LOW-TEMPERATURE-DEPENDENT RIBONUCLEASE IN CHROMATIN OF WINTER WHEAT SEEDLINGS

Kimiko Sasaki and Shoji Sasaki

Department of Botany, Faculty of Science,
Hokkaido University, Sapporo, Japan

Received August 3,1976

SUMMARY: A kind of ribonuclease having an optimum temperature of about 15°C was detected in chromatin of seedlings of a species of winter wheat, besides another kind having an optimum temperature of about 30°C. The optimum pH was about 8.0. The activity of ribonuclease was inhibited by bentonite and DNA, and was stimulated by cyclic AMP. The low-temperature-dependent activity was thermolabile.

Different kinds of RNase may be involved in the regulation of synthesis and function of RNA during cell cycle or life cycle of plants (1). In a previous paper (2), it was reported the possibility of presence of RNase which has optimum temperature of 15°C. Dévay reported the presence of a specific kind of RNase which is very active even at low temperature (0°C) and is synthesized as early as the first few hours of vernalization at 0°C (3,4).

In this paper, a low-temperature-dependent RNase present in chromatin of seedlings of a species of winter wheat is investigated and its physiological role is discussed.

MATERIALS AND METHODS

Plant materials: Seeds of winter wheat (Triticum aestrium L. vc. Mukakomugi) were sterilized for 20 min in 8% hypochlorite solution, washed 10 times with sterile water, then soaked in sterile water for 2 hr at 20°C. Soaked seeds were allowed to germi-

nate on one layer of wet filter paper in Petri dishes for 3 days at 24°C in darkness.

Preparation of purified chromatin: Purified chromatin was prepared from root-omitted seedlings by the method described in a previous paper (2).

Extraction and partial purification of RNase: Sonic extract was prepared from purified chromatin by the procedures as described below at 0°to 4°C. Purified chromatin prepared from about 20 g fresh weight of seedlings was sonicated for 1 to 2 min in 20 ml of a standard buffer consisted of 0.15 M Tris-HCl,pH 7.8, 0.1 mM EDTA, 5 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 10 mM $(NH_4)_2SO_4$, and 25% glycerol. The supernatant obtained after centrifugation at 20,000 x g for 30 min was dialyzed overnight against 10 mM $(NH_4)_2SO_4$-buffer solution consisted of 50 mM Tris-HCl,pH 7.5, 1mM DTT, 25% glycerol and 10 mM $(NH_4)_2SO_4$, then was used as "sonic extract", which contained RNA polymerase and poly(A) polymerase (2). In some cases, RNase was successively extracted with 10 mM, 0.15 M and 0.4 M $(NH_4)_2SO_4$-buffer solution, which were the same as above except concentration of $(NH_4)_2SO_4$, from the purified chromatin at 4°C under stirring for 10 to 20 min. After centrifugation at 20,000 x g for 60 min, each of the extracts was dialyzed overnight against 10 mM $(NH_4)_2SO_4$-buffer solution, and was used as 10 mM, 0.15 M or 0.4 M $(NH_4)_2SO_4$ extract, respectively. In one case, supernatant obtained from homogenate of seedlings by centrifugation at 100,000 x g for 60 min was dialyzed overnight against 10 mM $(NH_4)_2SO_4$-buffer solution, and was used as "cytoplasmic soluble enzyme".

Assay of RNase: Standard reaction mixture consisted of 50 mM Tris-HCl,pH 7.8, 10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT, 2 µg yeast sRNA, 0.1 µg [^{14}C]RNA (15 x 10^3 cpm), and purified chromatin (equivalent to 135 µg protein) or enzyme solution (35 µg protein) in a final volume of 0.25 ml. In some cases, a mixture of 0.4 mM each of ATP, CTP, GTP and UTP, 10 mM cyclic AMP (cAMP), 20 µg DNA (calf thymus), 5 µg bentonite, 5 µg polyvinylsulfate (PVS) or 10 mM $ZnSO_4$ was added. Reaction was allowed at a temperature range between 0° and 40°C for 10, 20 or 60 min. After the reaction, acid-insoluble fraction was collected on a glass-fiber filter (Whatman,GF/C), washed with 5% TCA including 10 mM sodium pyrophosphate and dried, after which radioactivity was counted. The activity of RNase was calculated from the differences of

radioactivities of acid-insoluble fractions before and after the reaction.

