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SHORT COMMUNICATION

THE INFLUENCE OF OZONE ON GROWTH AND RIBOSOMAL RNA IN PINTO BEAN PLANTS

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Abstract—A study was made of the relationship between the level of *r*RNA and the rate of growth in pinto bean plants treated with 0·35 ppm ozone for 20–40 min. Cytoplasmic ribosomes and a fraction containing both cytoplasmic and chloroplast ribosomes were initially isolated from primary leaves. The *r*RNA components of 25S, 23S, and 16S were extracted from these fractions and analysed by sucrose density gradient. The levels of cytoplasmic *r*RNA components, 25S and 16S, are not significantly affected by ozone. In contrast, ozone specifically decreases the relative level of 23S of chloroplast *r*RNA, as the rate of growth is inhibited.

INTRODUCTION

OZONE is one of the photochemical oxidants present in the smog complex. Atmospheric oxidants are known to cause growth retardation in plants[1] in addition to other physiological alterations. Ozone is estimated to be responsible for 85–90% of the total oxidizing potential of the polluted air.[1] It was reported that 90–170 ppm ozone inhibited the cellulose synthesis in cell walls[2] and the growth of oat coleoptile segments *in vitro*.[3] The authors of these studies related their findings to ozone-induced growth retardation in natural vegetation. However, the maximum concentrations of oxidants in the atmosphere near many metropolitan areas do not exceed an hourly average of about 0·60 ppm throughout the seasons.[4] Therefore, a new assessment has been needed.

Observations in our laboratory revealed that the treatment of 9-day-old pinto bean plants with an ozone concentration of 0·35 ppm for 40 min resulted in about 35% growth inhibition. Growth rate is known to be controlled by the rate of protein synthesis.[5] RNA content plays a vital role in protein synthesis.[6] Ribosomal RNA represents the bulk of total RNA of the cell.[7] An investigation, therefore, was made of a possible relationship between the *r*RNA level and the growth rate of ozone-treated pinto bean plants.

RESULTS AND DISCUSSION

Figure 1 shows that ozone inhibits the growth rate of pinto bean plants by about 35 per cent of the control under the conditions indicated. Such data demonstrate experimentally

[1] O. C. TAYLOR, *J. Occupational Med.* **10**, 485 (1968).
[2] L. ORDIN and B. P. SKOE, *Plant Physiol.* **39**, 751 (1964).
[3] L. ORDIN and B. PROPST, *Plant Physiol.* **36**, 326 (1961).
[4] U.S. Department of Health, Education, and Welfare, National Air Pollution Control Association Publication No. AP-63, Air Quality Criteria for Photochemical Oxidants, pp. 2–3 (1970).
[5] F. C. NEIDHARDT and B. MAGASANIK, *Biochim. Biophys. Acta* **42**, 99 (1960).
[6] J. INGLE and R. H. HAGEMAN, *Plant Physiol.* **39**, 730 (1964).
[7] J. BONNER, Ribosomes. In *Plant Biochemistry* (edited by J. BONNER and J. E. VARNER), pp. 21–37, Academic Press, New York (1965).

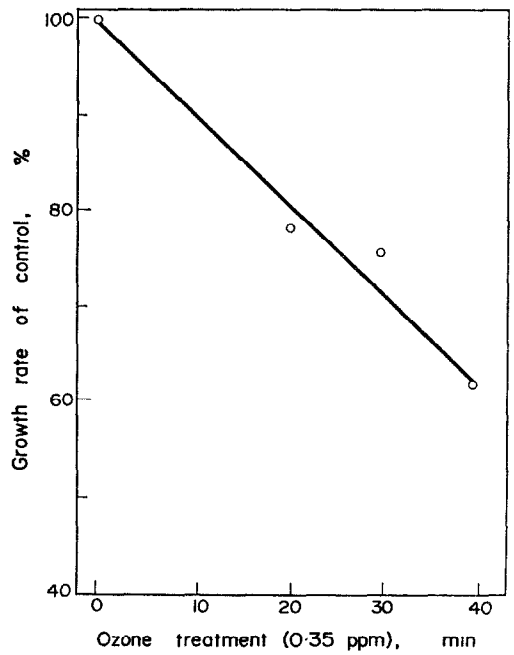

FIG. 1. GROWTH RATES OF 9-DAY-OLD PINTO BEAN PLANTS TREATED WITH OZONE (0·35 ppm) FOR VARIOUS PERIODS OF TIME. THE GROWTH RATE WAS DETERMINED BY MEASURING THE DISTANCE BETWEEN THE SHOOT APEX AND THE NODE POSITION OF PRIMARY LEAVES 3 DAYS AFTER THE OZONE TREATMENT. AN AVERAGE OF 70–78 MEASUREMENTS WERE MADE FOR EACH GROUP OF 3 DIFFERENT TREATMENTS AND CONTROL.

that the smog complex containing ozone can inhibit the growth of plants. The ozone-induced growth retardation was characterized by a low level of visible leaf symptom. This symptom was unlikely to be associated with cellular damage, since ion-leakage from the leaves was found to be low.

