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Short Communication

# Use of metal-chelate affinity chromatography and hydrophobic interaction chromatography for purification of placental protein 12

Aaron L. Berkovsky[a,*], Pavel P. Potapov[b]

[a]*Hematological Scientific Center, Russian Academy of Medical Science, Novozykovsky pr. 4a, Moscow 125167, Russia*
[b]*Yarinvest Medical Int'l Co., P.O. Box 34, Yaroslavl 150062, Russia*

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**Abstract**

Placental protein 12 was isolated from amniotic fluid. Albumin was removed by means of ion-exchange chromatography on DEAE-Sepharose and chromatography on Blue-Sepharose. Complete purification was obtained by metal-chelate affinity chromatography and hydrophobic interaction chromatography under mild conditions for desorption. Using this procedure large quantities of a highly purified preparation can be obtained in one run.

## 1. Introduction

Placental protein 12 (PP12) is one of the most important placental proteins [1–4]. For the study of its physicochemical properties and biological function, and for the development and production of the test-systems to be used for diagnosis of the pathologic phenomena of the reproductive system, large amounts of highly purified protein are needed. During our studies on the fractionation of fetal and placental proteins we developed a new technology for the purification of PP12 applying metal-chelate and hydrophobic interaction chromatography. The methods developed seem very suitable for preparative purposes. They are less costly than the previously used procedures for purification of PP12 [5–8].

* Corresponding author.

## 2. Experimental

### 2.1. Materials

DEAE-Sepharose CL-6B, Cibacron Blue 3GA-Sepharose CL-6B, epoxy-activated Sepharose 6B, and Phenyl-Sepharose CL-4B were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden).

Preparation of the nickel-chelate gel was essentially carried out as described by Porath *et al.* [9].

### 2.2. Samples

Amniotic fluid was obtained from patients undergoing legal abortion (4–5 months gestational period). Sodium azide and phenylmethylsulfonyl fluoride were added to a final

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concentration of 0.2% and 0.001 m*M*, respectively. Solutions used in all experiments contained the same concentration of inhibitors. The samples were desalted by ultrafiltration, and the pH adjusted to 5.2 with 0.5 *M* acetic acid. After standing overnight at 4°C the precipitate was removed.

### 2.3. Analytical procedure

The PP12 content was measured by radial double immunodiffusion in agar gel using rabbitt anti-PP 12 monospecific immune serum.

The purity of PP12 was ascertained by immunoelectrophoresis in an agarose gel containing antibodies against human serum [10]. SDS-polyacrylamide gel electrophoresis was carried out in 10% acrylamide [11]. The protein concentration was estimated from the absorbance at 280 nm.

### 2.4. Chromatography

All chromatographic experiments were performed at 20–22°C.

The material was applied to a DEAE-Sepharose column (gel volume 1 l) previously equilibrated with 0.025 *M* acetate buffer, pH 5.2. The column was washed with equilibrating buffer and 0.025 *M* acetate buffer, pH 4.5. When the absorbance at 280 nm was less than 0.01, PP12 was eluted with 0.1 *M* acetate buffer, pH 4.0 (volume 1.2 l). The collected salt-free fraction was concentrated to a volume of 200 ml and diluted with 0.01 *M* Na-phosphate buffer, containing 0.15 *M* NaCl, to pH 7.5. Then, the sample was passed through a column of Cibacron Blue–sepharose (gel volume 100 ml). The column was washed with 500 ml of the starting buffer. The material obtained was applied to a nickel-chelate column (gel volume 200 ml). The column was washed with phosphate-buffered saline and 0.01 *M* Na-phosphate buffer, pH 6.0, containing 1 *M* NaCl. Desorption of PP12 was carried out with 0.02 *M* Na-acetate buffer, pH 4.0, containing 1 *M* NaCl. The PP12 containing fraction was dialyzed against 0.01 *M* Na-phosphate buffer, pH 6.5. Ammonium sulphate was added to a final concentration of 1 *M*. The dialyzed material was applied to a phenyl-sepharose column (volume 200 ml) equilibrated with the same solution. The column was washed with phosphate buffer without ammonium sulphate. PP12 was eluted with 0.02 *M* Tris-HCl buffer, pH 9.0.

