Phytochemistry, 1972, Vol. 11, pp. 2443 to 2446. Pergamon Press. Printed in England.

RNA POLYMERASE ACTIVITY IN RESTING AND GERMINATING WHEAT SEEDS

BARBARA MAZUŚ and J. BUCHOWICZ

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

(*Received* 3 *December* 1971)

Key Word Index—*Triticum aestivum*; Gramineae; wheat; RNA polymerase; germination.

Abstract—RNA polymerase activity was measured in extracts of dry wheat seeds and in extracts of the grain germinated for various periods, up to 48 hr. Activity was found to be relatively high in the dry seed and to increase moderately with germination. These observations support the view that mature wheat seed may resume RNA synthesis immediately after suitable changes in the environment allow it to germinate.

INTRODUCTION

IT MAY be assumed *a priori* that synthesis of new RNA molecules must play an important role in processes leading to the rapid growth and differentiation of embryonic tissues. There is, however, considerable controversy about the occurrence of RNA synthesis in embryos of wheat and related cereals at the onset of germination. Some lines of evidence indicate that the synthesis of RNA is resumed immediately[1] or soon after[2–6] the mature seeds are exposed to the optimal germination conditions. Others, on the contrary, support the view that RNA synthesis is not essential for the immediate processes of germination[7–9] and is triggered only after 12 hr[10] or even longer periods[11] of imbibition.

One of the reasons for the restriction of RNA synthesis at the onset of germination could be a lack of enzyme activities necessary for the synthesis of RNA or its precursors. Studies on the activity of many enzymes involved in purine[12] and pyrimidine[13] nucleotide synthesis in germintaing wheat seeds have shown, however, that they are endowed with enzyme systems necessary to provide direct precursors of RNA immediately after imbibition starts. In an attempt to extend these studies, measurements of RNA polymerase (nucleosidetriphosphate: RNA nucleotidyltransferase, E.C. 2.7.7.6) activity in dry and germinating wheat seeds were carried out. Preliminary results have been presented earlier.[14]

[1] E. REJMAN and J. BUCHOWICZ, *Phytochem.* **10,** 2951 (1971).
[2] D. P. HOLDGATE and T. W. GOODWIN, *Phytochem.* **4,** 845 (1965).
[3] C. VAN DE WALLE and G. BERNIER, *Exptl Cell Res.* **55,** 378 (1969).
[4] R. DELTOUR, *Planta* **92,** 235 (1970).
[5] C. VAN DE WALLE, *FEBS Letters* **16,** 219 (1971).
[6] D. CHEN, G. SCHULTZ and E. KATCHALSKI, *Nature New Biol.* **231,** 69 (1971).
[7] A. MARCUS and J. FEELEY, *J. Biol. Chem.* **240,** 1675 (1965).
[8] D. CHEN and D. J. OSBORNE, *Nature, Lond.* **225,** 336 (1970).
[9] D. P. WEEKS and A. MARCUS, *Biochim. Biophys. Acta* **232,** 617 (1971).
[10] D. CHEN and D. J. OSBORNE, *Nature, Lond.* **226,** 1157 (1970).
[11] D. CHEN, S. SARID and E. KATCHALSKI, *Proc. Natl. Acad. Sci. U.S.* **60,** 902 (1968).
[12] C. E. PRICE and A. W. MURRAY, *Biochem. J.* **115,** 129 (1969).
[13] B. MAZUŚ and J. BUCHOWICZ, *Phytochem.* **11,** 77 (1972).
[14] B. MAZUŚ and J. BUCHOWICZ, *Abstr. Commun. 7th Meet. Eur. Biochem. Soc.* p. 195 (1971).

RESULTS

Identification of RNA Polymerase Activity in Resting Wheat Seeds

The enzyme extract obtained from dry wheat seeds catalysed incorporation of [^{14}C]UTP into the acid-insoluble fraction (Table 1). All four ribonucleoside-5′-triphosphates, denatured calf thymus DNA and Mn^{2+} ions were necessary for full activity, but Mn^{2+} ions could be replaced by Mg^{2+} if used in a ten-fold higher concentration. The substrate incorporation was linear with increasing concentrations of protein up to 1 mg/reaction mixture. The rate of the reaction was influenced by changes in pH and temperature showing optima at pH 8·4 and 30°. Under these optimal conditions, the total radioactivity of the acid-insoluble product increased linearly with time for the first 8 min of incubation. The reaction product could be solubilized by ribonuclease- or by KOH-catalysed hydrolysis. 80% of the incorporated radioactivity was recovered from the KOH-hydrolysate in the form of 2′(3′)-UMP.

