MULTIPLE FORMS OF A EUKARYOTIC RNA POLYMERASE II STIMULATION FACTOR

Michael E. DAHMUS

Department of Biochemistry and Biophysics, University of California, Davis, California 95616 USA

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1. Introduction

Eukaryotic cells contain multiple polymerase species which differ in their structure, function and localization. RNA polymerase II is responsible for the synthesis of heterogeneous nuclear RNA, a part of which is presumably the precursor of messenger RNA. For a current review on the structure and function of eukaryotic RNA polymerase see Chambon [1].

We have previously described the purification of a protein factor that stimulates the activity of purified Novikoff ascites RNA polymerase II [2]. This protein factor, designated HLF_2, does not stimulate the activity of *E. coli* RNA polymerase nor does it stimulate the activity of RNA polymerase II when denatured DNA is used as template. It appears to stimulate the initiation of RNA chains and alters the base composition of the RNA transcribed by purified RNA polymerase II [3]. This communication reports the resolution of a new protein factor that has a molecular weight identical to that of HLF_2 but can be resolved from HLF_2 by ion-exchange chromatography. Both factors stimulate the activity of DEAE-cellulose purified RNA polymerase II but only one factor stimulates highly purified RNA polymerase II. It is suggested that these two factors represent the active and inactive form of a single factor.

2. Materials and methods

Biochemicals: Unlabeled ribonucleoside triphosphates were purchased from P-L Biochemicals. [^{3}H]UTP was obtained from Schwarz-Mann.

Solutions: Buffer A contained 0.05 M Tris–HCl (pH 7.9), 5 mM $MgCl_2$, 0.1 mM EDTA, and 0.5 mM dithiothreitol.

Buffer B contained 0.05 M Tris–HCl (pH 7.9), 0.1 mM EDTA, and 0.5 mM dithiothreitol.

Cell Line: The Novikoff ascites tumor line was maintained by serial transplantation in female Sprague–Dawley rats. The cells were harvested and washed as previously described [2].

RNA polymerase assay: RNA polymerase was assayed as previously described [2]. Ascites DNA was prepared following the method of Dahmus and McConnell [4].

2.1. *Purification of RNA polymerase and stimulation factors*

RNA polymerase II was prepared by the procedure previously described [3]. Protein factors were prepared as described up to the step just prior to CM-cellulose chromatography [2] and further purified as described below. The sample (approx. 120 mg protein obtained from 50 g ascites cells) was applied to a 2.5 × 30 cm column of CM-cellulose equilibrated in buffer A. The column was developed with 2.5 column volumes of a linear gradient of 0–0.8 M NH_4Cl in buffer A. The peaks of stimulation activity that eluted at about 0.06 and 0.20 M NH_4Cl were then dialyzed against buffer B and separately chromatographed on a 0.9 × 12 cm phosphocellulose column equilibrated in buffer B. The columns were developed with five column volumes of a linear gradient of 0–0.8 M NH_4Cl in buffer B.

2.2. *Gel electrophoresis*

Polyacrylamide gel electrophoresis was carried out in sodium dodecyl sulfate as described by Weber and Osborn [5].

3. Results

The first step in the resolution of stimulation factors present in the flow-through material from the DEAE-cellulose column is chromatography on CM-cellulose. Treatment of sample to this point was as previously described [2]. The resolution of protein stimulation factors on CM-cellulose is shown in fig.1. The first peak of activity contains a heat stable factor and has been described in a number of systems [2,3,6–8]. The second peak of activity can be resolved into two peaks, designated HLF_1 and HLF_2 by chromatography on phosphocellulose (fig.2a). Chromatography of the third CM-cellulose peak on phosphocellulose results in a single peak of stimulation activity designated HLF_3 (fig.2b).

