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Bentazon Influence on Selected Metabolic Processes of Isolated Bean Leaf Cells

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Abstract. Time- and concentration-course studies were conducted to determine the effect of bentazon [3-isopropyl-1*H*-2,1,3,-benzothiadiazin-4(3*H*)-one 2,2-dioxide] on photosynthesis, RNA synthesis, protein synthesis, and lipid synthesis using enzymatically isolated leaf cells of red kidney bean (*Phaseolus vulgaris* L.). Photosynthesis and RNA synthesis were inhibited about 75% at 1 μM bentazon at the 30 min treatment period. This was the lowest concentration and shortest time that significantly inhibited any of these four processes. The degree of inhibition of photosynthesis was greater than the degree of inhibition of RNA synthesis at higher concentrations and/or longer time periods. At 10 μM bentazon, protein synthesis and lipid synthesis were also inhibited. Lipid synthesis was stimulated at 0.1 and 1 μM at 120 min.

Bentazon [3-isopropyl-1*H*-2,1,3-benzothiadiazine-4(3*H*)-one 2,2-dioxide] is a selective herbicide for the postemergence control of weeds in bean, corn, mint, peanut, pea, rice, and soybean fields. Absorption, translocation, selectivity, and metabolism of bentazon have been studied by several workers (Mine et al. 1975, Mahoney and Penner 1975, Penner 1975). The effect of this herbicide on the photosynthetic apparatus and photosynthesis was studied by Böger et al. (1977) and Mine and Matsunaka (1975).

Böger et al. (1977) reported that bentazon inhibits photosystem II activity in isolated chloroplasts. They also reported that this inhibition is similar to the reduction that occurs in cellular photosynthesis. They attributed this inhibition to a metabolic process that brings about the binding of bentazon (or an active derivative) to the thylakoids. Mine and Matsunaka (1975) found that bentazon also inhibits the Hill reaction in isolated chloroplasts and photosynthetic CO_2

fixation in different tested plants. Potter (1977) reported that bentazon inhibits photosynthesis in treated common cocklebur (*Xanthium pennsylvanicum* Wallr). leaves.

This study was conducted to further confirm the effect of bentazon on photosynthesis and compare these results to its effect on RNA, protein, and lipid synthesis.

Materials and Methods

The methods used in the present research were described by Ashton and Glenn (1982/1983). Cells of the primary leaves of 7-day-old red kidney bean (*Phaseolus vulgaris* L.) seedlings were isolated by the modified method described by Ashton et al. (1977). Tissues were macerated by medium containing 0.7 M sorbitol, potassium dextran sulfate, and Macerase/TM (Calbiochem). The isolated cells were washed in washing medium containing 0.65 sorbitol and inorganic salts and then incubated in an incubation medium containing 0.625 M sorbitol, inorganic salts, and MES buffer. All media were adjusted to pH 5.8. The cell preparation used for these assays contained 0.02–0.03 mg of chlorophyll/ml or 0.05–0.075 mg of chlorophyll/assay. Two-and-one-half ml of the cell suspension was placed in a 25-ml Erlenmeyer flask. The herbicide and radioactive substrate solutions were added to each flask at the rate of 0.05 ml and 0.1 ml, respectively. The final volume was 2.65 ml. The herbicide concentrations in the incubation media were 0.1, 1, 10, 100, and 1000 μM.

The assay methods for the processes studied were essentially the same as those used by Ashton et al. (1977) and Ashton and Glenn (1982/1983). The stoppered flasks were placed in a shaking water bath at 25°C and illuminated from above with fluorescent lamps (Westinghouse, warm white, 20 watts) with an intensity of 4,300 lux at the level of the flasks. Samples were removed after 30, 60, and 120 min and the radioactive product of each process isolated as previously described (Ashton et al. 1977) prior to liquid scintillation counting. Photosynthesis was assayed by incubating the cells with 5 μCi of $NaH^{14}CO_3$ containing 6 mM $NaH^{12}CO_3$. RNA synthesis was determined by measuring the incorporation of 5 μCi of [2^{14}C] uracil into RNA. Protein synthesis was determined by measuring the incorporation of 1 μCi of L-[U-^{14}C] leucine into protein. Lipid synthesis was determined by measuring the incorporation of 1 μCi of [1,2-^{14}C] sodium acetate into lipids. Radioactivity was determined by adding 10 ml of scintillation fluid consisting of 0.4% PPO and 0.01% POPOP in toluene to samples and counting them in a liquid scintillation spectrometer.

