HALOBACTERIUM CUTIRUBRUM RNA POLYMERASE: SUBUNIT COMPOSITION AND SALT-DEPENDENT TEMPLATE SPECIFICITY

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1. Introduction

We have described [1, 2] the partial purification and properties of DNA-dependent RNA polymerase from the extreme halophile *Halobacterium cutirubrum*. Since extremely halophilic bacteria have an intracellular ionic strength of about 5.9 and enzymes isolated from them usually require high salt concentrations for stability and activity [3], it was surprising that the RNA polymerase was inhibited by salt when calf thymus DNA was used as template. The present paper describes (1) the isolation and purification of two proteins that together form the active enzyme, and (2) the effect of salt concentration on the template specificity of the polymerase.

2. Materials and methods

DNA-dependent RNA polymerase was isolated from late log phase *H. cutirubrum* strain no. 9 cells as previously described [2]. After partial purification by acid precipitation, it was passed through a column (5 cm × 100 cm) of Bio-Gel P-60 polyacrylamide gel swollen and equilibrated in 2.5 M KCl, 1 M NaCl, 10 mM tris-HCl buffer, pH 8.6 ('high salt buffer') [2, 4]. The active fractions were chromatographed on a hydroxylapatite gel column (0.9 cm × 15 cm) previously equilibrated with high salt buffer. After the enzyme had been applied in high salt buffer (5 ml), a further 20 ml of the same buffer were passed through the column, followed by 0.1 M phosphate in high salt buffer. Fractions were collected and dialyzed against high salt buffer. The individual fractions were all inactive, but polymerase activity was restored when the protein peak (protein α) eluted with high salt buffer containing 0.1 M phosphate was mixed with the second of two peaks that were eluted during the preliminary washing step (protein β). Protein β was further purified by adsorption to hydroxylapatite (1 g/8 ml solution) followed by batchwise elution with 0.1 M phosphate in high salt buffer (5 ml). All steps after the harvesting of the bacteria were performed at 0–5°. The purified proteins α and β were both homogeneous when examined by polyacrylamide gel electrophoresis at several pH values and gel concentrations.

H. cutirubrum DNA was isolated and purified by a method adapted from the procedures of Marmur [5] and Loeb and Chauveau [6]. It had a two-step thermal denaturation curve similar to that previously described for *H. salinarium* DNA [7], and the T_ms indicated molar base ratios of 66% and 57% GC for the main component and the satellite respectively, in agreement with the published values [8]. The purified DNA contained less than 3% (w/w) of protein and 1% (w/w) of RNA, estimated by the microbiuret [9] and the RNA-specific cysteine-H_2SO_4 [10] procedures, respectively.

RNA polymerase activity was measured by the standard assay [2]. The rate of initiation of new polyribonucleotide chains was determined simultaneously by the method of Travers and Burgess [11] which measures incorporation of γ-^{32}P-ATP and –GTP into an acid-insoluble form. γ-Labelled ^{32}P-purine nucleotides were from International Chemical and Nuclear Corporation, Irvine, Calif., USA. Calf thymus DNA (Sigma Chemical Co., St. Louis, Mo., USA), T_4-coliphage DNA (Calbiochem, Los Angeles, Calif., USA), poly d(AT) (General Bio-

chemical, Chagrin Falls, Ohio, USA) or *H. cutirubrum* DNA was used as template. The standard assay medium (0.1 ml) contained: tris-HCl buffer, pH 8.6, 10 μmoles; ^{14}C- and γ-^{32}P-ATP, 10 nmoles; γ-^{32}P-GTP, 10 nmoles; CTP and UTP, 15 nmoles each; native DNA, 30 μg; $MgCl_2$, 10 μmoles; $MnCl_2$, 1 μmole; protein α and/or protein β, as indicated. Assay mixtures designated '+ salt' contained, in addition, KCl (150 μmoles) and NaCl (60 μmoles). Incubation was at 37° for 1 hr. Aliquots were then streaked on filter paper strips, which were washed, dried and counted by liquid scintillation as previously described [2]. All results were corrected for quenching, for ^{32}P counted in the ^{14}C-channel of the liquid scintillation counter and for incorporation into suitable controls.

3. Results and discussion

Proteins α and β were both essential for RNA polymerase activity at either high or low salt concentrations. Nearest neighbour analyses with poly d(AT) as template and ATP and UTP as substrates, were performed as described by Litvak, Carré and Chapeville [12], using equal amounts of α and β. The results showed that the template was copied correctly in the standard assay conditions.