Figure 2 (left half) shows the sedimentation patterns of cytoplasmic *r*RNA components from pinto bean plants treated with ozone. To estimate the concentrations of the different *r*RNA components, the areas under the various peaks were integrated. The relative levels of total *r*RNA (as per cent of control total *r*RNA) and the proportions of 25S and 16S components (as per cent of total *r*RNA in each experiment) are expressed in the upper right corner graphs. The 80S monomers from bean cytoplasm are made up of 25S and 16S *r*RNA components, whereas the corresponding values for *r*RNA of 70S chloroplast ribosomes are 23S and 16S.[8] As shown here, oxone does not change the relative levels of total cytoplasmic *r*RNA and its components (25S and 16S). In addition, the peaks corresponding to 25S and 16S *r*RNA are relatively sharp and distinctive. These data indicate that ozone does not degrade the two components of cytoplasmic *r*RNA. The *r*RNA contaminants from chloroplast ribosomes, if any, are assumed to be low, since there is no detectable 23S *r*RNA peak.

As shown in Fig. 2 (right half), the *r*RNA sample prepared from control 1100 *g* pellet shows three peaks (25S, 23S and 16S). The 16S peak is assumed to contain both cytoplasmic and chloroplast *r*RNA fractions. Ozone decreases the relative peak height of 23S *r*RNA

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

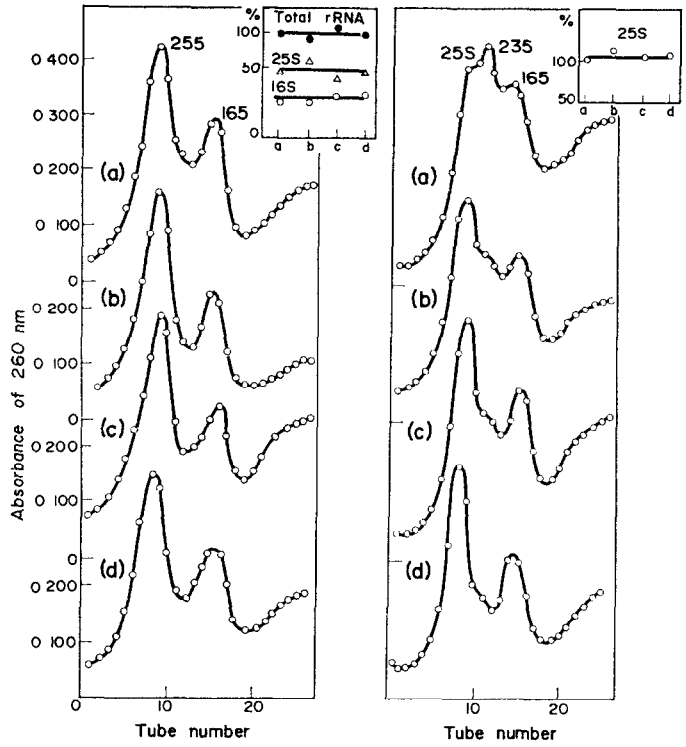

FIG. 2. SIZE DISTRIBUTION OF RIBOSOMAL RNA FROM CYTOPLASMIC RIBOSOMES (LEFT) AND THE SAMPLE CONTAINING BOTH CYTOPLASMIC AND CHLOROPLAST RIBOSOMES (RIGHT) AFFECTED BY OZONE. Nine-day-old pinto bean plants were treated with 0·35 ppm ozone for (*a*) 0 min for control, (*b*) 20 min, (*c*) 30 min, and (*d*) 40 min. The primary leaves were harvested 1·5 days after the ozone treatment. For the analysis of cytoplasmic *r*RNA, the volume of total ribosomes from 100 g fresh wt. of leaves was adjusted to 4 ml with buffer III (8) in each experiment. A fraction (0·2 ml) of sample from this total ribosomal suspension was analysed. For the analysis of *r*RNA components from the sample containing both cytoplasmic and chloroplast ribosomes, the total ribosomes from 100 g fresh wt. of leaves was analysed. Direction of sedimentation was from right to left. Per cents and distribution patterns of various *r*RNA components are single determinations and generally consistent with values obtained from replicate experiments.

compared with the constant level of 25S *r*RNA. To show the level of 25S *r*RNA more clearly, estimations were made by integrating the areas under the peaks (fraction number 1–9). These values are expressed as per cents of the control in the upper right corner graph.

