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EFFECT OF OZONE ON RIBOSOMES IN PINTO BEAN LEAVES

CHONG W. CHANG

Plant Science Research Division, Agricultural Research Service, USDA, Beltsville, Maryland 20705, U.S.A.

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Abstract—A study was made of cytoplasmic and chloroplast ribosomes in the primary leaves of pinto bean plants exposed to ozone. The isolated ribosomes were analysed by sucrose density gradient. Ozone at the levels of 0·35 ppm for 20–35 min does not change the concentrations of various sedimenting particles of the cytoplasmic ribosomes. Ozone at similar levels, however, specifically decreases the population of chloroplast ribosomes per unit fresh weight of leaves. The distribution pattern of these chloroplast ribosomes is characterized by the low concentration of the fast-sedimenting polysome particles concomitant with the low magnitude of other slow-sedimenting components. The kinetics of ribosome populations during leaf growth demonstrates that ozone does not influence the daily levels of different ribosomal components of cytoplasmic ribosomes. However, ozone prematurely decreases the concentrations of polysomes and other components of chloroplast ribosomes below control level at the early stage of leaf development. These findings are discussed to explain initiation of the premature senescence caused by ozone.

INTRODUCTION

OZONE is a major phytotoxicant present in the photochemical smog complex. This oxidant is known to accelerate senescence in plants.[1] It was reported that there is a fall in RNA and protein content and a decline in the capacity to synthesize these metabolic constituents during the naturally occurring ageing process.[2] Since protein synthesis is directly related to polysome content, it was suggested[3] that a decline in the polysome level may mark the initiation of the senescence. Srivastava and Arglebe[4] demonstrated that polysomes and ribosomes are lost in senescing barley leaves. Chang[5] recently also reported the dissociation of polysomes into smaller particles in corn roots aged by fluoride. However, no investigations have been made on how senescence caused by ozone affects the ribosome population of pinto bean plants.

The aims of the present study were to determine the particle distributions and the concentrations of various sedimenting components of cytoplasmic and chloroplast ribosomes in ozone-treated pinto bean leaves.

RESULTS AND DISCUSSION

As shown in Fig. 1, determinations were made of the sedimentation patterns of cytoplasmic ribosomes (A) and chloroplast ribosomes (B and C) from primary leaves of pinto bean plants. The maximum UV absorbance was found in fractions 23 and 26 of ribosomes from cytoplasm and chloroplasts, respectively. To further verify their different sedimentation rates, these two classes of ribosomes isolated (A and C) were mixed and analysed for a sedimentation profile. A clear resolution of the two strongest peaks is seen (D). The strongest

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[5] C. W. CHANG, *Can. J. Biochem.* **48,** 450 (1970).

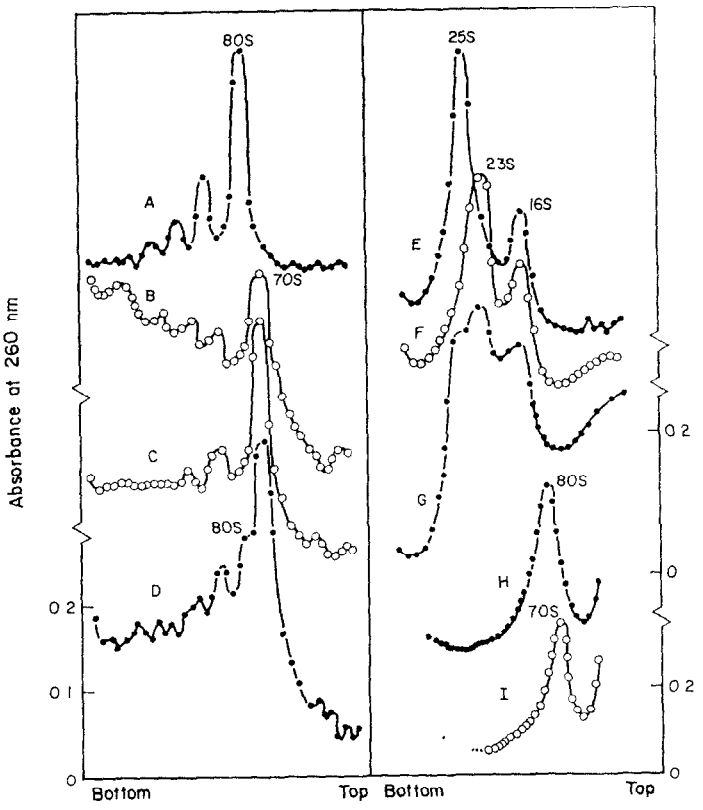

FIG. 1. SEDIMENTATION PATTERNS OF RIBOSOMES AND RIBOSOMAL RNA FROM BEAN CYTOPLASM AND CHLOROPLASTS.