Assay of poly(A) and RNA polymerases: Reaction medium consisted of 50 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM DTT, 0.4 mM each of ATP, CTP, and GTP, 4 μM UTP, 0.4 μM [^{3}H]UTP (10^5 cpm), and chromatin (135 μg protein) or enzyme solution (35 μg protein) in a final volume of 0.2 ml. Reaction was allowed at 0° to 40° for 20 or 60 min. After the reaction, radioactivity of acid-insoluble fraction was counted as described in assay of RNase. Poly(A) polymerase was assayed as described in a previous paper (2).

Chemicals: [^{3}H]UTP (27.6 Ci/mmole) and [^{14}C]RNA (1 μCi/mg) were purchased from New England Nuclear.

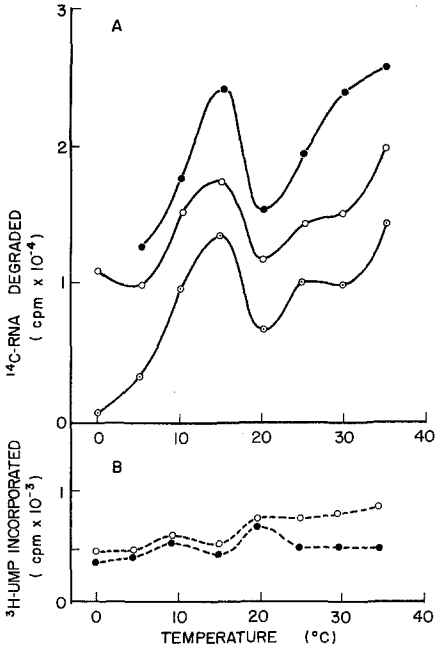

Fig. 1. Effect of reaction temperature on activities of RNase and RNA polymerase in chromatin. A, RNase: ⊙———⊙, without a mixture of ATP, CTP, GTP and UTP, for 20 min; o———o, with the mixture, for 20 min; ●———●, with the mixture, for 60 min. B, RNA polymerase: o-----o, 20 min; ●-----●, for 60 min.

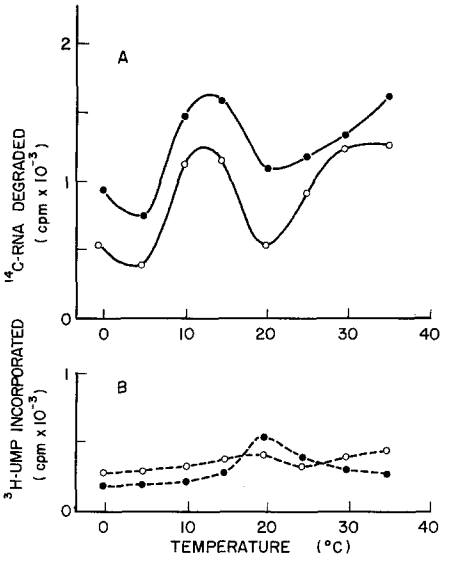

Fig. 2. Effect of reaction temperature on activities of RNase and RNA polymerase in sonic extract. A, RNase: o———o, with the mixture same as in Fig. 1, for 20 min; ●———●, withe the mixture, for 60 min. B, RNA polymerase: o-----o, 20 min; ●-----●, 60 min.

RESULTS AND DISCUSSION

The effect of pH on the activity of chromatin-bound RNase was investigated in acetate, phosphate, and Tris-HCl buffers at pH 4.5 to 8.8. The optimum pH was about 8.0.

The activity of RNase in chromatin and sonic extract was a function of temperature as shown in Figs. 1 and 2. Here it can be seen that two distinct temperature optima exist, one at about 15°C and a second at 30°C. The activity was found at even at 0°C. When the activity was measured for a long time using much RNA, an optimum temperature of 40°C was observed. These results suggest that low-temperature-dependent RNase is bound with chromatin of winter wheat seedlings, in addition to high-temperature-dependent one, and the low-temperature-dependent

RNase is much labile which is detected only by a micro-assay. Large amounts of the latter RNase was present in cytoplasmic soluble fraction, whereas only a small quantity of the former RNase was (Fig. 2). This result suggests that low-temperature-dependent RNase may be solubilized from chromatin during homogenation of seedlings. The enzyme was more efficiently extracted from chromatin by 0.4 M $(NH_4)_2SO_4$-buffer than with 10 mM or 0.15M $(NH_4)_2SO_4$-buffer as shown in Fig. 4.

