A Rapid and Simple Procedure for Purification of S-II, a Transcription Factor of Ribonucleic Acid Polymerase II

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(Received July 10, 1986)

We previously reported the purification of eukaryotic transcription factor S-II and its phosphorylated form S-II' from Ehrlich ascites tumor cells. In this work, we modified the previous purification procedure to make it quicker and simpler. By this improved procedure, involving a different solubilization method, and improved buffer solutions and centrifugation processes, S-II could be purified to homogeneity from 1 kg of frozen cells in about 10 d instead of 1 month. The purity, specific activity and total yield of S-II purified by the new method were comparable to those obtained by using the previous method.

Keywords—transcription; S-II; stimulatory factor; RNA polymerase II; Ehrlich ascites tumor cell

Introduction

Multiple factors have been found to participate in transcription mediated by ribonucleic acid (RNA) polymerase II.1,2) For elucidation of the mechanism of transcription, it is therefore indispensable to isolate and characterize these factors. Although chromatographic fractionations of these factors have been reported,1−7) none of the factors has yet been purified to homogeneity.

We have purified a stimulatory protein of RNA polymerase II, named S-II and its phosphorylated form S-II', and another related protein S-I(b) to homogeneity from Ehrlich ascites tumor cells.8−10) Experiments using antibody against S-II showed that S-II is one of the essential transcription factors for RNA polymerase II.11,12) Furthermore, S-II was demonstrated to form a stoichiometric complex with RNA polymerase II in the initiation step, and this complex was shown to be stable during the elongation step of transcription.13) Moreover, results suggested that the function of S-II is regulated by phosphorylation and dephosphorylation of the protein molecule.14−16) Therefore, for studies on the mechanism of eukaryotic transcription, S-II must be obtained in quantity. However, our previous method for purification of S-II was laborious and about 30 d were required to purify 1−2 mg of S-II from 1 kg of Ehrlich ascites tumor cells.

This paper describes a rapid, simple method for the purification of S-II. By modification of the solubilization procedure and several other conditions, almost the same amount and the same quality of S-II could be obtained from 1 kg of Ehrlich ascites tumor cells in about 10 d.

Materials and Methods

Cells, Buffer Solutions, and Chemicals—Ehrlich ascites tumor cells were grown and harvested as described
Buffer I was composed of 50 mm Tris–HCl, pH 7.9, 5 mm 2-mercaptoethanol, and 0.1 mm ethylenediaminetetraacetic acid (EDTA). Buffer II consisted of 10 mm Tris–HCl, pH 7.9, containing 5 mm 2-mercaptoethanol. Buffer III had the same composition as buffer I, but was supplemented with 5 mm MgCl₂. Buffer IV consisted of 50 mm Tris–HCl, pH 7.9, 5 mm 2-mercaptoethanol, and 0.1% (v/v) Triton X-100. Buffer V was the same as buffer IV except that the Tris–HCl concentration was reduced to 10 mm. Buffer VI was composed of 10 mm Tris–HCl, pH 7.9, 0.1 mm EDTA, 10 mm MgCl₂, 10 mm KCl, and 50% (v/v) glycerol.

Dimethylaminomethyl (DEAE)-cellulose (DE 52), phosphocellulose (P 11), and carboxymethyl (CM)-cellulose (CM 52) were purchased from Whatman. 3H-uridine triphosphate (UTP) was from Amersham Japan Corp.

Assay of Stimulatory Factor of RNA Polymerase II
This assay was done essentially as described before, using partially purified RNA polymerase II from Ehrlich ascites tumor cells and CsCl-purified Ehrlich deoxyribonucleic acid (DNA). One unit of stimulatory activity was defined as the amount that increased the activity of 10 units of RNA polymerase II to 11 units under the standard assay conditions.

Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Gel Electrophoresis and Protein Determination
SDS-polyacrylamide gel electrophoresis was performed by the method of Laemmli in 12.5% acrylamide gel. After electrophoresis, proteins were located by staining with Coomassie Brilliant Blue by the method of Fairbanks et al.

Results and Discussion

Purification of S-II
All subsequent procedures were performed at 0—4 °C unless otherwise stated.

Solubilization of S-II—About 1 kg of Ehrlich ascites tumor cells was homogenized in 11 of buffer I containing 0.2% (w/v) sodium deoxycholate in a Polytron homogenizer (Kinematica, Switzerland) at a power setting of 10 for 10 min. Then, 128 ml of saturated (NH₄)₂SO₄ solution was added, and the mixture was homogenized again under the same conditions for 15 min. Then 3.4 l of buffer I was added and the mixture was centrifuged for 30 min at 10000 × g. The supernatant was saved for the next step. The efficiency of solubilization of stimulatory factors was monitored by radioimmunoassay with antibody against S-II, since stimulatory activity could not be quantitated under the standard assay conditions due to contamination with other proteins in the preparation. Results showed that the efficiency of solubilization was comparable to that in the previous method (data not shown). Thus, the procedures of homogenization in a motor-driven homogenizer and sonication used previously could be replaced by disruption in a Polytron. We used a regular refrigerated centrifuge instead of an ultracentrifuge to obtain solubilized samples and did not add glycerol to buffers. These improvements of the procedure saved much time.

First (NH₄)₂SO₄ Precipitation—About 51 of supernatant was mixed with a half volume of saturated (NH₄)₂SO₄ solution and the mixture was stirred for 30 min. The resulting precipitate was removed by centrifugation for 50 min at 10000 × g, and the supernatant was collected. Solid (NH₄)₂SO₄ was added to the resulting clear supernatant at 540 g per liter, and the mixture was stirred for 1 h. Precipitated proteins were collected by centrifugation for 50 min at 10000 × g, dissolved in 100 ml of buffer II, and dialyzed against 51 of the same buffer for 18 h with two changes of the outer solution. Insoluble materials appearing during dialysis were removed by centrifugation for 30 min at 10000 × g, and the buffer was changed to buffer III by adding concentrated solutions of constituents.