## 3. Results and discussion

After the preliminary processing the sample (volume 2 l) contained *ca.* 120 g of total protein and *ca.* 60 mg of PP12. Ion-exchange chromatography was used for fractionation of blood serum proteins [12]. Most of the ballast proteins (mainly albumin) were removed at this stage without much loss of PP12 which was eluted with 0.1 *M* acetate buffer, pH 4.0 (volume 1.2 l). The eluted material contained 21 g of total protein and 54 mg of PP12. The use of milder conditions (0.025 *M* acetate buffer, pH 4.0) did not result in full elution of PP12. The best way to remove the remaining albumin was by chromatography on Blue-Sepharose [13] whereby *ca.* 20% of the applied protein was adsorbed. There was no measurable adsorption of PP12. Almost all PP12 eluted from the column with the starting buffer. When the column was washed with two volumes of 0.1 *M* phosphate buffer, containing 3 *M* NaCl, no PP12 could be detected in the effluent.

The sample (volume 700 ml) that was applied to the nickel-chelate column contained *ca* 17 g of total protein and 50 mg of PP12. Approximately 70% of the applied protein was either not adsorbed or was eluted under mild conditions (Fig. 1). Preliminary experiments have been carried out before the optimal conditions for desorption of PP12 were chosen. The fraction that eluted with the acetate buffer, pH 4.0, containing 1 *M* NaCl contained *ca.* 600 mg of total protein and 45 mg of PP12. No PP12 could be detected in the 0.05 *M* EDTA fraction.

About 90% of protein adsorbed to phenyl-sepharose (Fig. 2). Desorption of 60–70% of the ballast proteins occurred when the column was washed with buffer without ammonium sulphate, and most of the PP12 remained on the column. The conditions used for desorption of PP12 were

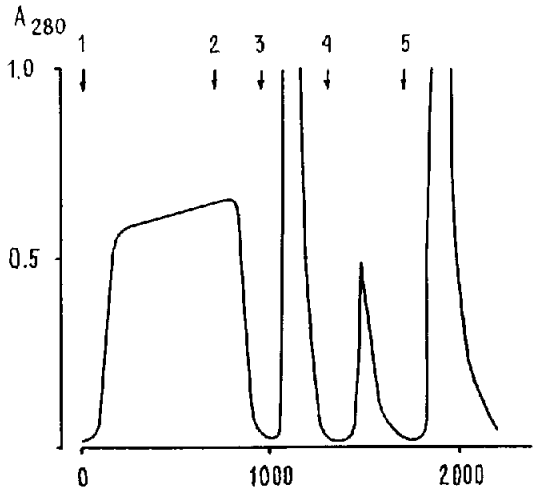


Fig. 1. Chromatography of PP12 containing fraction on nickel-chelate sepharose 6B. Gel volume 200 ml. (1) Application of PP12 in PBS (700 ml), pH 7.4, followed by elution with (2) PBS, pH 7.4, (3) 0.01 *M* Na-phosphate buffer, pH 6.0, containing 1 *M* NaCl, (4) 0.02 *M* Na-acetate buffer, pH 4.0, containing 1 *M* NaCl, and (5) PBS, pH 7.4, containing 0.05 *M* EDTA.

