Biological Properties of Amino Acid Conjugates of 2,4,5-Trichlorophenoxyacetic Acid

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The effect of L-amino acid conjugates of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) on cell elongation of *Avena sativa* coleoptile sections and on growth of soybean root callus tissue cultures has been evaluated at concentrations of 10^{-4} to 10^{-8} M. Most of the conjugates gave good stimulation of elongation in the *Avena sativa* test and were equivalent to the stimulation observed for 2,4,5-T. The most active 2,4,5-T conjugates based on their half-maximum concentrations were Val, Phe, Ile, Met, and Ala. In the soybean root callus tissue culture test, 2,4,5-T and all conjugates gave stimulation of growth at low concentration and inhibition of growth at high concentration (10^{-4} M). The most active conjugates (Phe, Cys, Leu, Ala, Ser, Trp, and Ile) were statistically equivalent to the stimulation exhibited by 2,4,5-T. The Glu and Asp conjugates, which have been isolated as metabolites of 2,4,5-T in soybean root callus tissue, demonstrated poor biological activity.

Soybean plant tissue cultures metabolize the herbicide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) to amino acid conjugates of which two have been identified as 2,4,5-T-Glu and 2,4,5-T-Asp (Arjmand et al., 1978b). Amino acid conjugates of 2,4-dichlorophenoxyacetic acid (2,4-D) and indole-3-acetic acid (IAA) strongly stimulated the elongation of *Avena* coleoptile sections and the growth of soybean callus tissue (Fueng et al., 1974, 1977) and show inhibition at high concentrations. Therefore, the ability of amino acid conjugates of 2,4,5-T to stimulate cell elongation of *Avena sativa* coleoptile sections and to stimulate growth of soybean root callus tissue cultures was examined at concentrations of 10^{-4} to 10^{-8} M.

EXPERIMENTAL SECTION

Oat seeds, *Avena sativa* L. (var. Gary Penfield), were dehusked and surface sterilized in 5% hydrogen peroxide for 20 min and rinsed three times with distilled water. The sterilized seeds were spread over moist filter paper in glass trays covered with glass plates and kept in the dark at room temperature (25–27 °C) for 24 h. The germinating grains were then exposed to red light for 4 h (25-W ruby red photographic lamp) at a distance of 46–61 cm and then allowed to germinate in the dark for 48 h. The 3-day-old seedlings with coleoptiles 23–30 mm long were selected from which one 6.5-mm coleoptile section was excised 2 mm from the apex (Wang et al., 1968). Ten randomly selected coleoptile sections were transferred to each petri dish containing 10 mL of the basal medium (2% sucrose in 0.01 M KH_2PO_4) with or without 2,4,5-T or the conjugate at selected concentrations. The segments were incubated for 20 h in darkness (25–27 °C) and then the length was measured to the nearest 0.1 mm.

The auxin dependent callus tissue used in these studies was originally derived from soybean root (*Glycine max* [L.] Merrill var. Amsoy). Stock callus cultures were grown on an agar medium of Miller (1963) with 3% sucrose, kinetin (0.5 mg/L), and naphthaleneacetic acid (NAA 2.0 mg/L) under continuous low intensity fluorescent light at 25 °C for about 5 weeks. For bioassay, four clumps (about 5–10 mg each) of callus tissue were asceptically inoculated into each of four 125-mL Erlenmeyer flasks which contained 50 mL of the agar medium (0.7% agar) as stated for stock cultures except NAA was replaced with varying concentrations (10^{-4}–10^{-8} M) of 2,4,5-T or 2,4,5-T-amino acid conjugate dissolved in 50 μL of ethanol or less. The tissues were allowed to grow under fluorescent light (ca. 40 foot candles) for a period of 4 weeks at 25–26 °C. The callus tissues were then removed from the nutrient agar and blotted briefly, and the fresh weight was determined.

The preparation and purification of these amino acid conjugates have been previously described (Arjmand et al., 1978a). Each conjugate was pure when analyzed by gas–liquid chromatography and high-pressure liquid chromatography. No free 2,4,5-T was detected in these samples. The following conjugates were examined: Gly, Ala, Pro, Ser, Val, Thr, Leu, Ile, Cys, Asp, Glu, Met, Phe, and Trp.