(A), Cytoplasmic ribosomes. (B), chloroplast ribosomes analysed immediately after a clarifying spin; (C), chloroplast ribosomes analysed after clarified ribosomes were kept in a cold room at 2–4° overnight; (D), a mixture of (A) plus (C); (E), ribosomal RNA from cytoplasm; (F), ribosomal RNA from chloroplasts; (G), a mixture of (E) plus (F); (H), cytoplasmic ribosomes and (I), chloroplast ribosomes treated with 0·2 μg/ml crystalline bovine ribonuclease for 5 min at 1–4°.

The ribosomal RNA was prepared by the procedure of Kurland* with minor modifications. The ribosomes sample was exposed to 0·5% sodium dodecyl sulfate (in 0·01 M tris-HCl, pH 7·6, 0·001 M $MgCl_2$) at 15° for 5 min and analysed. See materials and methods for other analytical procedures.

peak shown in (A) and that shown in (B or C) therefore are assumed to be of 80*S* and 70*S*, respectively. Investigations were made of a possibility that chloroplast polysomes contained contaminating cytoplasmic polysomes composed of 80*S* monosomes. The possibility that cytoplasmic polysomes were also contaminated with chloroplast polysomes composed of 70*S* monosomes was studied. It was shown that 80*S* ribosomes from pinto bean cytoplasm are made up of 25*S* and 16*S* *r*RNA fractions, whereas the corresponding values for *r*RNA of 70*S* chloroplast ribosomes are 23*S* and 16*S*.[6] Therefore, analyses were made of *r*RNA components of these two classes of ribosomes. The size distribution of *r*RNA fractions (E) from cytoplasmic ribosomes shows only two sharp peaks (25*S* and 16*S*). The sedimentation pattern of rRNA fractions (F) from chloroplast ribosomes also shows only two distinctive peaks (23*S* and 16*S*). The mixture of *r*RNA components (E plus F) reveals three peaks

* C. G. Kurland, *J. Mol. Biol.* **2**, 83 (1960).

[6] E. STUTZ and H. NOLL, *Proc. Natl. Acad. Sci.* **57**, 774 (1967).

(25*S*, 23*S* and 16*S*) (G). The data above indicate that the polysome components of cytoplasmic ribosomes (A) and chloroplast ribosomes (B) do not contain detectable levels of *r*RNA contaminants. The sedimentation profiles of cytoplasmic and chloroplast ribosomes treated with ribonuclease were determined (H and I, respectively). These treatments affect immediate destruction of heavier particles and result in concomitant increase in monomers. These observations verify the polysome nature of these preparations. Similar claims were supported by the assays of polysomes with ribonuclease in published reports.[6,7] The breakdown products of disintegrated polysomes also show up as single peaks of monomers in the sedimentation patterns shown in (H) and (I). These data reconfirm that each class of ribosomes prepared is highly purified.

Figure 2 presents the sucrose gradient distribution profiles of cytoplasmic and chloroplast ribosomes from ozone-treated pinto bean leaves. To estimate the concentrations of different sedimenting components of chloroplast ribosomes, the areas under the peaks were integrated by a procedure patterned after that of an earlier report.[5] As seen in Fig. 2 (left half), ozone does not significantly change the concentrations of various sedimenting particles of cytoplasmic ribosomes. In contrast, the data in Fig. 2 (right half) show that ozone causes the