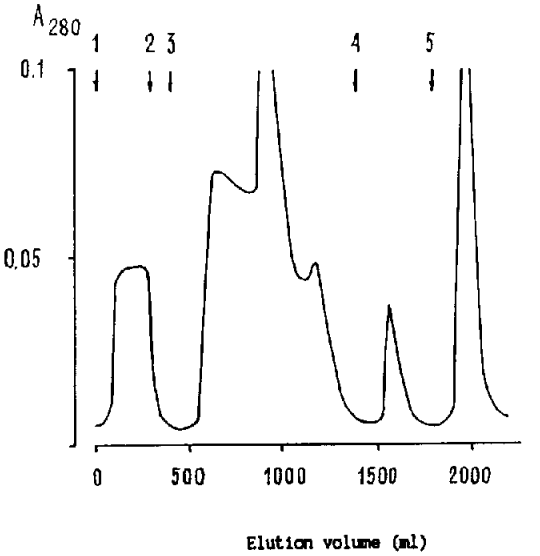


Fig. 2. Chromatography of PP12 containing fraction on phenyl-sepharose CL-4B. Gel volume 200 ml. (1) Application of PP12 in 0.01 *M* Na-phosphate buffer, pH 6.5, containing 1 *M* ammonium sulphate (300 ml), followed by elution with (2) 0.01 *M* Na-phosphate buffer, pH 6.5, containing 1 *M* ammonium sulphate, (3) 0.01 *M* Na-phosphate buffer, pH 6.5, (4) 0.02 *M* Tris-HCl buffer, pH 9.0, and (5) PBS, pH 6.5, containing 10% butanol.

comparatively mild, and *ca.* 50% of the applied PP12 remained on phenyl-sepharose. The remaining PP12 was eluted together with a large amount of ballast proteins when detergents or organic solvents were used. Under such conditions the probability that the native properties of the protein are altered increases. The fraction that eluted with Tris-buffer, pH 9.0, contained 20 mg of 95% pure PP12.

SDS gel electrophoresis was carried out and the purified PP12 preparation gave a single electrophoretically distinct component with a molecular mass of 28 kDa (Fig. 3). The preparation did not react with antiserum to normal human serum in an immunoelectrophoresis assay.

A summary of the purification of PP12 according to the methods described above is shown in Table 1.

The advantage of the method described here is its potential for the processing of large amounts of biological material. Under the experimental conditions used here large quantities of highly

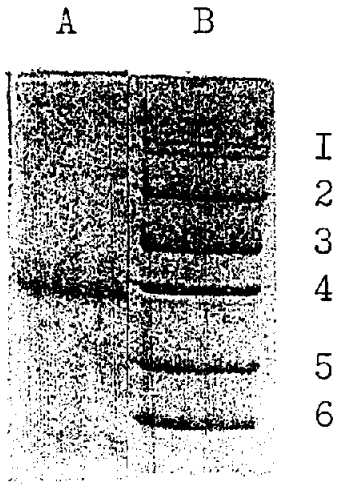


Fig. 3. Electrophoresis in polyacryamide gel in the presence of sodium dodecyl sulphate. (A) Purified PP12 preparation; (B) Standard proteins. (1) Phosphorylase b, $M_r$ 94 kDa; (2) bovine serum albumin, $M_r$ 67 kDa; (3) ovalbumin, $M_r$ 43 kDa; (4) carbonnic anhydrase, $M_r$ 30 kDa; (5) soybean trypsin inhibitor, $M_r$ 20.1 kDa; (6) lactoalbumin, $M_r$ 14.4 kDa.

Table 1
Summary of PP12 purification procedure

| Purification step | Total protein ($A_{280}$) (mg) | PP12 amount (mg) | Recovery (%) | Purification factor[a] |
|---|---|---|---|---|
| Starting material | 120 000 | 60 | 100 | – |
| DEAE-sepharose | 21 000 | 54 | 90 | 5.1 |
| Blue-sepharose | 17 000 | 50 | 83 | 5.9 |
| Ni-chelate-sepharose | 600 | 45 | 75 | 150 |
| Phenyl-sepharose | 21 | 20 | 33 | 1900 |

[a] Related to the purity of starting material.

purified material can be obtained in one run. Moreover, in the present procedure the chemical activity and the physico-chemical properties of the protein remain unchanged, since the protein it is not exposed to denaturating agents or extreme pH. Another advantage is that chromatographic adsorbents can be easily regenerated and used several times. In this respect, the method presented here for the isolation and purification of PP12 may be particularly useful.

## 4. Acknowledgements

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