THE ROLE OF BASIC PROTEINS IN THE DNA DEPENDENT RNA POLYMERASE REACTION

Evidence that RNA polymerase subunits are distinct from fraction S protein

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1. Introduction

The previously defined fraction S of calf thymus contains a basic protein factor which efficiently stimulates the activity of the eucaryotic α-amanitin sensitive RNA polymerase B (or II) on a native DNA template under defined in vitro conditions [1,2]. At present, this is the only stimulatory factor extractable from animal tissues, for which formation of a complex between factor and the sensitive enzyme has been reported [2]. This complex formation appears to be specific in so far, as it is restricted to the RNA polymerase B. Neither the *E.coli* RNA polymerase, which is poorly stimulated by fraction S, nor the fraction S insensitive RNA polymerase A (or I) of calf thymus bind stimulatory factor to a measurable extent (Stein, unpublished results).
A rough determination of the molecular weight of fraction S activity has recently demonstrated a striking similarity between the molecular weight of fraction S and that of one of the small RNA polymerase B subunits, reported to be in the range of 25 000 daltons [3,4]. This finding, together with the aforementioned affinity between the fraction S activity and the RNA polymerase B, raised the question of whether the fraction S activity may not simply represent an easily dissociable enzyme subunit which thus could in some cases become separated from the actual enzyme molecules. Further support for this hypothesis was provided by recent findings, that mammalian enzymes in fact may lose a part of their smaller subunits; this was demonstrated for the RNA polymerase A of calf thymus [5] as well as of yeast [6].

Since we do not have any detailed knowledge regarding the nature of the stimulatory activity of fraction S, the possible subunit origin of that fraction was more carefully studied. The experiments summarized here, suggest a clear difference between the molecular weight of fraction S and those of each of the smaller RNA polymerase subunits. This indicates that fraction S activity does not represent a temporarily free subunit of the RNA polymerase B. This may also throw some light on the role of this stimulatory protein, which thus may be regulatory rather than restorative.

2. Materials and methods

RNA polymerase B of calf thymus was purified primarily in four steps carried out at 4°C. Calf thymus tissue was homogenized in a low salt buffer as previously described [1]. The homogenate after being cleared by centrifugation (100 min, 12 000 rev./min, Sorvall GSA rotor) was treated batchwise with DEAE cellulose (Whatman DE 52) in homogenization buffer at 0.1 M $(NH_4)_2SO_4$. The stimulatory activity did not absorb to the DEAE under these ionic conditions and was further handled as given below. The enzymatic activity, which was absorbed, could be eluted (after extensive washing of the DEAE cellulose with the absorbing buffer) by 0.4 M $(NH_4)_2SO_4$ in the MS 30 buffer of Kedinger et al. [7]. The eluate was further absorbed onto a phosphocellulose column (Whatman P 11) in MS 30 buffer, diluted to 0.05 M $(NH_4)_2SO_4$. It was eluted

from that column by 0.15 M $(NH_4)_2SO_4$ in the same buffer and chromatographed on DEAE Sephadex essentially according to [3]. Peak fractions of the enzymatic activity eluting from the column were concentrated with $(NH_4)_2SO_4$ and spun in a sucrose gradient (5–20%) in MS 30 buffer with 0.1 M $(NH_4)_2SO_4$ at 65 000 rev./min for 18 h. Fractions containing the enzymatic activity were found to coincide with a protein peak and were clearly separation from the bulk of contaminating protein. These fractions were used in the following experiments. Their α-amanitin sensitivity was more than 90% at 1 μg/ml of α-amanitin.

Preparation of RNA polymerase B specific stimulatory activity (fraction S) started with the material not absorbed to the DEAE cellulose as mentioned above. It was concentrated by a 45–60% $(NH_4)_2SO_4$ precipitation, dialysed against a buffer containing 0.01 M Tris–HCl pH 7.0, 5 mM β-mercaptoethanol, 10% glycerol (TGM-buffer) in the presence of 0.03 M KCl, and thereafter applied to a SP-Sephadex column at about 2 mg protein/ml column volume. After extensive washing of the column with the absorption buffer, the stimulatory activity was eluted by increasing the KCl concentration to 0.1 M KCl in the TGM buffer and then given directly onto a hydroxyapatite column equilibrated with 0.01 M phosphate buffer, pH 6.0, 5 mM β-mercaptoethanol, 10% glycerol. Stimulatory activity was eluted from that column by a gradient from 0.1–0.35 M phosphate buffer, pH 6.0, with the other components as above. At this step, stimulatory activity eluted usually as a single, slightly asymmetric peak which coincided with a small protein peak. The active fractions were pooled, dialysed extensively against TGM buffer with 0.03 M KCl and stored at −70°C. (fraction S). If necessary, they were once more concentrated by $(NH_4)_2SO_4$ precipitation.

