INCREASE IN CHROMATIN ASSOCIATED NUCLEASE ACTIVITY OF EXCISED BARLEY LEAVES DURING SENESCENCE AND ITS SUPPRESSION BY KINETIN*

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Received July 3, 1968

Summary. No significant changes in the composition of chromatin were found when the excised barley leaves were floated on water or on kinetin solution in the dark. However, increases in the activities of chromatin associated ribonuclease and deoxyribonuclease in leaves floated on water were noted and kinetin which retarded senescence drastically suppressed the increases in these enzymes.

Introduction. Senescence in first seedling leaf of barley (Srivastava and Atkin, 1968; Atkin and Srivastava, 1968) is manifested in declines in the level of chlorophyll, RNA and DNA and alterations in P^{32} incorporation into RNA. However, if the excised leaves are floated on kinetin solution the senescence is retarded (Srivastava, 1967). Since these results suggested that changes in chromatin may occur during senescence the studies on chromatin from barley leaves during senescence were started.

During the determination of melting curves of chromatin or on allowing the chromatin to stand at room temperature the precipitation of chromatin from senescent leaves was noted. Examination of the precipitate and the incubation of chromatin with DNA and RNA substrates revealed the presence of both deoxyribonuclease and ribonuclease activities. Since activities of the above

* Supported by National Science Foundation Grant GB-4720X and the United States Atomic Energy Commission Contract # AT(30-1)37221.

enzymes in chromatin from intact or excised leaves of barley increased during senescence it was considered desirable to examine the effect of kinetin on these changes. This paper reports the results of these experiments which have been repeated at least three times.

Materials and Methods. Barley (Hordeum vulgare L. var. Wolfe) plants were grown in soil in pots on greenhouse benches and given 16 hours of light per day. When the plants were 7 days old the first seedling leaves were excised, washed with deionized distilled water and floated in Pyrex trays on water or on 10 mg/l of kinetin solution (1 litre/25 g leaves) in the dark. The freshly excised leaves from 7 day old plants and the leaves which had been floating on water or on kinetin solution for 12 to 96 hours were used for the preparation of the chromatin.

Preparation of chromatin. The chromatin (from 25-30 g leaves) was prepared and purified by ultra-centrifugation (2 hr. at 70,000 x g) through 1.7 M sucrose essentially as described by Huang and Bonner (1962). The transparent chromatin pellet obtained by ultracentrifugation was washed by suspending in 0.05 M tris, pH 8.0 buffer containing 0.01 M mercaptoethanol and centrifuging at 30,000 x g for 30 minutes. The washed chromatin pellet was dispersed in 0.01 M tris, pH 8.0 buffer (25 ml) and dialysed overnight against the same buffer at 1° C. Following dialysis the chromatin was used for the assay of ribonuclease and deoxyribonuclease activities and for the chemical analysis.

Assay of ribonuclease and deoxyribonuclease. For the estimation of ribonuclease activity 1-2 ml of the chromatin was incubated with 2 ml of 1 mg/ml Torula s-RNA (Calbiochem) in 0.05 M tris-HCl pH 7.5 for 0, 1, 2 and 4 hours at 40° C. For the estimation of deoxyribonuclease 1-2 ml of the chromatin was incubated with 2 ml of 0.05 M tris-HCl pH 7.5, 2 ml of 1 mg/ml DNA Na (Calbiochem) and 0.1 ml of 4 mg/ml $MgCl_2$ for 0, 1, 2 and 4 hours at 40° C. At the end of incubation time the undigested nucleic acid was precipitated with 15 ml of chilled $HClO_4$ ethanol mixture (5% $HClO_4$ in absolute ethanol). The contents were chilled at -20° C for 2-3 hours and then centrifuged at

30,000 x g for 30 minutes. The OD at 260 mμ of the supernatants for 1, 2 and 4 hours incubations was read against blank (zero time). Ribonuclease or deoxyribonuclease activity was expressed as enzyme units per mg of DNA in the chromatin. One enzyme unit was defined as an increase of OD 1.0 in 1 ml of solution for an incubation period of 1 hour.

Chemical analysis of chromatin. The histones from chromatin were extracted with 0.5 N HCl and estimated by the procedure of Lowry et al., (1951). The residue left after HCl extraction was completely solublized in 0.3 N NaOH. The protein estimation on an aliquot of the NaOH solution gave the amount of residual protein. Another aliquot of the NaOH solution was made 5% with respect to trichloroacetic acid, heated at 90° C. for 15 minutes, and centrifuged. DNA and RNA were estimated on the supernatant obtained by the diphenylamine (Burton, 1965) and orcinol (Markham, 1955) procedures respectively.

Results. No significant changes in the composition of the barley leaf chromatin during senescence or during kinetin treatment were noted (Table 1) and the ratio of histone to DNA was close to one in all chromatins.