Because of an abundance of DEAE-cellulose purified RNA polymerase II, this partially purified polymerase fraction was routinely used to monitor stimulation activity. However, if highly purified RNA polymerase II was used to monitor stimulation activity no activity was detected in the region of HLF_3. The effect of HLF_2 and HLF_3 on the activity of partially purified and highly purified RNA polymerase II was, therefore, further examined. Increasing amounts of either HLF_2 or HLF_3 were assayed in the presence of a fixed amount of either DEAE-cellulose purified

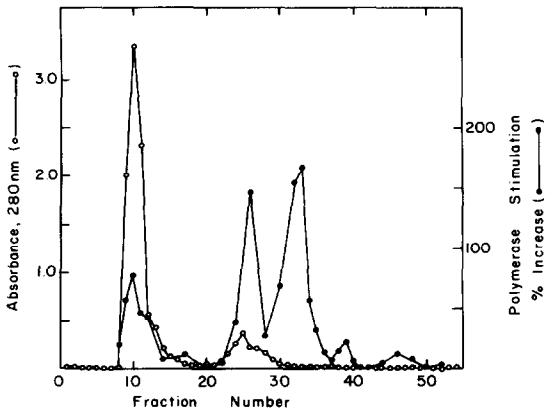

Fig.1. Resolution of protein factors by CM-cellulose chromatography. The sample was applied to a 2.5 × 30 cm column of CM-cellulose as described in Materials and methods. Fractions of 7.5 ml were collected. Aliquots of 0.02 ml were assayed for stimulation activity in the presence of a fixed amount of DEAE-cellulose purified ascites RNA polymerase II. Enzyme alone incorporated 24 pmol UMP in 30 min.

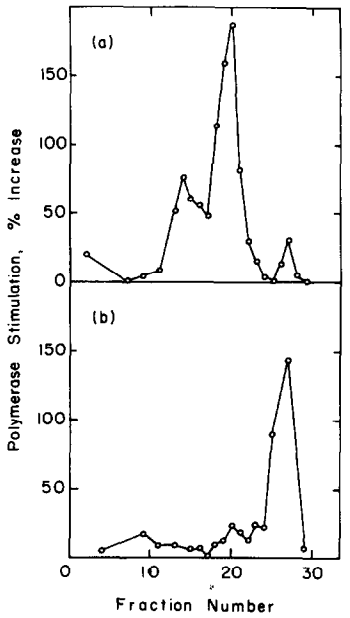

Fig.2. Chromatography of CM-cellulose purified stimulation factors on phosphocellulose. (a) Chromatography of the second CM-cellulose peak on a 0.9 × 12 cm column of phosphocellulose as described in Materials and methods. Fractions of 1 ml were collected and 0.02 ml aliquots were assayed in the presence of a fixed amount of DEAE-cellulose purified RNA polymerase II. Enzyme alone incorporated 11 pmol UMP in 30 min. (b) Chromatography of the third CM-cellulose peak on phosphocellulose. Conditions were identical as those used in (a) except that stimulation activity was assayed in the presence of a higher concentration of RNA polymerase II. Enzyme alone incorporated 22 pmol UMP in 30 min.

or highly purified RNA polymerase II. As shown in fig.3, HLF_2 stimulated the activity of RNA polymerase II irrespective of the stage of polymerase purification. HLF_3, however, stimulated only the DEAE-cellulose purified enzyme.

The fact that HLF_3 stimulated only the less pure fraction of RNA polymerase suggests that the stimulation may have resulted from an indirect effect on polymerase activity such as would arise from the inhibition of RNase or protease activity associated with the DEAE-cellulose purified enzyme. We found no evidence for the presence of such inhibitors in the HLF_3 fraction.