Table 1 shows the results of a study of the template specificity of the enzyme in the presence and absence of salt. In the presence of 1.5 M KCl and 0.6 M NaCl, the enzyme was active with *H. cutirubrum* DNA and calf thymus DNA was accepted as template to a limited extent only. However, in the absence of salt the template specificity was reversed: the enzyme was active with calf thymus DNA, as previously reported [2], and no significant transcription of *H. cutirubrum* DNA occurred. T_4-DNA was without template activity either with or without salt, whereas the alternating copolymer poly d(AT) was a template at both high and low ionic strength. However, *H. cutirubrum* DNA at high ionic strength was almost twice as effective as any other template studies so far. It was also found, in separate experiments, that a total salt concentration of 2.5 M or higher was necessary for optimum activity with *H. cutirubrum* DNA and the rate of reaction fell rapidly as the salt concentration was reduced below 2 M. These results explain our earlier observations that *H. cutirubrum* DNA-dependent RNA

Table 1
Template specificity of *H. cutirubrum* RNA polymerase.

DNA	Salt	Incorporation (nmoles)		Ratio ^{14}C/^{32}P
		^{14}C-ATP	γ-^{32}P-ATP and -GTP	
HC	+	3.03	0.164	18.8
HC	–	0.01	0.004	–
CT	+	0.20	0.013	15.4
CT	–	1.77	0.098	18.1
T_4	+	0.01	0.003	–
T_4	–	0.02	0.005	–
d(AT)	+	1.56	0.070	22.3
d(AT)	–	1.59	0.071	22.4

Chain elongation (^{14}C-ATP incorporation) and initiation (γ-^{32}P-ATP and -GTP incorporation) were measured in the standard assay (Materials and methods) with and without 1.5 M KCl, 0.6 M NaCl; 160 ng each of both protein α and protein β were used in all cases. Abbreviations: CT, calf thymus; d(AT), polydeoxy(adenylate-thymidylate) copolymer; HC, *H. cutirubrum*; T_4, T_4-coliphage.

Table 2
Effect of changes in relative concentration of proteins α and β on DNA-dependent RNA polymerase activity.

Protein (ng)		Incorporation (nmoles)		Ratio ^{14}C/^{32}P
α	β	^{14}C-ATP	γ-^{32}P-ATP and -GTP	
Exp. 1				
40	0	0	0	–
40	16	0.351	0.020	17.5
40	32	0.672	0.037	18.1
40	40	0.722	0.040	18.0
40	48	0.730	0.041	17.8
40	80	0.711	0.040	17.8
Exp. 2				
0	40	0	0	–
16	40	0.370	0.020	18.5
32	40	0.784	0.040	18.4
40	40	0.900	0.050	18.0
48	40	0.910	0.050	18.2
80	40	0.920	0.050	18.4

Chain elongation (^{14}C-ATP incorporation) and initiation (γ-^{32}P-ATP and -GTP incorporation) were measured in the standard assay system using *H. cutirubrum* DNA as template in the presence of 1.5 M KCl, 0.6 M NaCl and the indicated amounts of proteins α and β.

polymerase was more active in the absence of salt, since the template specificity of the enzyme is profoundly affected by changes in the ionic strength of the assay medium.

The effect of varying the concentration of either protein α or protein β in the presence of a constant amount of the other component was then examined, using *H. cutirubrum* DNA as template in high-salt conditions (table 2). It can be seen that a plateau of activity was attained when the weight (and hence molar) ratio of the two proteins was 1:1. Further, the ratio of chain elongation to chain initiation ($^{14}C/^{32}P$ ratio) remained constant for all molar ratios of proteins α and β (table 2) and was only affected slightly by the nature of the template (table 1) whenever significant incorporation was detected. It is therefore probable that proteins α and β are essential subunits that combine in the conditions of the assay to form the active enzyme and that neither of them is an independent initiation factor similar to the σ-factor of the *E. coli* enzyme [13, 14]. Chain initiation might be a function of either protein or of both together; further study will be necessary to clarify this point.

When protein β was stored in the absence of salt, it lost its activity irreversibly within 20 hr, and the loss was accompanied by a change in electrophoretic mobility. The inactive protein was incapable of yielding an active polymerase when mixed with protein α. In contrast, the latter retained 50% of its potential activity after storage for 20 days in the absence of salt.

The molecular weights of proteins α and β were estimated by gel filtration and density gradient centrifugation in high salt buffer by the methods of Louis et al. [4], and found to be 18,000 in both cases. A mixture of α and β had the same molecular weight, so they do not associate in high salt buffer. It will be of interest to investigate the nature of their functional association during chain initiation and polymer synthesis.

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