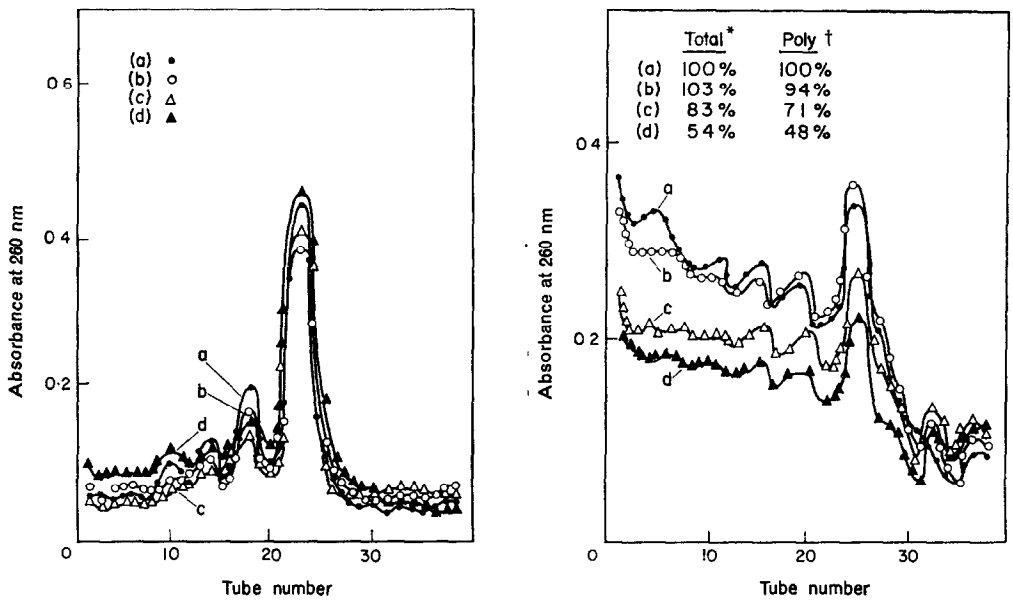

FIG. 2. SUCROSE GRADIENT SEDIMENTATION PROFILES OF CYTOPLASMIC RIBOSOMES (LEFT HALF) AND CHLOROPLAST RIBOSOMES (RIGHT HALF).

Isolated from the primary leaves of (a) control and ozone-treated pinto bean plants. Nine-day-old plants were treated with 0·35 ppm ozone for (b) 20 min, (c) 25 min and (d) 35 min. The primary leaves were harvested 2 days after ozone treatment. For cytoplasmic ribosomes, the volume of total ribosomes from 70 g fr. wt. of leaves was adjusted to 4 ml with buffer III[6] in each experiment A fraction of sample (0·15 ml) from this total ribosomal suspension was analysed. For chloroplast ribosomes, total amount of sample was analysed. Direction of sedimentation is from right to left. The levels of total ribosomal components and polysomes as percents of controls are averages of two analyses.

* Total ribosomal components (fractions 1–39), † Polysomes (fractions 1–21).

[7] J. R. RAWSON and E. STUTZ, *Biochim. Biophys. Acta* **190**, 368 (1969).

reduction of polysome populations as well as low amounts of other lighter components in chloroplast ribosomes per unit fresh weight of leaves. These changes are related to the treatments with the oxidant. This distribution pattern is similar to that of senescing barley leaf ribosomes.[4] The RNA of ribosomes produced by the breakdown of polysomes is unstable in barley leaves,[4] in *Lupinus albus* hypocotyl[8] and probably also in the present experimental material, pinto bean leaves. This observation of the specific effect of ozone on chloroplast ribosomes is compatible with electronmicroscope observations of pinto bean leaves exposed to ozone.[9] The latter studies showed that chloroplast stroma changed before other subcellular components. A previous report also indicates that light is an influential factor for the physiological responses of plants to ozone.[10] This implies a close association between the action of ozone and the light-dependent organelle, chloroplast. This present finding supports this implication.

Investigations also were made of the sedimentation profiles of cytoplasmic and chloroplast ribosomes from control and ozone-treated pinto bean leaves of increasing ages. To estimate the concentrations of the various sedimenting particles, the areas under the peaks were integrated. The integrated values were than expressed in graphic forms shown in Fig. 3 in an attempt to visualize the kinetics in the levels of these components.

Figure 3 shows that the components of cytoplasmic and chloroplast ribosomes in control plants are composed mainly of polysomes and monosome particles. The polysome population of cytoplasmic ribosomes shows a progressive decline as a function of age. In contrast, the polysome concentration of chloroplast ribosomes increases up to the age of 11 days, and decreases thereafter. Since the levels of polysome populations of two major sites of protein synthesis decrease concurrently after plants are 11 days old, this age is assumed to be the stage when natural senescence begins in pinto bean leaves. By these kinetic observations, the view[3] that a decline in the polysome level may mark the intiation of senescence is further strengthened.

