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RNA POLYMERASE FROM OPAQUE-2 AND NORMAL *ZEA MAYS* ENDOSPERM

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Abstract—RNA polymerase from Opaque-2 and normal maize showed qualitative differences during endosperm development. DEAE-Sephadex column chromatography indicated the presence of one and three RNA polymerases respectively at 15 and 25 days post-pollination. The polymerases from Opaque-2 and normal endosperms at 15 days post-pollination showed considerable differences in Mn^{2+} optimum. The optimum Mn^{2+} for normal polymerase was ten times higher than for Opaque-2 polymerase. The polymerase activity from endosperms at 15 days post-pollination was due to nucleoplasmic RNA polymerase II.

INTRODUCTION

The presence of multiple forms of DNA-dependent RNA polymerase in several eukaryotes [1–6] suggest that specific genes or a group of genes might be transcribed by different enzymes. This has been supported by the fact that one enzyme is localized within the nucleolus where ribosomal RNA synthesis is restricted, while another is in the nucleoplasm [1,6–8] where DNA like RNAs are formed. In our earlier studies [9] on the probable mechanism of suppression of zein synthesis in Opaque-2 maize endosperm it was suggested that the Opaque-2 gene exerts a regulatory control of *m*RNA synthesis. In the present studies RNA polymerases from developing endosperms of normal and Opaque-2 maize have been purified and characterized with respect to metal ion requirements, and sensitivity to inhibitors.

RESULTS AND DISCUSSION

In order to determine the presence of multiple polymerases and to observe changes in polymerases during endosperm development, the RNA polymerase from normal and Opaque-2 endosperm was fractionated on DEAE-Sephadex. RNA polymerase was eluted from the column in one peak at a salt concentration *ca* 0·22 M from 15-day-old normal and Opaque-2 endosperms, while the endosperms at 25 days post-pollination showed one major peak of activity and two minor peaks. The activity of RNA polymerase from 15-day-old endosperms was considerably higher than that from 25-day endosperms. Due to the lability of RNA polymerases from 25 days post-pollinated endosperms, these could not be characterized.

The characteristics of RNA polymerase from 15 day post-pollinated normal and Opaque-2 endosperms are shown in Table 1. Both polymerases showed an absolute requirements for DNA, metal ion and nucleoside triphosphates. Higher salt concentration reduced the activity slightly in Opaque-2 but more in normal. At the optimum Mn^{2+} the polymerase activity was more than three fold greater than the activity at optimum Mg^{2+}. Actinomycin-D inhibited polymerases almost completely, while rifampicin had no inhibitory effect. Both polymerases preferred denatured DNA. Thus normal and Opaque-2 polymerases are similar in these properties to other purified RNA polymerases from other sources [6,10–14]. The sensitivity of the polymerase to α-amanitin and higher activity with Mn^{2+} compared to Mg^{2+} indicates that it is a nucleoplasmic enzyme, similar to RNA polymerase II found in other eukaryotes [4,10,14–17]. The lack of com-

Table 1. Characteristics of normal and Opaque-2 maize endosperm RNA polmerase

Assay system	Enzyme source Normal (relative activity)	Opaque-2 (relative activity)
Complete	100*	100†
−CTP, GTP, ATP	<1	1
−DNA	0	0
−Mn, −Mg	2·2	2·6
$-Mn^{2+}$ + Mg^{2+}, 1 mM	21·3	5·4
$-Mn^{2+}$ + Mg^{2+}, 5 mM	30·5	31·0
$-Mn^{2+}$ + Mg^{2+}, 10 mM	15·6	26·3
+Calf-thymus DNA Native	13·0	40·0
$+(NH_4)_2SO_4$, 50 mM	54·8	85·4
+Actinomycin-D (10 μg/ml)	<1	1
+Rifampicin (80 μg/ml)	102	102
+α-Amanitin (0·1 μg/ml)	9·6	2·8
+RNase A + T_1 (8 μg/ml + 10 units) Preincubation	56	61
+RNase A + T_1 (8 μg/ml + 10 units) Postincubation 60° for 15 min	8	10

The enzyme from 15 day old endosperms purified on DEAE-Sephadex was assayed as given in experimental.

* Value actually found was 198 pmol UMP incorporated/mg protein/30 min.

† Value actually found was 217 pmol UMP incorporated/mg protein/30 min.

Table 2. Effect of Mn^{2+} concentration on RNA polymerase activity from normal and Opaque-2 maize endosperm

$MnCl_2$ (mM)	Relative activity Normal	Opaque-2
0	1·0	<1·0
0·01		10·5
0·05		81·1
0·1	19·1	100·0
0·5	95·1	73·4
1·0	100·0	49·0
2·0	77·0	31·3

plete inhibition by RNase suggests that some of the product may be associated with the DNA template [10]. The enzymes from normal and Opaque-2 endosperms, though similar in elution patterns, were found to differ in their Mn^{2+} requirements. The optimum Mn^{2+} concentration for normal polymerase was 10 times higher than for Opaque-2 polymerase (Table 2). This may account for the differential rate of RNA synthesis rather than the type of RNA synthesized, depending on the *in vivo* conditions.

EXPERIMENTAL

The endosperms from self-pollinated ears of normal inbred line Fla 3H 94-f and its Opaque version were hand ground in liquid N_2 in the presence of buffer I (50 mM Tris–HCl, pH—7·9; 0·1 mM EDTA; 0·5 mM PMSF; 5 mM $MgCl_2$; 10 mM mercaptoethanol; 0·4 M $(NH_4)_2SO_4$; 25% glycerol). Slurry was passed through Mira-cloth and the homogenate obtained was sonicated 3 × for 20 sec each time. The sonicated extract was centrifuged at 144000 *g* for 1 hr and the supernatant collected. To this an equal vol sat $(NH_4)_2SO_4$ (pH, 8) was added slowly, stirred at 4° for 45 min and centrifuged at 10000 *g* for 10 min. The ppt solubilised in buffer II (50 mM Tris–HCl, pH—7·9; 0·1 mM EDTA; 1 mM $MgCl_2$; 10 mM mercaptoethanol; 25% glycerol) was freed of salts by passing it through Sephadex G-50 (7 × 2·5 cm) equilibrated with buffer III (50 mM Tris, pH—7·9; 10 mM mercaptoethanol; 0·1 mM EDTA; 0·5 mM $MgCl_2$; 10 mM $(NH_4)_2SO_4$; 25% glycerol). Proteins eluted in the void vol were layered on a DEAE-Sephadex A-25 column (4 × 2·5 cm) equilibrated with buffer III. Proteins eluted by a linear gradient of $(NH_4)_2SO_4$ (0·1 M–1 M) in buffer III were monitored at 283 nm and 1·8 ml fractions were collected. The overall purification procedure resulted in a 637-fold increase in sp. act. (pmol UMP incorporated/mg protein/30 min.) The complete reaction mixture contained in a reaction vol of 0·25 ml: 0·2 mM each of ATP, GTP, CTP; 20 μg single stranded calf thymus DNA; 1 mM Mn^{2+}; TES, 50 mM; mercaptoethanol, 10 mM; H^3-UTP, 2 μCi (15·5 μCi per mMol); Enzyme, 50 μl; $(NH_4)_2SO_4$ 50 mM. For the Opaque-2 polymerase assay the reaction mixture contained 0·1 mM Mn^{2+} instead of 1 mM. The assay was carried out at 27° for 30 min. At the end of the incubation period the reaction was terminated, the reaction mixture filtered on glass fibre filters, washed and counted according to the method of Ref. [18]. Protein was estimated by the method of Ref. [19].

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