The reduced level of 23S *r*RNA in chloroplasts may be associated in part with the direct action of ozone on the protein components of chloroplast ribosomes. Ozone is known to oxidize a number of amino acids in aqueous solution[9] and to inhibit sulfhydryl enzymes.[10] Cysteine is the amino acid most susceptible to ozone.[9] Ozone might destroy the integrity of polysome and monosome particles by reaction with the sulfhydryl group of ribosomal proteins, since 100S and 70S particles of *E. coli* ribosomes can be completely dissociated by the actions of sulfhydryl reagents such as *p*-chloromercuribenzoic acid and iodine.[11]

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

Chang[12] has recently demonstrated that ozone specifically decreases the population of chloroplast ribosomes, but not that of cytoplasmic ribosomes. The sucrose gradient distribution pattern of these chloroplast ribosomes is characterized by a low concentration of polysome and monosome particles without any concomitant accumulation of subunits. A similar distribution pattern is also seen in senescing barley leaves.[13] The RNA of ribosomes produced by the breakdown of polysomes is unstable and is lost *in vivo* in barley leaves[13] and possibly in the present experimental material, pinto bean leaves. The reduction of *r*RNA components observed in this study therefore is attributed to a loss of chloroplast ribosomes. The different responses of the two classes of *r*RNA to ozone are likely to be due to the different amino acid residues of the two classes of ribosomal proteins and/or their different geometric configurations.

EXPERIMENTAL

Pinto bean plants (*Phaseolus vulgaris* L.) were raised in a carbon-filtered greenhouse. Treatment of plants with ozone was conducted in a fumigation chamber equipped with provisions for light, temperature, and relative humidity control. Ozone was generated electrolytically and forced into the fumigation chamber. Compressed tank oxygen was passed through a twin-type ozonizor. Ozone concentration was regulated by the rate of oxygen flow. The concentration of ozone in the fumigation chamber was determined by continuous monitoring with an ozone meter and a strip chart recorder. The ozone absorbing reagent was 1% KI in 0·1 M neutral phosphate buffer.[14]

Two ribosome samples, cytoplasmic ribosomes and a fraction containing both cytoplasmic and chloroplast ribosomes, were prepared in a cold room at 1–4°. The first samples were isolated by the procedure of Stutz and Noll.[8] The second samples were prepared by a similar procedure,[8] but with the following minor modifications. 100 g of leaf material was homogenized in a Waring blender for 30 sec with 3 ml of cold buffer I (0·7 M sucrose, 0·1 M tris HCl, pH 7·5, 0·005 M $MgCl_2$, 0·05 M KCl, 0·005 M 2-mercaptoethanol) per g fresh leaf weight. The homogenate was filtered through 2 layers of gauze, and the filtrate centrifuged for 2 min at 600 *g*. Centrifugation at 1100 *g* for 15 min yielded the chloroplast pellets associated with cytoplasmic ribosomes. This pellet was washed once by suspending in 30 ml of buffer I, and the previous centrifugation was repeated (1100 *g* pellet). The supernatant was combined with the 1100 *g* supernatant fraction (cytoplasmic ribosomes were prepared from this fraction). The rest of the ribosome isolation from the 1100 *g* pellet was essentially that of Stut and Noll.[8] From each 1100 *g* pellet prepared from similar amounts of control materials, a mixture of two types of ribosomes was isolated. The components of *r*RNA then were extracted and analysed for size distribution according to the procedures as described in the text. The relative peak heights of 25S *r*RNA from cytoplasmic ribosomes and 23S *r*RNA from chloroplast ribisomes were practically constant. The yield of ribosomes was estimated by the absorbance at 260 nm of an aliquot of each ribosome suspension prepared. These materials were kept at −60° until analysed. The extractions of *r*RNA then were made from replicate ribosomes, the yields of which varied not more than about 7 per cent. The preparation of *r*RNA from ribosomes by the procedure of Kurland,[15] with minor modifications, was quantitative, since the ribosomes sample exposed to the modified conditions (0·5% sodium dodecyl sulfate at 15° for 5 min) was directly analysed for *r*RNA components. The size distribution of *r*RNA components was determined by the procedure described earlier.[16]

[12] C. W. CHANG, *Phytochem.* **10,** 2863 (1972).
[13] B. I. S. SRIVASTAVA and C. ARGLEBE, *Plant Physiol.* **42,** 1497 (1967).
[14] D. H. BYERS and B. E. SALTZMAN, *Am. Ind. Hyg. Assoc. J.* **19,** 251 (1958).
[15] C. G. KURLAND, *J. Molec. Biol.* **2,** 83 (1960).
[16] C. W. CHANG, *Can. J. Biochem. Physiol.* **48,** 450 (1970).

Key Word Index—Phaseolus vulgaris; Leguminosae; ozone effects; growth; *r*RNA; chloroplast *r*RNA.