The data presented in Figure 1 show that young leaves from 7 day old plants had very low ribonuclease and deoxyribonuclease activities. In leaves floated on water the activities of these enzymes started to increase 12 to 24 hours after excision and rapidly increased 48 to 96 hours after excision. In leaves floated on kinetin solution the increases in the activities of both of these enzymes were drastically suppressed. Kinetin (0.01, 0.1, 1.0 or 10.0 mg/l) when added to in vitro assay mixture, however, had no effect on the activities of the above chromatin associated enzymes. Also, estimations of nuclease activities in chromatin from water floated leaves in the presence of chromatin from young or kinetin floated leaves simply gave addititive results indicating the absence of any nuclease inhibitors in the chromatin.

Discussion. The isolation procedure for the chromatin excludes contamination from soluble enzymes or cellular organelles. The great reproducibility in quantitative terms of data of Figure 1 emphasizes the absence of chance con-

Table 1.

The composition of chromatin from fresh barley leaves and from excised barley leaves floated on water or on 10 mg/l of kinetin solution in the dark.

Sample	Hours of floating	Chromatin components (% of total mass) DNA	Histone	Residual protein	RNA
Fresh leaves	0	36.2 ± 1.0*	35.4 ± 2.5	20.3 ± 2.7	8.2 ± 1.8
Leaves floated on water	12	36.5	33.8	22.7	6.8
	24	34.8	38.6	18.7	7.9
	48	34.1	32.8	23.7	9.4
	96	31.5	31.4	26.5	10.5
Leaves floated on kinetin	12	34.8	34.8	24.4	5.8
	24	34.2	36.4	23.9	5.3
	48	32.7	32.8	27.9	6.5
	96	36.7	33.0	21.0	9.2

* Standard deviation (n = 5).

tamination. Also, in contrast to changes in chromatin associated nucleases (Fig. 1) the soluble ribonuclease declines and soluble deoxyribonuclease changes little up to 4 days in water and kinetin floated leaves (Srivastava and Ware, 1965). The pH optima of the soluble nucleases are between 5.5 - 6.0 and of chromatin associated nucleases between 7.0 - 7.5. Furthermore, both ribonuclease (Marushige and Bonner, 1966) and neutral deoxyribonuclease (Swingle et al., 1967) have been found associated with chromatin from mammalian cells. It may therefore, be concluded that the nuclease activity associated with chromatin in barley leaves is real and does not represent a contamination.

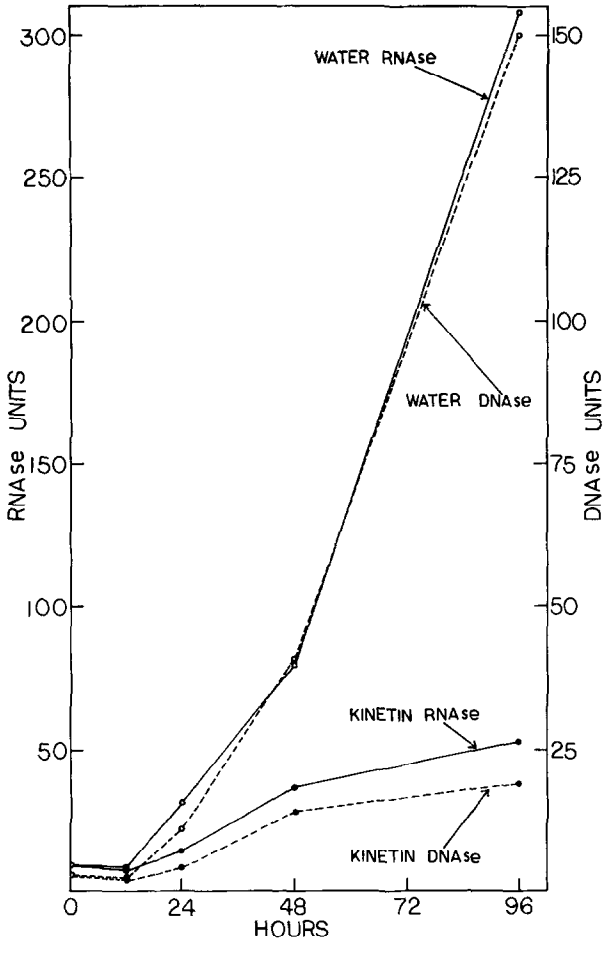

Figure 1. The changes in chromatin associated deoxyribonuclease and ribonuclease activities of excised barley leaves floated on water or on 10 mg/l of kinetin solution in the dark.

The association of ribonuclease and deoxyribonuclease with chromatin, their increase during aging, and their suppression by kinetin not only indicate the processes involved in the aging and in the mechanism of action of hormone but they also point out how these enzymes may be involved in the regulation of genetic activity.

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