TRANSFER RNA-PRIMED OLIGOADENYLATE SYNTHESIS IN MAIZE SEEDLINGS. III. DEOXYOLIGONUCLEOTIDE PRIMERS.

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Summary. Single stranded DNA can replace tRNA as the nucleic acid component required for ATP incorporation by the adenylating enzyme from maize seedlings. Preliminary data suggest that deoxyoligonucleotides serve as primers for addition of AMP moieties to 3' hydroxyl termini. The strict requirement for manganese is abrogated by desalting the enzyme.

We have been trying to discern the physiological role of the adenylating enzyme isolated from corn tissue (Walter and Mans, 1970). Although no definitive metabolic function for analogous activities from mammalian tissue (Klemperer, 1965) and bacteria (August et al., 1962) has been elucidated, a structural relationship between poly A polymerase and RNA polymerase in E. coli has been indicated (Terzi et al., 1970). In this communication I report the results of preliminary experiments that suggest the maize adenylating enzyme is either an RNA polymerase or a component of a complex of proteins that has RNA polymerase activity.

In purification of the soluble RNA polymerase from maize seedlings (Stout and Mans, 1967), the adenylating enzyme was found in proteins precipitated at lower $(NH_4)_2SO_4$ levels (25 to 35% saturated) than the salt precipitated proteins rich in polymerase (35 to 50% saturated $(NH_4)_2SO_4$). Upon chromatography on DEAE-cellulose, adenylating enzyme was eluted at 0.2 M Tris-HCl whereas the polymerase was eluted at 0.5 M salt. Therefore, adenylating enzyme and RNA polymerase II (Benson, 1971) are readily separable.

The adenylating enzyme formed aggregates in saturated $(NH_4)_2SO_4$ that were

excluded from Sephadex G-200. However, if the same enzyme was desalted on Sephadex G-50, it eluted slightly after bovine serum albumin on Sephadex G-200. The apparent decrease in molecular weight on disaggregation of the enzyme resulted in a 3-fold increase in specific activity.

As detected originally (Walter and Mans, 1970) adenylating enzyme required tRNA, ATP and manganese for the addition of AMP moieties to the 3' hydroxyl terminus of the primer (Mans and Walter, 1971). The disaggregated enzyme utilized here catalyzed the accumulation of acid-insoluble material when presented with single-stranded DNA, ATP and magnesium (Fig. 1). Although without effect after 15 min incubation, deoxyribonuclease I after 60 min enhanced the activity of the adenylating enzyme twelve-fold on native DNA; presumably, by generating single-stranded DNA and 3' hydroxyl termini (Laskowski, 1961). The behavior of adenylating enzyme on several synthetic polymers of deoxyribonucleotides suggests that like tRNA single-stranded DNA is utilized as a primer rather than

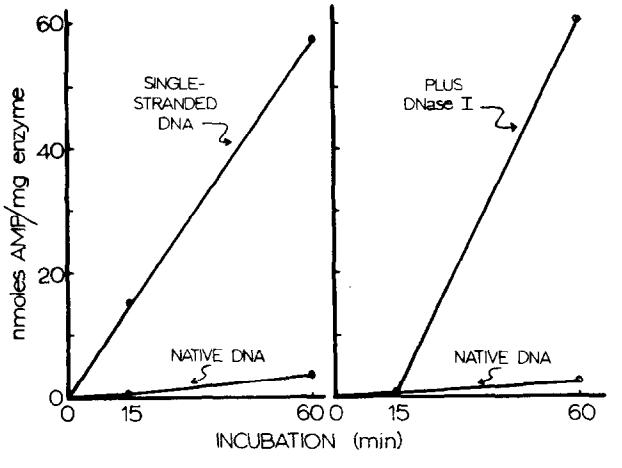

Fig. 1. A reaction mixture (0.2 ml) containing 20 µmoles Tris-HCl (pH 8.4), 2 µmoles DTT, 2.5 µmoles $MgCl_2$, 0.25 µmoles [8-^{14}C]ATP (3.5 mc/mmole), 80 µg adenylating enzyme and 0.83 A_{260} native calf thymus DNA or 0.90 A_{260} single-stranded calf thymus DNA (resolved from double-stranded on hydroxyl apatite) where indicated was incubated at 30°. Where indicated, 5 µg deoxyribonuclease I (Worthington Biochemicals) was added at zero time. Aliquots were removed at times indicated and acid-insoluble radioactivity determined on filter paper disks as described previously (Walter and Mans, 1970).

as a template (Table I). Poly dT and poly dA primed ATP incorporation. In the absence of TTP, poly dA:dT and poly d(AT) primed ATP incorporation.

TABLE I

DEOXYOLIGONUCLEOTIDE SPECIFICITY OF ADENYLATING ENZYME

Reaction mixtures as described in Fig. 1 but containing 0.5 μmoles $MnCl_2$, 0.8 A_{260} of the indicated nucleic acid and 0.12 μmoles TTP where indicated, were incubated at 30° for 60 min and acid-insoluble radioactivity determined.

Primer	Specific Activity (nmoles AMP/mg enzyme)	
	without TTP	with TTP
poly dT	9.2	--
poly dA	34.4	29.5
poly dA:dT	37.2	31.1
poly d(AT)	22.2	13.0

Since deoxyoligonucleotides stimulated AMP incorporation in the presence of magnesium as well as manganese, the metal requirement of the desalted enzyme primed with tRNA was re-examined. In the presence of magnesium tRNA served as a primer and the addition of salt inhibited the reaction (Table II). In contrast, addition of salt to a reaction mixture containing manganese stimu-

TABLE II

METAL AND SALT REQUIREMENTS FOR RNA

PRIMER ACTIVITY WITH ADENYLATING ENZYME

Reaction mixtures as described in Fig. 1 but containing 1.0 μmoles of $MnCl_2$ or 2.5 μmoles $MgCl_2$, 0.95 A_{260} *E. coli* tRNA, poly U or poly A and 10 μmoles $(NH_4)_2SO_4$ as indicated were incubated at 30° for 60 min and acid-insoluble radioactivity determined.

Primer	Specific Activity (nmoles AMP/mg enzyme)	
	with $MgCl_2$	with $MnCl_2$
tRNA	174	72
tRNA with $(NH_4)_2SO_4$	68	234
Poly A	2.4	0.6
Poly U	12.9	7.5

lated ATP incorporation. Poly U also stimulated ATP incorporation in the presence of magnesium. Activity, barely detectable with poly A in the presence of manganese, was significant with desalted enzyme in the presence of magnesium.

In summary: thus alteration in the specificity of the nucleic acid required to prime ATP incorporation as a function of divalent metal ion and salt concentration and the behavior of the enzyme on DEAE-cellulose chromatography suggests that the maize adenylating enzyme may be a part of a protein complex that functions physiologically as an RNA polymerase, perhaps polymerase I (Roeder and Rutter, 1970). Alternatively, the adenylating enzyme may function directly in the synthesis of adenylate-rich regions of mRNA.

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