As shown also in Fig. 3 (left half), ozone does not markedly influence the daily concentrations of the various sedimenting components of cytoplasmic ribosomes. By contrast, ozone decreases considerably, the polysome population of chloroplast ribosomes per unit fresh weight of leaves (Fig. 3, right half). The reduction from the control value occurs the first day after ozone treatment. This depression reaches the maximum (about 40% of the control) the second day. Thereafter, the rate of reduction decreases. The fact that the polysome level of chloroplast ribosomes decreases well before the stage of natural senescence (age of 11 days) may account for the initiation of ozone-induced senescence, since this reduction would depress the level of protein synthesis[3] below that of the control. A unit weight of chloroplast ribosomes is known to be 10–20-fold more active in protein synthesis than a unit weight of cytoplasmic ribosomes.[11] Ozone, therefore, may initiate the premature senescence by decreasing the polysome level of chloroplast ribosomes after the plants are exposed to this oxidant.

This communication demonstrates clearly the differential responses of the two classes of ribosomes in pinto bean leaves to ozone, one of the photochemical smog elements. There are at least several possible explanations for the decreased polysome population of chloroplast ribosomes observed in this study: (a) ozone (an oxidant with a redox potential of +2·07 V)

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[9] W. W. THOMSON, W. M. DUGGER, Jr. and R. L PALMER, *Can. J. Bot.* **44,** 1977 (1966).
[10] O. C. TAYLOR, W. M. DUGGER, JR., E. A. CARDIFF and E. F DARLEY, *Nature, Lond* **192,** 814 (1961).
[11] N. K. BOARDMAN, R. I. B. FRANCKI and S. G. WILDMAN, *J. Molec. Biol.* **17,** 470 (1966).

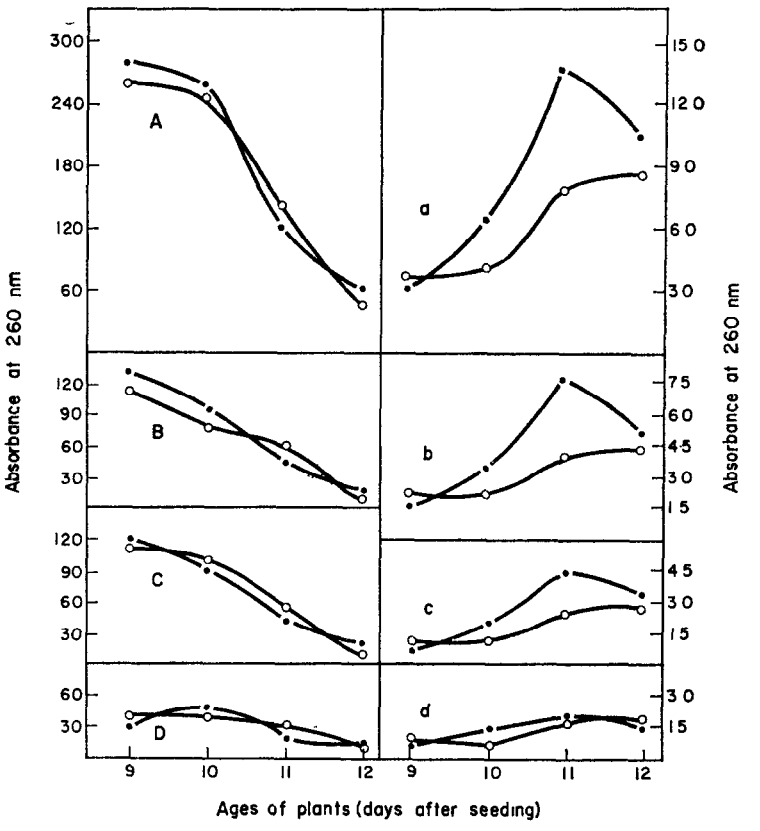

FIG. 3. KINETICS IN THE LEVELS (TOTAL ABSORBANCE AT 260 nm FROM 70 g fr. wt. OF LEAVES) OF CYTOPLASMIC AND CHLOROPLAST RIBOSOME COMPONENTS FROM THE PRIMARY LEAVES OF CONTROL AND OZONE-TREATED PINTO BEAN PLANTS.

The left half represents cytoplasmic ribosome components: (A), total components, fractions 1–39; (B), polysomes, fractions 1–19; (C), monosomes, fractions 20–25; (D), subunits, fractions 26–39.

