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Short Communication

THE APPLICATION OF FPLC CHROMATOGRAPHY (MONO Q) FOR THE PURIFICATION OF WHEAT RNA POLYMERASES II AND III

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Abstract: Transcription is the main step in the regulation of gene expression. To study this process *in vitro*, it is necessary to obtain highly purified RNA polymerases. Here, we describe a method of RNA polymerase purification using a Mono Q FPLC column. Using Mono Q column chromatography accelerates the purification process and separates RNA polymerase II from RNA polymerase III with good yield.

Key Words: Wheat, Transcription, RNA Polymerase II and III, Mono Q Column, FPLC

INTRODUCTION

DNA dependent RNA polymerases are key enzymes in the transcription process. Three types of RNA polymerases occur in eucaryotic cells [1]. For adequate transcription, a number of transcription factors are needed [2-5]. Some transcription factors are necessary for the initiation of transcription [6], and others for the elongation of the RNA chain [7] and the termination of transcription. There is, however, limited information available about plant transcription factors [4]. The basic methodology for the study of transcription factors requires working with purified homologous RNA polymerases. Some of these factors could interact with the DNA matrix [8], others with RNA polymerase [7]. The application of RNA polymerases is connected with the *in vitro* transcription of specific DNA sequences [9, 10]. This is a very important research field with a wide use of different polymerases. Recently, information has becme available about plant transcription systems. For efficient work using

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Abbreviations used: EDTA - methylenediaminetetraacetic acid; TRIS - 2-amino-2-(hydroxymethyl)-1,3-propanediol; cpm - counts per minute

in vitro transcription systems, purified RNA polymerases and a well-defined source of transcription factors are necessary [11].

Although RNA polymerase was isolated for the first time in the seventies, there are still significant problems that exist connected with the purification and storage of polymerases. All the currently applied methods are based on the methods of Jendrisak and Burgess [12]. This method was modified in our laboratory to purify RNA polymerases II and III [13], but the purification efficiency was not sufficient, especially during the last stage of purification. The development of protein purification techniques enables the application of FPLC for RNA polymerase preparation. Here, we describe a purification procedure for RNA polymerases II and III on a Mono Q column, and the estimation of their activity.

METHODS

Isolation and purification of RNA polymerases

Wheat germs were obtained from a local mill. Initial steps of the RNA polymerase preparation, isolation, extraction and precipitation with Polymin P and ammonium sulfate were carried out as described by Jendrisak and Burgess [12] with modifications [13]. Ammonium sulfate precipitate (fraction F1) was dissolved in 500 ml TEPG buffer (50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.1 mM PMSF, 25% glycerol) and mixed for 2 hours with 60 ml DEAE-Sepharose CL6B resin. The DEAE-Sepharose with the adsorbed proteins was loaded onto the column (2x20 cm). Unbound proteins were eluted with TEPG buffer containing 0.25 M ammonium sulfate. The fractions with RNA polymerases activity were eluted from the column with a 0.5-1.0 M ammonium sulfate linear gradient in the same buffer. The samples containing RNA polymerases activity were pooled, dialyzed against TEPG buffer with 0.075 M ammonium sulfate (fraction F2) and applied on a DEAE-Cellulose column (2x20 cm) equilibrated with the same buffer. Unbound proteins were eluted with the loading buffer. Fractions containing RNA polymerases activity were eluted with a 0.3 M ammonium sulfate linear gradient in TEBG buffer (50 mM Tris-HCl pH 7.9, 1 mM EDTA, 0.1 mM β-mercaptoethanol, 25% glycerol). The fractions containing RNA polymerase activity were pooled, concentrated using Sephadex G-100 and dialyzed against TEBG buffer with 50% glycerol (fraction F3).

Chromatography of RNA polymerases on a Mono Q column

Fraction 3 was loaded in TEBG buffer on a Mono Q column equilibrated with the same buffer. The proteins were recovered from the column by a linear gradient of 0.04-0.3 M ammonium sulfate in TEBG buffer. One ml fractions were collected. Each fraction was tested for RNA polymerases II or III activity. The fractions containing RNA polymerases II or III were pooled separately. RNA polymerase II (fraction F4 II) was frozen in liquid nitrogen and stored at -70°C. RNA polymerase III (fraction F4 III) was concentrated by ultrafiltration using an Ultrafree-MC centrifugal filter (Millipore) and stored at -70°C.