3. Results

Fraction S, when purified as given in Materials and methods exhibits a specific stimulatory activity (on a weight basis of protein) about 200–300-fold higher than that of the original crude extract. Upon polyacrylamide electrophoresis of fraction S according to Weber and Osborn [9], a major protein fraction with

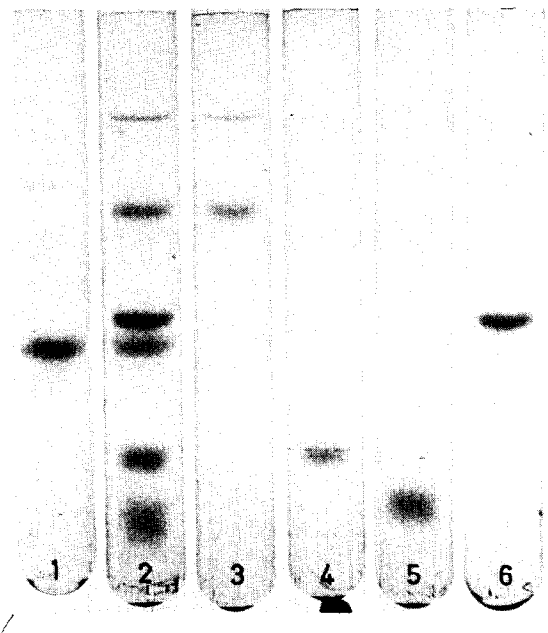

Fig.1. Sodium dodecyl sulfate polyacrylamide electrophoresis of fraction S as compared to marker proteins in 10% polyacrylamide gels according to Weber and Osborn [9]. The tubes contain, fraction S (5 μg) gel, 1,2; ovalbumin, gel 3,2; myoglobin, gel 4,2; cytochrome *c*, gel 5,2; carbonic anhydrase, gel 6,2.

a fairly uniform size distribution is consistently seen (fig.1). The molecular weight of this fraction, as determined by this method, is 26 500 with reference to the marker proteins (fig.2). When fraction S is sub-

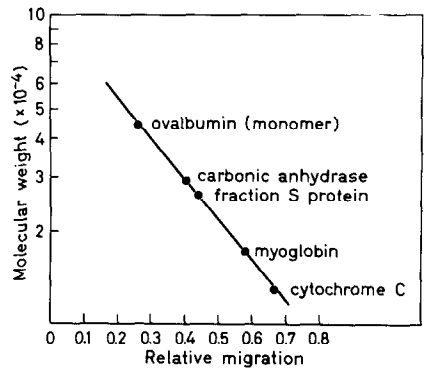

Fig.2. Molecular weight estimation of the fraction S protein according to its mobility relative to the reference proteins listed in fig.1. The relative migration of the proteins was determined according to [9].

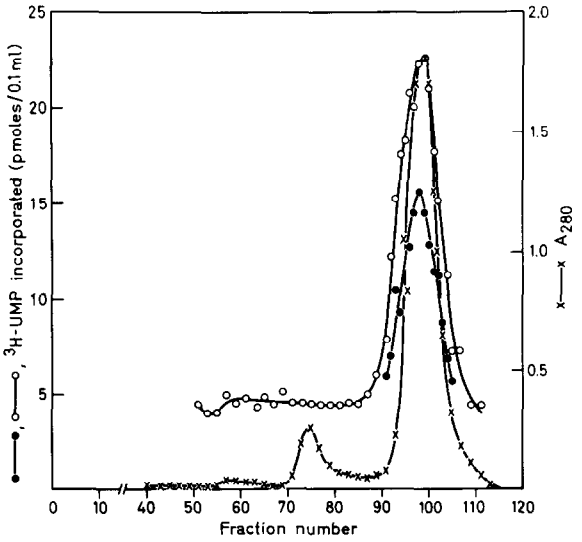

Fig.3. Gel chromatography of fraction S on Sephadex G-100 (superfine). Elution of fraction S (25 mg) from the column (2.5 × 40 cm) was carried out with 0.01 M Tris–HCl, pH 7.0, 5 mM β-mercaptoethanol. 0.5 ml portions were collected and protein distribution determined by A_{280} nm – adsorption of every second column fraction (×——×) Activity tests were performed as given in [8] with 3 μl of every second column fraction. (○——○) and, where indicated, with 2 μl of each column fraction (■——■).