Chlorophyll content was determined by the method of Vernon (1960). One ml of cell suspension was added to 4 ml of acetone, mixed, and centrifuged, and the optical density of the supernatant was measured. The rate of each process was calculated as disintegrations per min/mg chlorophyll. Six replicates were used for each treatment and the data were statistically analyzed.

Results

The effects of bentazon on photosynthesis, RNA synthesis, protein synthesis, and lipid synthesis, using isolated leaf cells of red kidney beans, are presented

in Table 1. All data were calculated as dpm/mg chlorophyll. Percent inhibition relative to the control is calculated for each process at each time period. Negative values indicate stimulation instead of inhibition.

The lowest concentration (0.1 μM) did not inhibit any of the metabolic processes at any incubation periods. However, this concentration (0.1 μM) significantly stimulated lipid synthesis at the 120 min incubation period. At the highest concentration (1.0 mM) and longest incubation period (120 min) all four metabolic processes were essentially completely blocked. Intermediate concentrations and incubation times gave intermediate values for the various metabolic processes. In general, the control values of each process increased linearly with time.

Photosynthesis was markedly inhibited by 1 μM bentazon; 76, 86, and 89% at 30, 60, and 120 min, respectively. At the higher concentrations and all incubation times, the inhibition of photosynthesis was significantly greater. At 10 μM and above, photosynthesis was essentially blocked (98 to 99% inhibition) at all incubation times.

RNA synthesis was significantly reduced at 1 μM bentazon with all incubation periods; 75, 82, and 89% at 30, 60, and 120 min, respectively. However, with increasing concentrations, the degree of inhibition of RNA synthesis did not increase significantly. This is in contrast to the results for photosynthesis, protein synthesis, and lipid synthesis; these processes were inhibited more with increasing concentrations of bentazon.

Protein synthesis was significantly reduced at 10 μM bentazon at the 60 and 120 min incubation periods, 21 and 22% inhibition, respectively. Although the percentage inhibition was slightly greater (26%) at 30 min, this was not statistically different from the control. At 100 and 1000 μM bentazon protein synthesis was further reduced, about 45 and 99% inhibition, respectively, at all three incubation periods.

Lipid synthesis was significantly reduced by bentazon at 10 μM with all incubation periods, 53, 60, and 71% at 30, 60, and 120 min, respectively. At 100 and 1000 μM bentazon, lipid synthesis was further reduced, about 90 and 98% inhibition, respectively, at all three incubation periods. At 120 min lipid synthesis was significantly stimulated 14 and 21% at 0.1 and 1.0 μM, respectively.

Discussion

Photosynthesis and RNA synthesis were inhibited about 75% at 1.0 μM bentazon at the 30 min treatment period. Since this is the lowest concentration that significantly inhibited any of the four metabolic processes studied at a given time of exposure, either photosynthesis or RNA synthesis could be considered to be the primary metabolic site of action of bentazon. This does not take into account the possibility that the primary site of action of bentazon may be some metabolic site that has not yet been investigated. Further definition of the relative sensitivity of photosynthesis and RNA synthesis would require shorter exposure times and/or a concentration series between 0.1 and 1.0 μM bentazon. However, the greater inhibition of photosynthesis at concentrations higher than 1.0 μM and exposure periods longer than 30 min, rel-

Table 1. The effect of bentazon on selected metabolic processes at three incubation periods.[a]