Determination of the DNA dependent activity of RNA polymerases II and III

The activity of RNA polymerases was studied according to the method described by Fabisz *et al.* [13]. Protein concentration was determined using Bradford's method [14].

Polyacrylamide gel electrophoresis

Protein gel electrophoresis was conducted in(on) a discontinuous polyacrylamide gel with SDS (8% stacking gel, 15% resolving gel), according to Laemmli [15]. The gels were silver stained according to the method described by Oakley *et al.* [16].

RESULTS AND DISCUSSION

The initial steps of the purification procedure (ammonium sulfate and Polymin P precipitations) were performed according to Jendrisak and Burgess [12] with modifications [13]. The first chromatography step on DEAE-Sephadex was also performed as described previously [13]. The most important modification of the purification procedure was the chromatography on a Mono Q column. During the initial step of purification (fraction F1), some inhibitors of RNA polymerase or RNases were removed. The most interesting step was the chromatography on a Mono Q column. Despite the losses of activity during this operation (the relatively low yield at this step), a very high degree of purification of both polymerases (II and III) was achieved. Polymerase II was eluted from Mono Q with 0.25 M and RNA polymerase III with 0.35 M ammonium sulfate gradient (Fig. 1) as identified by the α-amanitin test (Fig. 2). The stepwise gradient of polymerase elution was estimated by a preliminary gradient elution of the enzyme from the column. The purification results are summarized in Tab. 1.

Tab. 1. The steps of RNA polymerases II and III purification.

Purification step (Fraction)	Volume [ml]	Protein [mg]	Activity [cpm/5 μl]	Protein concentration [mg/ml]	Purification coefficient	Yield [%]	Total activity [μCi/10 min.]	Specific activity [μCi/10 min.]
F1	1600	6723	528	4.2		100	250.3	0.037
F2	1000	3120	4211	3.12	10.74	498.46	1247.7	0.39
F4 II	18	2.71	20236	0.15	516.76	44.44	539.6	199.12
F4 III	0.6	1.98	16776	3.3	18.83		14.9	7.53

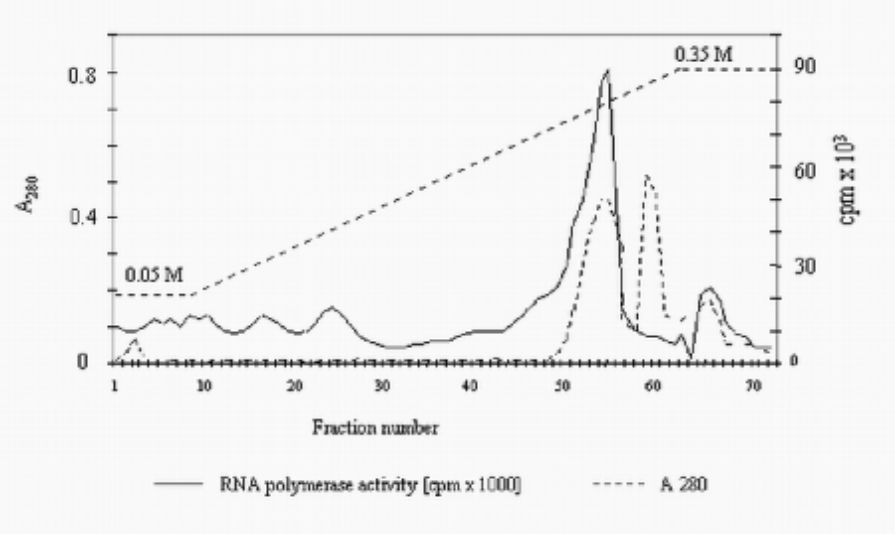

Fig. 1. Chromatography of RNA polymerases on a Mono Q column. Fraction F3 showing RNA polymerase activity was loaded on a Mono Q column. The proteins were eluted by a linear gradient of ammonium sulfate. One ml fractions were collected and the activity of the polymerases was measured as described [13].