RESULTS AND DISCUSSION

All the amino acid conjugates of 2,4,5-T which were tested stimulated the elongation of *Avena* coleoptile sections (Table I). The compounds are arranged according to their decreasing half-maximum elongation concentration. The optimum elongation response for 2,4,5-T and for the five most active derivatives was found at 10^{-6} M (Ala, Val, Ile, Met, and Phe). At 10^{-5} M the Gly, Ser, Pro, Thr, Asp, Glu, and Trp conjugates exhibited optimum response. The conjugates did not show any stimulation or inhibition of elongation at 10^{-4} M except for the Asp and Glu conjugate which did give a statistically significant increase. It should be mentioned that since the exact optimum was not determined, the half-maximum elongation concentrations are only approximations and are calculated from the semilog plot of the elongation of *Avena* sections vs. the concentration of the conjugate. The most active conjugates of 2,4,5-T based on their half-maximum concentrations were Val, Phe, Ile, and Met which were also among the most active 2,4-D conjugates (Feung et al., 1974) and indole-3-acetic acid conjugates (Feung et al., 1977).

The results of the stimulation of growth of soybean root callus tissue by 2,4,5-T, 2,4-D, NAA, and 12 amino acid conjugates of 2,4,5-T are presented in Table II. All conjugates behaved like auxins and stimulated growth at low concentrations and inhibited growth at higher concentrations. The conjugates are arranged according to their maximum stimulation of growth at 10^{-7} M. Nine of the twelve conjugates exhibited their maximum growth stimulation at 10^{-7} M which was also maximum for 2,4,5-T. The best stimulation was observed for the Phe, Cys, Leu, Ala, Ser, Trp, and Ile conjugates. They did not statistically differ from the strong stimulation of growth demonstrated

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Table I. Elongation of *Avena* Coleoptile Sections Induced by Amino Acid Conjugates of 2,4,5-T

2,4,5-T or 2,4,5-T conjugate	elongation, mm[a]					half-max. concn, M
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	
control (no additive)	2.2a	2.2a	2.2a	2.2a	2.2a	
Asp	4.4b	7.0b	4.3b	3.4a	3.9b	1.6×10^{-6}
Ser	2.4a	6.2b	4.8b	2.6a	2.6a	5.4×10^{-7}
Thr	2.0a	6.9b	4.9b	3.9b	2.7a	5.0×10^{-7}
Glu	3.9b	6.1b	5.2b	2.0a	2.1a	4.3×10^{-7}
Pro	2.9a	6.5b	5.3b	2.7a	3.2a	4.3×10^{-7}
Gly	2.7a	6.3b	5.5b	3.1a	2.4a	3.2×10^{-7}
Leu	2.0a	5.5b	5.8b	2.8a	3.4a	2.5×10^{-7}
Trp	3.0a	6.4b	5.0b	4.0b	3.9b	2.0×10^{-7}
2,4,5-T	2.9a	5.5b	6.5b	4.0b	3.6b	2.0×10^{-7}
Ala	2.3a	5.0b	5.0b	3.2b	3.2a	1.6×10^{-7}
Met	2.2a	4.7b	5.7b	3.5a	3.0a	1.4×10^{-7}
Ile	1.8a	5.5b	7.0b	4.4b	4.0b	1.4×10^{-7}
Phe	2.6a	5.3b	5.8b	4.0b	3.6a	1.0×10^{-7}
Val	2.1a	5.6b	5.9b	4.2b	3.8a	6.0×10^{-8}

[a] Means in the same concentration (column) followed by the letter b are statistically greater (5%) than control.

Table II. Fresh Weight of Soybean Root Callus Tissue Induced by Amino Acid Conjugates of 2,4,5-T

compound	weight of tissue, mg[a]			
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M
control (no additive)	437.6a	437.6ab	437.6a	437.6a
2,4-D	130.5b	919.1b	3104.9efg	2769.5bcd
NAA		2498.6c	3771.8g	3142.7bcd
2,4,5-T	65.1b	687.3abc	3680.7fg	6049.5fg
2,4,5,T Glu	163.0b	466.8ab	884.6ab	831.0ab
2,4,5-T Met	96.1b	443.9ab	1288.8abcd	1223.1abc
2,4,5-T Val	78.3b	394.9ab	1410.0abcd	1262.1abc
2,4,5-T Thr	118.4b	367.9ab	894.9ab	1269.0abc
2,4,5-T Asp	153.8b	341.7ab	1007.3abc	1403.7abc
2,4,5-T Ile	55.1b	459.0ab	2604.1ef	4630.4def
2,4,5-T Trp[b]	97.2b	798.1bc	3887.9g	4713.4def
2,4,5-T Ser		221.3a	1480.5bcd	5060.7efg
2,4,5-T Ala		255.1a	2067.0cde	5129.2efg
2,4,5-T Leu[b]	60.5b	342.3ab	2618.4eg	6045.0fg
2,4,5-T Cys[b]	45.3b	330.0ab	3645.5fg	6447.1fg
2,4,5-T Phe		270.5a	2300.2de	7073.0g

[a] Average weight of the four pieces of tissue per flask. Means in the same concentration (column) followed by the same letter are not significantly (5%) different. [b] No ethanol was used. In all other flasks 50 μL of ethanol was used.

