondii*. These micro-organisms show close antigenic similarity and high cross-reactivity.

The repeated use of the immunosorbent does not cause a loss in its binding capacity.

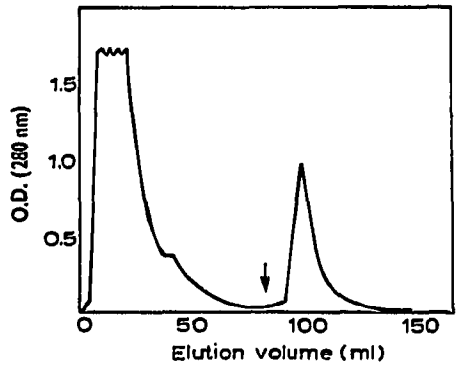

Fig. 2. Separation of anti-*Candida albicans* antibodies on cross-linked *Candida albicans* mannan with 2 *M* magnesium chloride solution.

REFERENCES

1 C. E. Ballou, *J. Biol. Chem.*, 245 (1970) 1197.
2 S. Suzuki and H. Sunayama, *Jap. J. Microbiol.*, 12 (4) 413.
3 D. Šikl, L. Masler and Š. Bauer, *Collect. Czech. Chem. Commun.*, 35 (1969) 2965.
4 Ľ. Kuniak and R. H. Marchessault, *Stärke*, 24 (1972) 110.
5 J. Šandula and A. Vojtková-Lepšíková, *Folia Microbiol.*, 19 (1974) 67.
6 H. O. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
7 E. A. Kabat and M. M. Mayer, *Experimental Immunochemistry*, Thomas, Springfield, Ill., 1964.