TABLE 1. REQUIREMENTS OF RNA POLYMERASE EXTRACTED FROM DRY WHEAT SEEDS

Assay system	UTP incoporation (*p*-mol/assay)
Complete	13·5
ATP omitted	4·5
CTP omitted	4·0
GTP omitted	3·9
Spermidine omitted	4·1
Mn^{2+} omitted	3·1
Mn^{2+} omitted; Mg^{2+}, 1·3 μmol, added	2·7
Mn^{2+} omitted; Mg^{2+}, 13 μmol, added	11·4
Calf thymus DNA omitted	4·8
Complete, native calf thymus DNA used	7·0
Enzyme omitted	< 1·0
Complete, boiled enzyme used	< 1·0
Complete, zero time	< 1·0

The complete assay and incubation conditions were as described in the Experimental.

All these observations are consistent with the view that a DNA-dependent RNA polymerase is present in, and may be extracted in active state from, the dry wheat seeds. To assess whether the level of this activity was increased on germination, a comparison was made of extracts obtained from dry and germinating wheat seeds.

Changes in RNA Polymerase Activity During Germination

The extracts obtained from dry seeds and from seeds germinated for various periods of time (up to 48 hr) showed comparable levels of RNA polymerase activity (Table 2). Some slight and irregular changes during the first 12 hr of imbibition and a rather moderate increase during the next 36 hr was observed. At the end of the period, the total activity of RNA polymerase, expressed as nmol of the precursor incorporated per seed, was only twice of that found in the initial material. A similar tendency was found when RNA polymerase activity was measured in the absence of the exogenous DNA template. The fact that ribonuclease activity, tested under conditions of the RNA polymerase assay, was

low and essentially constant during the period investigated (Table 3) indicates that the pattern of changes found for RNA polymerase was not affected markedly by activity of the hydrolase.

TABLE 2. CHANGES IN TOTAL ACTIVITY OF RNA POLYMERASE IN WHEAT SEED DURING GERMINATION

Germination time (hr)	Concn of protein in the extract (mg/ml)	UTP incorporation (*p*mol/seed) Complete assay	DNA omitted
0	5·3	13·4	4·9
1	6·2	14·5	6·4
2	6·2	12·6	6·4
4	5·8	13·3	5·4
6	5·6	15·0	6·2
12	5·7	10·4	4·3
24	6·0	18·8	11·5
48	6·5	22·3	12·0

The incubation conditions were as described in the Experimental section.

TABLE 3. LEVELS OF RIBONUCLEASE ACTIVITY IN RNA POLYMERASE PREPARATIONS EXTRACTED FROM DRY AND GERMINATING WHEAT SEEDS

Germination time (hr)	Ribonuclease activity* at pH 5·0	at pH 8·4
0	68·5	10·2
2	70·5	11·0
4	71·0	10·5
6	70·0	10·3
12	68·0	10·5
24	60·0	11·0
48	98·0	13·0

* Absorptivity × 100 of the acid-soluble product solution. See the Experimental section for details.

Thus, it is evident that the level of RNA polymerase activity in extracts from resting seeds is comparable to that of seeds germinated for up to 48 hr.

DISCUSSION

As in the case of enzymes involved in purine[12] and pyrimidine[13] nucleotide synthesis, RNA polymerase activity was found to be present in resting wheat seeds. Only moderate increase in activity occurred during germination which indicates that there is no need for polymerase synthesis to initiate DNA transcription. The striking parallelism between the changes in RNA polymerase activity tested in the presence and absence of exogenous DNA leaves little doubt that no drastic changes in template availability occur at the early stage of germination. In this respect wheat seems to differ markedly from hazel, where a rapid increase in DNA-template availability was found to be associated with the breaking of seed dormancy.[15]

The measurements of enzyme activities seem to indicate that mature wheat seed is potentially able to resume RNA synthesis immediately after appropriate changes in the environment allow it to germinate. The previously reported data[1] on the incorporation of labeled orotate, uracil and uridine *in vivo* have shown that this synthesis may actually occur at the onset of induction of the germination process.

Although no investigations have been made in relation to germination, RNA polymerase has been found in dormant pea seeds[16] and isolated from young pea,[17] maize[18] and soybean[19] seedlings.

[15] B. C. JARVIS, B. FRANKLAND and J. H. CHERRY, *Plant Physiol.* **43**, 1743 (1968).
[16] G. R. BARKER and M. RIEBER, *Biochem. J.* **105**, 1195 (1967).
[17] J. BONNER, R. C. HUANG and N. MAHESHWARI, *Proc. Natl. Acad. Sci. U.S.* **47**, 1548 (1961).
[18] E. R. STOUT and R. J. MANS, *Biochim. Biophys. Acta* **134**, 327 (1967).
[19] T. J. O'BRIEN, B. C. JARVIS, J. H. CHERRY and J. B. HANSON, *Biochim. Biophys. Acta* **169**, 35 (1968).