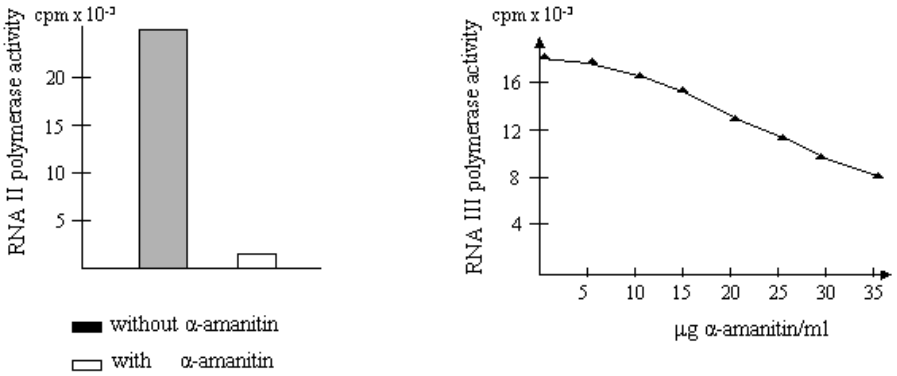

Fig. 2. The effect of α-amanitin on RNA polymerase activity. Left, the effect of α-amanitin on RNA polymerase II activity. The analysis was performed after the addition of 5 μg α-amanitin/ml (white bar) and without α-amanitin (black bar). Right, the effect of α-amanitin on RNA polymerase III activity. Other conditions as described in the Methods.

This level of purification of the enzyme by a one-step procedure was never previously achieved. We also used other column chromatographies, e.g. affinity chromatography on DNA-Cellulose or ionic chromatography with other exchangers like CM-Cellulose or DEAE-Sephacel, but none of those yielded such a high level of purification. The relatively low yield of the chromatography

step could be due to the very high degree of purity of the protein. After the Mono Q purification step, 18 proteins were found in the RNA polymerase II fraction and 12 proteins in the RNA polymerase III fraction, as shown by gel electrophoresis (Fig. 3).

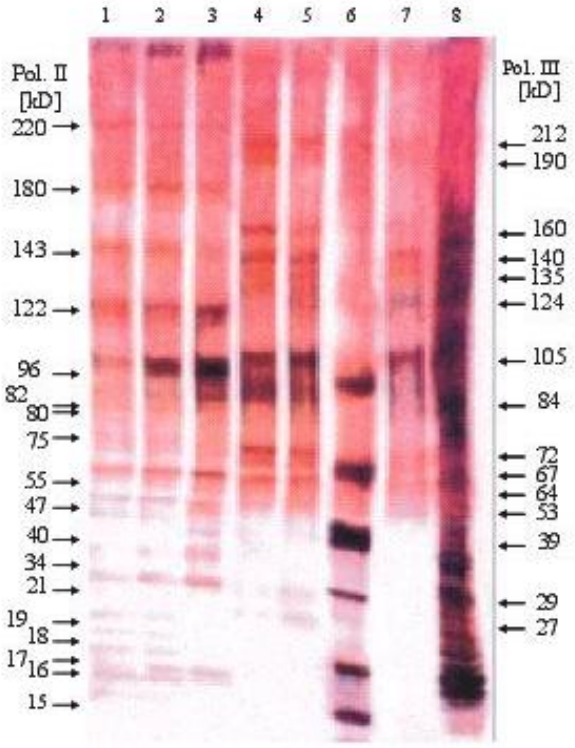

Fig. 3. Polyacrylamide gel electrophoresis of the RNA polymerases II and III fractions. Electrophoresis of the proteins was performed in(on) a discontinuous 8-15% gel and silver stained. Lane 1-3: fractions 51-56 obtained after Mono Q chromatography with RNA polymerase II activity; lanes 4, 5 and 7: fractions 66-69 obtained after Mono Q chromatography with RNA polymerase III activity; lane 6: molecular weight marker (95, 67, 43, 30, 20, 14 kDa); lane 8: crude extract after ammonium sulfate precipitation.

When the gel was stained with Coomassie Brilliant Blue, the number of RNA polymerase II protein bands decreased to 12. The next purification steps that led to a decreased number of bands also reduced the activity of the enzyme.

The last step of the protocol involved freezing polymerase III preparation in liquid nitrogen after concentration by ultrafiltration through an Ultrafree-MC centrifugal filter (Millipore). After the enzyme is concentrated by ultrafiltration, it does not display any loss of activity, even when stored for a 10-month period.

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