DEAE-Cellulose Column Chromatography and Second (NH₄)₂SO₄ Precipitation—The dialyzed material was applied to a column of DEAE-cellulose (7.5 × 25 cm) equilibrated with buffer III at a flow rate of 180 ml/h, and the column was then washed with 21 of buffer III. Unadsorbed fractions with optical densities at 280 nm of more than 0.5 were collected and combined. Then 1.5 volumes of saturated (NH₄)₂SO₄ solution was added to this unadsorbed fraction with thorough mixing. Then solid (NH₄)₂SO₄ was added at 300 g per liter of the mixture with stirring for 1 h. The resulting precipitate was collected by centrifugation for 50 min at 10000 × g, dissolved in 50 ml of buffer II, and dialyzed against 51 of buffer II for 10 h with two changes of the outer solution. Insoluble materials that appeared
during dialysis were removed by centrifugation for 30 min at 10000 × \( g \), and the clear supernatant was collected.

**Ethanol Precipitation**—The supernatant was mixed with a half volume of ethanol that had been kept at \(-20^\circ\text{C}\), and stirred for 1 min. The mixture was kept at \(-20^\circ\text{C}\) for 1 h to complete protein precipitation, and then centrifuged for 10 min at 2000 × \( g \). The resulting pellet was suspended in 100 ml of buffer IV, and homogenized in a glass homogenizer by 10 strokes of a Teflon pestle. The homogenate was centrifuged for 30 min at 10000 × \( g \), and the clear supernatant was collected.

**Phosphocellulose Column Chromatography**—The soluble fraction thus obtained was loaded on a column of phosphocellulose (2.5 × 10 cm) equilibrated with buffer IV. Then the column was washed successively with one column volume of buffer IV, three column volumes of buffer IV containing 0.1 M NaCl, and five column volumes of buffer IV containing 0.3 M NaCl. S-I(b) activity was detected in the eluate with 0.3 M NaCl, indicating that S-I(b) was also solubilized by this procedure. S-II and S-II‘ were then eluted with 800 ml of a linear gradient of 0.3—0.8 M NaCl in buffer IV. Fractions of 5 ml were collected and 5 \( \mu \)l of each fraction was assayed for activity to stimulate RNA polymerase II. S-II and S-II‘ were eluted as a single peak with 0.45 M NaCl. About 50 ml of active fraction was dialyzed against 51 of buffer V for 10 h with two changes of the outer solution.

**CM-Cellulose Column Chromatography**—The resulting dialyzate was loaded on a column of CM-cellulose (1.5 × 8 cm) equilibrated with buffer V. The column was washed with three column volumes of buffer V, and adsorbed materials were eluted with 600 ml of a linear gradient of 0—0.1 M NaCl in buffer V. Fractions of 3 ml were collected and the stimulatory activity of 5 \( \mu \)l of each fraction was assayed. As shown in Fig. 1, S-II‘ and S-II were eluted with 35 and 45 mm NaCl, respectively, as found previously. However, the amount of S-II‘ relative to S-II was less than with the previous method and it varied from preparation to preparation. Since the recovery of S-II was about the same, the new method was probably less efficient for solubilization of S-II‘. Active fractions of S-II and S-II‘ were pooled separately, dialyzed

![Fig. 1. CM-Cellulose Column Chromatography of S-II and S-II’](image1)

Active fractions from the phosphocellulose column were combined and loaded on a column of CM-cellulose as described in the text. The RNA polymerase II stimulatory activity of each fraction (thick line) and the NaCl concentration (thin line) are indicated. The broken line indicates the amount of uridine monophosphate (UMP) incorporated with RNA polymerase II alone.

![Fig. 2. SDS-Polyacrylamide Gel Electrophoresis of the Final Preparations of S-II’ and S-II](image2)

Active fractions of S-II’ (lane 1) and S-II (lane 2) from the CM-cellulose column were pooled and analyzed by SDS-polyacrylamide gel electrophoresis. The molecular weights determined with reference to various standard marker proteins are shown in kilodaltons.
against buffer VI, and stored at \(-20^\circ\text{C}\).

**Evaluation of the New Purification Method**

As shown in Fig. 2, at the CM-cellulose step, S-II was almost homogeneous, but S-II’ was not still pure, when pooled fractions of S-II and S-II’ from CM-cellulose step were analyzed by SDS-polyacrylamide gel electrophoresis. Therefore, with this new method after the CM-cellulose step we could omit the second DEAE-cellulose column chromatography, a step that was essential for obtaining homogeneous S-II in the previous method. The purity of S-II’ at the CM-cellulose step was always lower than that obtained by the previous method. Therefore, we concluded that this new procedure is not suitable for purification of S-II’. A summary of a typical purification of S-II is shown in Table I. The specific activity and yield of purified S-II were comparable with those obtained by the previous method. Thus, we conclude that the new method for purification of S-II is better than the previous one. Starting from the same amount of frozen cells, it was possible to save more than two weeks by improving the procedure for solubilizing stimulatory proteins and omitting the second DEAE-cellulose column chromatography. Use of a regular refrigerated centrifuge instead of an ultracentrifuge and omission of glycerol from buffer solutions also made the purification procedure much easier.

We believe that this new method for purifying S-II should be useful in studies on the regulation of eukaryotic transcription and transcription factors.

**Acknowledgement**

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

**References**