EXPERIMENTAL

Germination. 100-seed samples of wheat (*Triticum aestivum*, ssp. vulgare, var. lutescens Al., cv. Kutnowska) were germinated under conditions described previously[13] for periods varying from 0 to 48 hr, as specified. In each case, the whole sample was used for enzyme extraction. Germination percentage was 95.

Enzyme extraction. A sample of 100 dry seeds was ground and homogenized with 10 ml of ice-cold 0·05 M Tris-HCl buffer, pH 8·4, containing 1 μmol of 2-mercaptoethanol. Germinated seeds were treated in a similar manner but the volume of buffer used was reduced by a value corresponding to the amount of H_2O taken up by the sample. The homogenate was centrifuged at 15 000 *g* for 10 min at 0° and the supernatant fraction was treated with 400 mg of activated charcoal. The charcoal was then filtered off and the resulted filtrate used as the enzyme source. The 15 000 *g* pellet was occasionally tested for RNA polymerase activity after resuspending it in the original buffer volume and was always found to have negligible activity.

Enzyme assay. Conditions of the assay were similar to those described by Stout and Mans.[18] The complete system contained in a final volume of 0·3 ml: Tris-HCl buffer, 50 μmol (pH 8·4); heat-denatured calf thymus DNA, 100 μg; each of ATP, CTP and GTP, 0·2 μmol; [4-^{14}C]UTP, 0·5 μCi (21 μCi/μmol); $MnCl_2$, 1·3 μmol; spermidine, 1·2 μmol; and the enzyme extract, 0·1 ml (concentrations of protein in the extracts used for each experiment are given in Table 2). The mixture was incubated at 30° for 5 min. The acid-precipitable material was then collected on filter paper disk[18] and used for the measurement of radioactivity. Data on protein concentration in the enzyme extracts reported here and on the changes in fr. and dry wt of standardized seed samples during the period of germination reported previously[13] give the possibility for recalculation of the results on other bases.

Ribonuclease activity. The enzyme extracts were tested for ribonuclease activity by measuring the conversion of exogenously added commercial RNA to acid-solulbe products. The incubation was carried out for 1 hr either under conditions of the RNA polymerase assay (pH 8·4) or at pH 5·0, under conditions optimal for ribonuclease activity.[20] In both cases 0·5 mg of highly-polymerized wheat germ RNA was used as the substrate. The reaction was stopped by adding 4·7 ml of 0·3 M $HClO_4$. Control experiments were performed in which the reaction was stopped at 0 time. After centrifugation absorptivity of the acid-soluble fraction was measured at 260 nm through a 1-cm light path. Ribonuclease activity is expressed as a difference in the absorptivity between solutions derived from incubated and control samples.

Control of microbial contaminations. Although the seeds were treated with 2% NaOCl and germinated under sterile conditions, the possibility of bacterial contamination was not entirely eliminated. Such contaminations would certainly be removed from the homogenate at the early stage of enzyme preparation, by centrifugation at 15 000 *g*. However, to make completely sure that bacteria did not contribute to the observed UTP incorporation, a test for leucine incorporation was employed as an index of microbial activity. If bacteria were present they should incorporate both RNA and protein precursors. No incorporation of [1-^{14}C]leucine could be, however, observed under conditions of the RNA polymerase assay. In addition, rifampicin (10 μg/assay) was found to be without effect on the observed UTP incorporation, thus indicating the absence of procaryotic RNA polymerase activity.

Other procedures. The radioactive product of the reaction catalysed by RNA polymerase extract, was solubilised from the acid-precipitable material by treatment either with bovine pancreatic ribonuclease or with 0·3 M KOH at 37° for 18 hr. 2′(3′)-UMP was isolated from the alkaline hydrolysate by methods described previously.[21] Protein was determined by the method of Lowry *et al.*[22] Radioactivity was measured in Packard liquid scintillation counter.

Chemicals. All reagents, including [4-^{14}C]uridine-5′-triphosphate, ammonium salt (The Radiochemical Centre, Amersham, Bucks., U.K.) were of commercial origins.

Acknowledgements—This work was supported by The Polish Academy of Sciences within the project 09.3.1 and by Grant No. FG-Po-262 from The United States Department of Agriculture, Agricultural Research Service.

[20] M. R. MCDONALD, in *Methods in Enzymology* (edited by S. P. COLOWICK and N. O. KAPLAN), Vol. 2, p. 427, Academic Press, New York (1955).
[21] J. BUCHOWICZ, I. REIFER and J. MAKOWSKI, *Acta Biochim. Polon.* **8**, 377 (1961).
[22] O. H. LOWRY, N. J. ROSENBROUGH, A. L. FARR and R. J. RANDAL, *J. Biol. Chem.* **193**, 265 (1951).