# PURIFICATION OF THE GALACTOSE-BINDING HEMAGGLUTININ OF *PSEUDOMONAS AERUGINOSA* BY AFFINITY COLUMN CHROMATOGRAPHY USING SEPHAROSE

N. GILBOA-GARBER, L. MIZRAHI and N. GARBER
*Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel*

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## 1. Introduction

Hemagglutinins from different sources vary in their reaction with components of cell membranes [1, 2] and other biological materials [3–13]. This variation in specificity was used for investigation of the biochemical nature of the combining residues and for purification of the hemagglutinins by means of affinity column chromatography. Sephadex column chromatography was employed for purification of concanavalin A [14, 15] and of the hemagglutinin from *Helix pomatia* [16, 17] and Sepharose was reported by Tomita et al. [18] to be very efficient for the purification of some galactose-binding plant hemagglutinins while ineffective for others.

We have recently described a new galactose-binding hemagglutinin, separated from cells of a pyocyanin-producing strain of *Pseudomonas aeruginosa* [19]. This hemagglutinin, which resembled certain phytohemagglutinins in several properties, was partially purified employing a procedure based on its relative resistance to heating and to low pH and on its precipitation by ammonium sulfate [20].

The present communication describes the further purification of the bacterial hemagglutinin by affinity chromatography on Sepharose column. The results show that the hemagglutinin of *Pseudomonas* interacts specifically with Sepharose and is easily eluted from it by D-galactose. The final hemagglutinin yield and the high purification obtained indicate the efficiency of this treatment.

## 2. Materials and methods

### 2.1. *Preparation of the bacterial hemagglutinin*

A subcellular preparation of the hemagglutinin-producing strain of *Pseudomonas aeruginosa* was obtained as previously described [19, 20]. Partial purification of the hemagglutinin (purification factor about 13) was obtained by heating 50 ml of the crude extract at 70° for 15 min, centrifugation and precipitation of the protein from the supernatant fluid (Fraction I) at 60% saturation of ammonium sulfate. The sediment was resuspended in 10 ml phosphate buffered saline at pH 7 (Fraction II).

### 2.2. *Chromatography of the hemagglutinin on Sepharose*

10 ml of Fraction II were applied on to a column (2.5 × 40 cm) of Sepharose 4B (Pharmacia). The column was washed with 0.01 M potassium phosphate, 0.15 M sodium chloride and 10 ml fractions were collected. The protein concentration in these fractions was measured according to Lowry et al. [21] and the hemagglutinating activity was determined against papain-treated human erythrocytes [19]. After disappearance of the protein in the effluents and further washing of the hemagglutinin-containing Sepharose, the buffered saline was substituted by 0.3 M D-galactose solution and another 20 fractions of 10 ml each were collected. The galactose containing fractions were dialysed overnight against phosphate-buffered saline and for another day against distilled water (in order to remove the galactose which interferes with the determinations of protein and

agglutinating activity). The protein content and the agglutinating activity in the dialysates were determined as described and the samples containing the purified hemagglutinin were combined (Fraction III).

2.3. *Polyacrylamide disc gel electrophoresis*

Samples of the different fractions containing about 3–6 mg protein per ml were analyzed by means of polyacrylamide disc gel electrophoresis. The procedure employed was essentially similar to that described by Davis [22]. 0.1 ml of the hemagglutinin preparation was mixed with 0.1 ml of 20% sucrose solution and half of the mixture was applied to the stacking gel (7%, pH 8.9). A constant current of 5 mA per tube was employed and the stacking gels were stained by Amido Black.

## 3. Results and discussion

The hemagglutinin of *Pseudomonas aeruginosa* which was purified about 13–16-fold by heating at 70° followed by ammonium sulfate precipitation, was found to be retained on the Sepharose column. No agglutinating activity was found in the protein-containing saline effluent (fig. 1). Such a specific adsorption was reported by Tomita et al. [18] for the galactose-binding plant hemagglutinins of *Ricinus communis*, *Momordia charantia* and *Abrus precatorius* as opposed to the hemagglutinin of *Sophora japonica*. The last hemagglutinin was not retained on the Sepharose in spite of its ability to bind galactose [18]. The specificity of the interaction of the bacterial hemagglutinin with the Sepharose was indicated by the ease of its elution with D-galactose as a competitive inhibitor (fig. 1).