We have previously reported that purified HLF_2 has a mol. wt. of 85 000 [2]. Polyacrylamide gel electrophoresis of fractions from the CM-cellulose

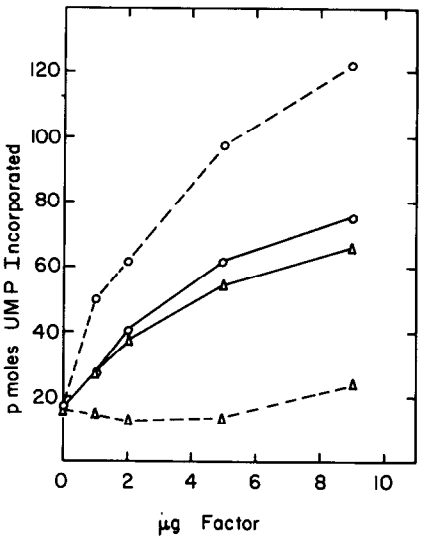

Fig.3. Effect of HLF_2 and HLF_3 on the activity of DEAE-cellulose purified and highly purified RNA polymerase II. Each reaction contained about 0.01 units of RNA polymerase II either in the form of DEAE-cellulose purified enzyme (o——o and o– – –o) or in the form of highly purified polymerase (^——^ and ^– – –^). Reactions which contained HLF_2 are indicated with solid lines (o——o and △——△); reactions which contained HLF_3 with broken lines (o– – –o and △– – –△). Reactions were carried out in the standard assay system. Protein was determined according to the method of Lowry et al. [9]. Both HLF_2 and HLF_3 were purified through phosphocellulose.

column in the presence of sodium dodecyl sulfate showed the presence of a protein with similar molecular weight coincident with the HLF_3 peak of activity. Phosphocellulose purified HLF_2 and HLF_3 were therefore analyzed by electrophoresis in sodium dodecyl sulfate polyacrylamide gels. The major protein associated with HLF_3 had a mobility indistinguishable from that of HLF_2 (fig.4). The major bands were not resolved when both HLF_2 and HLF_3 were electrophoresed in the same gel.

4. Discussion

The activity of highly purified ascites RNA polymerase II is stimulated 5–10-fold by a protein factor of mol. wt. 85 000 [2]. This factor appears to stimulate the initiation of RNA chains and alters the base composition of the RNA transcribed by RNA polymerase II using deproteinized DNA as a template [3].

HLF_2 and HLF_3 appear to be of identical mol. wt. as indicated by the fact that they cannot be resolved

Fig.4. Sodium dodecyl sulfate gel electrophoresis of phosphocellulose purified HLF_2 and HLF_3 on 5% polyacrylamide gels. Gel #1 contained *E. coli* RNA polymerase which was used as a standard; gel #2, HLF_2; and gel #3 HLF_3.

by sodium dodecyl sulfate polyacrylamide gel electrophoresis, but appear to differ in charge at pH 7.9, as indicated by the fact that they can be resolved by ion-exchange chromatography. This suggests that HLF_2 and HLF_3 may possess a similar primary sequence but differ in their extent or type of modification. If we assume that only one form is active in the stimulation of RNA polymerase II these results can be explained as follows: HLF_2 is the active form of factor and can, therefore, stimulate either the highly purified or less pure RNA polymerase II. HLF_3 is inactive and must be converted to HLF_2 before polymerase stimulation can occur. A component present in the DEAE-cellulose purified polymerase fraction catalyzes to conversion, thereby accounting for the ability of HLF_3 to stimulate only the impure polymerase fraction. This proposal is also consistent with the observation that the relative amounts of HLF_2 and HLF_3 vary somewhat from preparation to preparation even though the total activity recovered is quite constant.

This proposal would superimpose an additional level of control upon the activity of RNA polymerase II. Even though the physiological significance of HLF_2 has not yet been established, the fact that the stimulation of purified RNA polymerase II is enzyme specific, requires native DNA, and alters the base composition of the products argues against a variety of trivial explanations. Therefore, if HLF_2 plays a fundamental role in the regulation of RNA polymerase II activity in vivo, the change that it could bring about, acting in a catalytic manner, could be of very broad magnitude. This factor would have the potential to function as a member of a 'cascade' chain of amplification.

Acknowledgements

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