The right half represents chloroplast ribosome components: (a), total components, fractions 1–39; (b), polysomes, fractions 1–21; (c), monosomes, fractions 22–28; (d), subunits, fractions 29–39.

An average of two replicates was made to determine the concentration of each ribosomal component.

(●) Control, (○) Ozone.

is reported to oxidize a number of amino acid residues,[12] including the sulfhydryl group[13] of proteins. Cysteine is most susceptible to ozone in comparison with other amino acids in aqueous solution.[12] Ozone may destroy the integrity of polysome particles by the reaction with the sulfhydryl group of ribosomal proteins. This possibility is based upon the previous finding[14] that 100*S* particles of *E. coli* ribosomes can be completely dissociated by *p*-chloromercuribenzoate or mild iodine oxidation. To test these possibilities, preliminary experiments were conducted by treating isolated chloroplast ribosomes with ozone (0·55 μmole/min/ml

[12] J. B. MUDD, R. LEAVITT, A. ONGUN and T. T. MCMANUS, *Atmos. Environ.* **3**, 669 (1969).
[13] G. W. TODD, *Physiol. Plant.* **11**, 457 (1958).
[14] J. H. WANG and A. T. MATHESON, *Biochim. Biophys. Acta* **138**, 296 (1967).

ribosomes) and *p*-chloromercuribenzoate (3×10^{-4} M). About 40% of the total polysomes were disintegrated by these treatments. The different responses of the two classes of ribosomes to ozone may be attributed to the different amino acid residues of these ribosomal proteins[15] and/or their different configurations. (b) The maintenance of polysome complex is reported to require energy.[16] Ozone may well oxidize the reduced form of NADP. This nucleotide participates in the energy generating system in the chloroplast.[17] The influence of ozone on the polysome population of chloroplast ribosomes could also be mediated through a nonribosomal factor of impaired energy production.

EXPERIMENTAL

Pinto bean plants (*Phaseolus vulgaris* L.) were raised in a carbon-filtered greenhouse. A 16-hr photoperiod was maintained with 16 K lx from incandescent light sources. Temperature was about 29° during the day and about 21° at night. Relative humidity of about 85 per cent was maintained during the day. Primary leaves of 9–12-day-old control and treated plants were harvested in the cold room at 1–4°.

Treatment of plants with ozone was conducted in a fumigation chamber. Fluorescent lamps provided 17·5 K lx at plant height. Temperature was maintained at about 29°. Relative humidity ranged between 80 and 85 per cent. Ozone was generated electrolytically and forced into the fumigation chamber. Compressed tank oxygen was passed through a twin-type ozonizer. Concentration of ozone in fumigation chamber was determined by continuous monitoring with an ozone meter and a strip chart recorder.

Cytoplasmic and chloroplast ribosomes were isolated by the procedure of Stutz and Noll,[6] except for the following modification. The 1100 *g* pellet was washed twice with buffer I[6] and pelleted by repeating previous centrifugation. The volume of buffer I was 1·2 ml/g fresh leaf weight for the first washing and 0·4 ml for the second. The sample suspension in buffer I was stirred with a glass rod for 4 min each time before centrifugation. The sedimentation pattern of chloroplast ribosomes isolated by Stutz and Noll[6] shows high peaks of 80*S* contaminant, 50*S* and 30*S*. However, no peak of the 80*S* contaminant and extremely low levels of breakdown subunits are shown in the distribution profile of chloroplast ribosomes (Fig. 1B) isolated by the modified procedure. Extensive preliminary analyses were conducted to standardize the isolation procedure. These experiments indicated that ribosomal yields from replicate samples did not vary more than about 7% (or ±3·5% variation from the mean) as estimated by the values of total absorbance (260 nm) determined from the yields.

The ribosomes isolated by the modified procedure were clarified by centrifugation at 14,500 rev/min for 15 min. The determination of sedimentation profile was conducted by the procedure as described in an earlier report[5] except for 10–34% linear sucrose gradient in buffer III[6] and 4 hr centrifugation in a SW 25·1 rotor.

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[16] C. Y. LIN and J. L. KEY, *J. Molec. Biol.* **26,** 237 (1967).
[17] P. KARLSON and C. H. DOERING, *Introduction to Modern Biochemistry*, pp. 282, Academic Press, New York (1963).