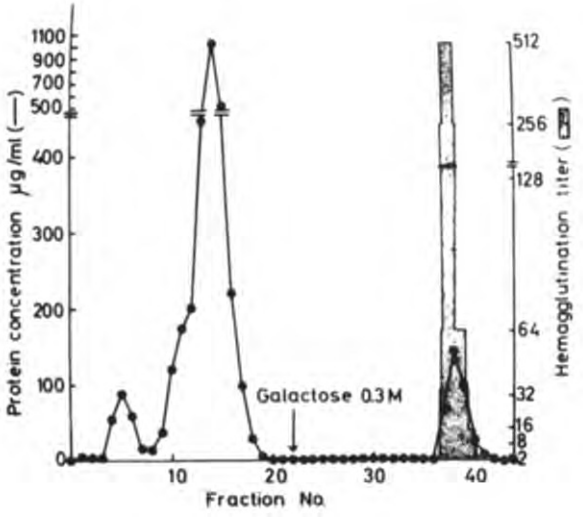


Fig. 1. Chromatography of the hemagglutinin of *Pseudomonas aeruginosa* on Sepharose 4B column (2.5 × 40 cm). The arrow indicates the addition of 0.3 M galactose.

The Sepharose column chromatography was found to be very efficient in the purification of the hemagglutinin. The pattern obtained in the polyacrylamide disc gel electrophoresis indicated that the eluate contained a single protein band as compared to more than ten bands in the partially purified preparation after ammonium sulfate precipitation (fig. 2).

The purification of the bacterial hemagglutinin in a high yield (table 1) by means of the Sepharose column chromatography compares favorably with the results obtained with concanavalin A adsorbed on Sephadex [14, 15] and with *Momordia charantia* adsorbed on Sepharose [18]. However, as the initial concentration of the hemagglutinin in the crude bacterial extract was only about 0.5% of the total protein

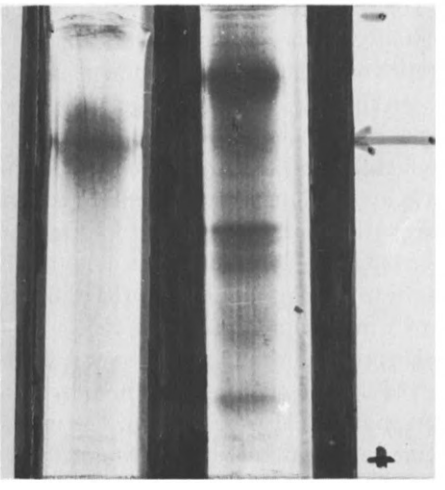

Fig. 2. Polyacrylamide disc gel electrophoresis of the partially purified hemagglutinin preparation – Fraction II (right gel) and of the purified preparation – Fraction III (left gel). 160 µg of protein in 0.1 ml of 10% sucrose solution were applied on to the gels.

Table 1
Purification of the hemagglutinin from *Pseudomonas aeruginosa.*

| Isolation Stage | Volume (ml) | Protein (mg/ml) | Total protein (mg) | Agglutinating activity | Purification factor | Approximate yield (%) |
|---|---|---|---|---|---|---|
| Crude | 50 | 15.5 –16.5 | 775–825 | 1024 | – | – |
| Fraction I | 45 | 2.32 | 104.4 | 1024 | 6.5–7 | 90 |
| Fraction II | 10 | 4.75 | 47.5 | 4096 | 13 –16 | 80 |
| Fraction III | 40 | 0.08– 0.09 | 3.6 | 1024 | 170 –210 | 80 |

The agglutinating activity represents the highest dilution (in 0.2 ml volume) giving detectable agglutination of papain-treated human erythrocytes. Fraction I is that obtained after heating at 70°, Fraction II – after ammonium sulfate precipitation and Fraction III is the eluate obtained from the Sepharose column after addition of D-galactose.

while the initial concentrations of the plant hemagglutinins were about 3–25% [14–18], the total purification factor of the bacterial hemagglutinin was about 170–210-fold as compared to 4–33-fold reported for the plant hemagglutinins [14–18]. From 50 ml of a crude bacterial extract containing about 775 mg of protein only 3.6 mg of purified hemagglutinin were obtained at conditions of about 80% yield.

The high recovery and purity obtained, indicate the efficiency of the Sepharose column chromatography for the purification of the hemagglutinin obtained from *Pseudomonas aeruginosa.*

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