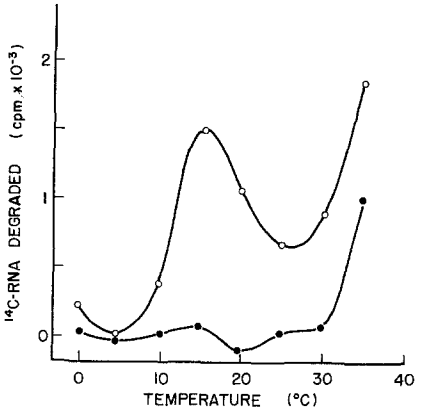

Fig. 3. Effect of reaction temperature on RNases in cytoplasmic soluble fraction and chromatin. o——o, cytoplasmic soluble fraction; •——•, chromatin. Without a mixture of XTPs, for 20 min.

It was observed two peaks of activities of poly(A) and RNA polymerases at 5°to 10°C and 30°C (Figs. 1-4). The reason for the drop at 15°C for polymerase activities may be due to the presence of low-temperature-dependent RNase in chromatin and also in sonic extract.

As shown in Fig. 5, the activity of RNase at low temperature was more thermolabile than that at high temperature. This re-

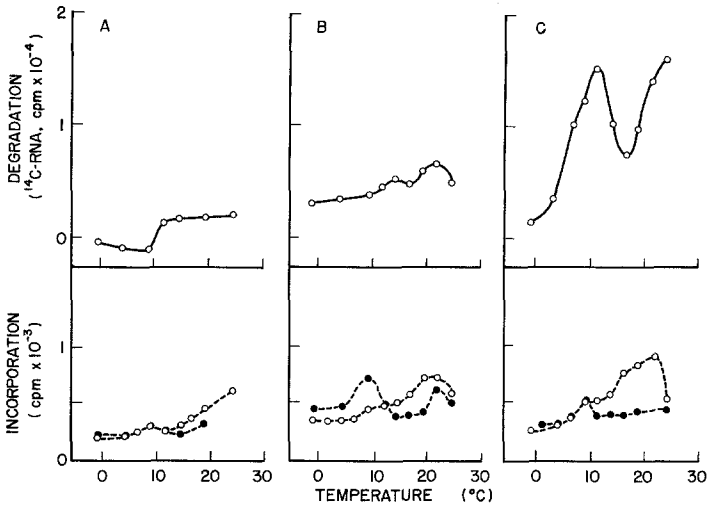

Fig. 4. Effect of reaction temperature on activities of RNase, RNA polymerase, and poly(A) polymerase in $(NH_4)_2SO_4$ extracts. o———o, RNase, with a mixture of XTPs; o-----o, RNA polymerase; •-----•, poly(A) polymerase. A, 10 mM $(NH_4)_2SO_4$ extract; B, 0.15 M extract; C, 0.4 M extract. Reaction time, 10 min.

sult suggests that there are at least two RNases having different optimum temperatures in chromatin of winter wheat.

Fresh chromatin had very low activities of RNA polymerase and RNase (Table 1). The activity of RNA polymerase was increased by aging of chromatin for a short time (10 min at 30°C or 1 to 3 days at 0° to 4°C) and was decreased by aging for a long time (more than 3 days), while that of RNase was increased by the aging for a long time (Table 1). These results suggest that certain factor(s) bound with chromatin is very labile and functions as regulator for synthesis and degradation of RNA.

The activity of RNase in 3 days-aged chromatin was detected in the presence of a mixture of ATP, CTP, GTP and UTP (Fig. 1).