Process	Concentration (μM)	Radioactivity 30 min (dpm/mg chl.)	(%[b])	60 min (dpm/mg chl.)	(%)	120 min (dpm/mg chl.)	(%)
Photosynthesis	0	1,752,395a	0	3,461,988d	0	6,199,352e	0
	0.1	1,906,555a	−9	3,334,777d	4	6,002,598e	3
	1	420,981b	76	495,791b	86	701,913b	89
	10	36,311c	98	29,492c	99	48,104c	99
	100	34,801c	98	28,334c	99	40,767c	99
	1000	36,919c	98	19,515c	99	30,627c	99
RNA synthesis	0	24,399a	0	54,254c	0	119,345d	0
	0.1	22,230a	9	54,825c	−1	106,988d	10
	1	6,207b	75	9,723be	82	12,813be	89
	10	6,086b	76	6,717b	88	8,314b	93
	100	7,107b	71	6,705b	88	7,149b	94
	1000	6,628b	73	5,275b	90	8,846b	93
Protein synthesis	0	639,305a	0	1,842,661d	0	4,643,583g	0
	0.1	606,561a	5	1,798,420d	2	4,193,197g	10
	1	584,456a	9	1,686,139d	8	4,353,532g	6
	10	473,968ab	26	1,455,853e	21	3,603,033h	22
	100	345,320b	46	1,018,499f	45	2,640,290i	43
	1000	10,895c	98	18,151c	99	26,627c	99
Lipid synthesis	0	292,253a	0	507,559d	0	1,082,711e	0
	0.1	297,859a	−2	541,016d	−7	1,239,072f	−14
	1	251,992a	14	483,293d	5	1,310,184f	−21
	10	136,704b	53	205,032b	60	316,479g	71
	100	26,798c	91	58,622ch	88	112,882h	89
	1000	5,209c	98	15,245c	97	10,747c	99

[a] Values within the same column or line followed by a common letter are not significantly different at the 5% level, according to Duncan's multiple range test.

[b] % = percent inhibition relative to the control; negative values indicate a stimulation.

ative to RNA synthesis, suggests that photosynthesis may have the dominant role in the action of bentazon. Other researchers have also reported that bentazon inhibits photosynthesis. Ratzlaff and Fischer (1973), Mine and Matsunaka (1975), and Potter (1977) reported that bentazon inhibits photosynthetic CO_2 fixation. Mine and Matsunaka (1975) found that bentazon also inhibits the Hill reaction. Böger et al. (1977) reported that bentazon inhibits photosystem II activity in isolated chloroplasts.

The data clearly show that protein synthesis and lipid synthesis are less sensitive to inhibition by bentazon than are photosynthesis or RNA synthesis in this system. A 10-fold higher concentration of bentazon is required to detect any inhibition of protein synthesis or lipid synthesis relative to photosynthesis or RNA synthesis. However, *in vivo,* the effect of bentazon on all four processes may contribute to the ultimate herbicidal action, since they are all significantly or markedly inhibited at 10 μM. This relatively low concentration is well within that which is generally considered to be a physiological range.

Bentazon stimulates lipid synthesis within a limited range of concentrations and exposure times as do certain other herbicides that inhibit photosynthesis (Ashton et al. 1977). At higher concentrations and/or longer exposure times, they usually inhibit lipid synthesis. In regard to concentration and time, the stimulation of lipid synthesis usually occurs concurrently with the inhibition of photosynthesis with each of these herbicides in this system. These other herbicides include atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], bromacil (5-bromo-3-*sec*-butyl-6-methyluracil), monuron [3-(*p*-chlorophenyl)-1,1-dimethylurea], and paraquat (1,1′-dimethyl-4,4′-bipyridinium ion); in general the degree of lipid stimulation with these herbicides is greater than that of bentazon. The relationship between photosynthesis inhibition and lipid synthesis with these compounds remains obscure. However, it might be that excess CO_2 form $NaHCO_3$ stimulates the transcarboxylase enzyme and increases the level of malonyl-CoA from acetyl-CoA. Malonyl-CoA plays a very important role in lipid synthesis, and at least six different enzyme activities involving malonyl-CoA have been observed (Stumpf 1976). In the push and pull of metabolic control, the activities of these enzymes must be balanced. The key enzyme in the synthesis of malonyl-CoA is acetyl-CoA carboxylase, and one of the rate-limiting steps in lipid synthesis is controlled by its activity. Perhaps these compounds interfere with the control mechanism of acetyl-CoA carboxylase or one of the other enzymes. It is also conceivable that the increase in lipid synthesis is associated with a membrane repair mechanism, since these compounds also damage membranes (Ashton and Crafts 1981).

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