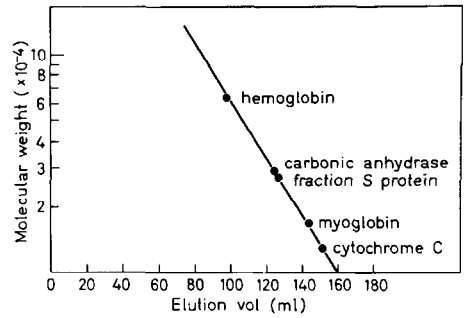

Fig.4. Molecular weight estimation of the fraction S protein by gel chromatography according to its elution behaviour from the Sephadex G-100 column of fig.3. Reference proteins, listed in fig.4, were run under the same conditions as fraction S.

jected to molecular sieve chromatography on a Sephadex G-100 gel, a rather homogeneous protein distribution is again observed, with one main symmetrical peak eluting from that column (fig.3). According to this method, the apparent molecular weight of the main protein constituent of fraction S is determined to be 27 500 (fig.4). Electrophoresis of several fractions of the main peak eluting from the Sephadex G-100 column reveals that the protein peak is also homogeneous under the electrophoretic conditions of Panyim and Chakley [10] (results not shown); this suggests that we are dealing here with only one protein, which will be referred to as the fraction S protein. The close correspondence between its concentration and the observed stimulatory activity in the column fractions of fig.3 further suggests that the fraction S protein may be identical with the stimulatory factor. This view is supported by the fact, that the purification schedule of fraction S, as given in Materials and methods, normally does not include a separation on a molecular weight basis. Therefore the correspondence between the stimulating activity and the protein concentration upon gel chromatography can hardly be accidental.

As mentioned above, the molecular weight of the fraction S is close to that of one of the small subunits of the RNA polymerase B of calf thymus, leading to the question of whether both peptides may not be in fact identical. According to previous determinations, the molecular weight of the enzyme subunit in question was estimated to be 25 000 dalton [3,4]. This suggests a molecular weight difference of roughly 2000 daltons between the fraction S protein and the 25 000 dalton subunit of the RNA polymerase B. This small difference may not be real however, especially since the molecular weight specifications for this enzyme subunit are not fully consistent [3,4,11]. To unequivocally prove or disprove whether a molecular weight difference exists between both peptides, it appeared necessary to directly compare their mobility under identical electrophoretic conditions, e.g., within one and the same gel. Figure 5 shows the results of such an experiment, where fraction S and RNA polymerase B of calf thymus are subjected to polyacrylamide electrophoresis both alone and together. RNA polymerase B here shows the typical banding pattern with the two (or possibly three) big subunits scarcely separated in the 9% gel and with three well separated small subunits. It is also seen that the fraction S protein exhibits about the

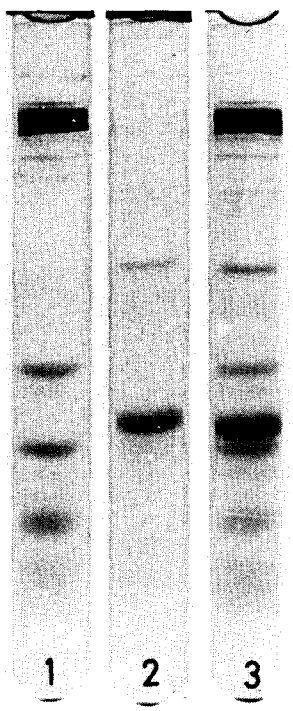

Fig.5. Sodium dodecyl sulfate polyacrylamide electrophoresis of calf thymus RNA polymerase B and fraction S alone, as well as in combination in 9% polyacrylamide gels according to Weber and Osborn [9]. Gel 1 contained 20 μg of RNA polymerase B purified as given in Materials and methods: gel 2, 3 μg of fraction S; gel 3, 20 μg of RNA polymerase B and 5 μg of fraction S.

same mobility as the middle of the small enzyme subunits. Upon combination of fraction S and RNA polymerase B however, a difference can clearly be seen between the mobility of the fraction S protein and that of the 25 000 dalton subunit. Consistent with the molecular weight determinations, the enzyme subunit in question moves slightly faster than the fraction S protein.

This results allows a clear cut distinction in the molecular weight of the fraction S protein and the 25 000 dalton subunit of the responsive enzyme, which implies that both peptides are basically different. It then appears likely, that the stimulatory effect of fraction S represents more than a mere restoration of an incomplete enzyme molecule. Nevertheless this possibility cannot be ruled out completely at the moment, since the fraction S protein may still act as a substitute for one of the subunits of the RNA polymerase B, for the 25 000 dalton peptide in special. In this case further similarities between fraction S and the subunit in question would have to be expected. Whether they do exist must await further investigation.

Acknowledgements

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