The activities of RNase and RNA polymerase were stimulated by cAMP, more intensely at 15°C than at 30°C (Table 2). The

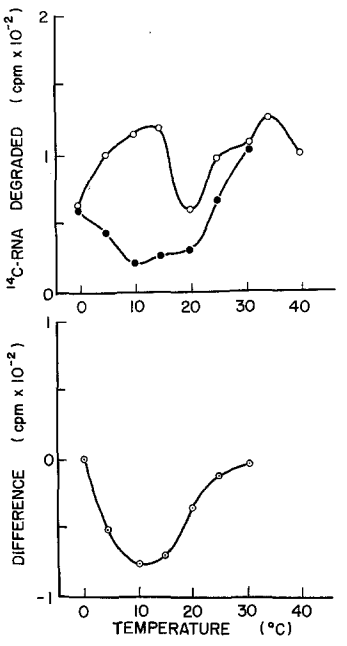

Fig. 5. Effect of heat-treatment of chromatin on RNase activity. RNase activities were assayed for 10 min at different temperatures before and after preincubation of chromatin at 30°C for 30 min. o———o, activity before preincubation; ●———●, activity after preincubation; ⊙———⊙, difference between the activities before and after preincubation.

Table 1. Effect of aging of chromatin on activities of RNase and RNA polymerase

Aging at 0°-4°C (days)	Activity			
	RNase [cpm, [^{14}C]RNA degraded/20 min]		RNA polymerase [cpm, [^{3}H]UMP incorporated/20 min]	
	15°C	30°C	15°C	30°C
0			222	195
1	380	300	12250	16986
4	1477	1591	12250	12204
6	2859	3593	1894	1394

Table 2. Effects of cyclic AMP, bentonite, and DNA on activities of RNase and RNA polymerase in chromatin and in sonic extract.

Enzyme source	Addition	Activity			
		RNase [cpm, [^{14}C]RNA degraded/20 min]		RNA polymerase [cpm, [^{3}H]UMP incorporated/20 min]	
		15°C	30°C	15°C	30°C
Chromatin	None	1211	2216	0	37
	cAMP(20 mM)	2079			
	DNA (10 µg)	771		780	2305
Sonic extract	None	7339	20722	58	175
	cAMP(20 mM)	12175	20755		
	DNA (10 µg)	2313	17210	880	1308
	cAMP(20 mM) + DNA(10 µg)			1780	1360
Sonic residue	None	1427	1551	10720	16922
	cAMP(20 mM)	1658	1821	13613	19365
	DNA (10 µg)	1005	1092	21440	21600
Chromatin	None	1379	3200		
	Bentonite (10 µg)	1110	1266		
Sonic extract	None	1458	1492		
	Bentonite (10 µg)	1018	1066		

activity of RNase was inhibited by DNA, more intensely at 15°C than at 30°C. Effects of PVS and $ZnSO_4$ were obscure. These findings suggest that RNase in chromatin and in sonic extract is modified by cAMP to be an activated form and by DNA to be an inactive form. Mendelson and Anderson (5) reported that RNA polymerase Ib was stimulated and RNA polymerase II was inhibited by cAMP. Actual reasons for activation by cAMP and for inactivation by DNA are unknown at the present time. Differences in

sensitivities to cAMP and DNA of RNase at 15° and 30°C suggest that different RNases having different optimum temperatures are present in chromatin.

The activity of RNase in chromatin was more thermolabile at low temperature than at high temperature (Fig. 5). From this result, it is suggested that low-temperature-dependent RNase functions only at low temperature, and has an important role in growth and development at low temperature and in vernalization process of winter wheat as discussed by Dévan (3,4).

Further purification and characterization of chromatin-bound RNase are required for the determination of properties and physiological role of RNase, especially low-temperature-dependent RNase which is detected by micro-analysis.

REFERENCES

1. Barnard, E. A. (1969) Annu.Rev.Biochem. 38, 677-732.
2. Sasaki, K. and Tazawa, T. (1973) Biochem. Biophys. Res. Commun., 52, 1440-1449.
3. Dévay, M. (1965) Acta Agr. Hung., 14, 93-97.
4. Dévay, M. (1966) Acta Agr. Hung., 15, 85-94.
5. Mendelson, L. S. and Anderson, K. M. (1973) Biochim. Biophys. Acta 299, 576-587.