Table III. Fresh Weight of Soybean Root Callus Tissue as Affected by Various Amounts of Ethanol

μL of ethanol per flask	weight of tissue, mg[a]	
	without auxin	with 10^{-5} M NAA
0	532.7 a	7571.6 a
50	437.6 a	2979.6 b
100	571.8 a	657.3 c
200	61.7 b	234.2 c
400	43.4 b	
500	41.9 b	159.3 c

[a] Average weight of the four pieces per flask. Means in the same concentration (column) followed by the same letter are not significantly different (5%).

by 2,4,5-T, but they did stimulate growth better than 2,4-D or NAA. Surprisingly, the Glu and Asp conjugates gave poor stimulation of growth and elongation. These two conjugates have been identified as the major and only significant amino acid conjugate metabolites of 2,4,5-T metabolism by soybean root callus tissue (Arjmand et al., 1978b). The Glu and Asp conjugate of 2,4-D and IAA have been shown to strongly stimulate growth and elongation (Feung et al., 1974, 1977). Also the Glu conjugate of 2,4-D was metabolized to free 2,4-D and other amino acid conjugates in soybean cotyledon callus tissue. Perhaps the Glu and Asp conjugates of 2,4,5-T are not readily converted to free 2,4,5-T and for this reason exhibit poor biological activity. Thus, the metabolic formation of the Glu and Asp conjugates by root callus tissue may in this case be thought as a detoxification step.

In earlier experiments 2,4,5-T and conjugates did not show strong growth stimulating properties. We have determined that the low biological response was due to inhibition by ethanol. Recently, Davis et al. (1978) have shown that ethanol is quite toxic to plant suspension tissue cultures. Since the conjugates of 2,4,5-T were introduced in the callus bioassay test in an ethanol solution we conducted an experiment to determine the effects of various levels of ethanol in the culture flasks with and without NAA (Table III). With NAA (10^{-5} M) even 50 μL of ethanol per 50 mL of media showed significant inhibition of growth of the callus tissue. In the absence of auxin, inhibition of growth was only observed after 200 μL of ethanol were used. Therefore, the choice and quantity of solvent used to introduce test chemicals with plant tissue cultures should be given careful consideration. The results of stimulation of growth presented in Table II also reflects the inhibition exhibited by the 50 μL of ethanol used to introduce most conjugates.

LITERATURE CITED

Arjmand, M., Hamilton, R. H., Mumma, R. O., *J. Agric. Food Chem.* **26**, 898 (1978a).

Arjmand, M., Hamilton, R. H., Mumma, R. O., *J. Agric. Food Chem.* **26**, 1125 (1978b).

Davidonis, G. H., Hamilton, R. H., Mumma, R. O., *Plant Physiol.* **62**, 80 (1978).
Davis, D. G., Wergin, W. P., Dusababek, K. E., *Pestic. Biochem. Physiol.* 8, 84 (1978).
Feung, C. S., Mumma, R. O., Hamilton, R. H., *J. Agric. Food Chem.* **22**, 307 (1974).
Feung, C. S., Hamilton, R. H., Mumma, R. O., *Plant Physiol.* **59**, 91 (1977).
Miller, C. O., *Modern Methods Plant Anal.* **6**, 194 (1963).
Wang, H. C., Hamilton, R. H., Deering, R. A., in "Biochemistry and Physiology of Plant Growth Substances", Wightman, R., Setterfield, G., Ed., Runge Press, Ottawa, Canada, 1968, p 685.

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Translocation and Accumulation of Seven Heavy Metals in Tissues of Corn Plants Grown on Sludge-Treated Strip-Mined Soil

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Metal translocation studies for Zn, Mn, Cu, Pb, Cr, Cd, and Hg were conducted for corn (*Zea mays* L.) plants grown on strip-mined soil amended with anaerobically digested sewage sludge (25 dry tons/acre). Differential metal accumulation rates in the seven tissues analyzed showed that generally the highest metal concentrations occur in the leaves and roots and the lowest in the grain and cob. With the exception of Mn and Hg, metal concentrations increased in tissues as a result of sludge application. The greatest increases were for Cd where mean tissue concentrations (ppm) for unamended and sludge-grown conditions respectively were roots: 0.062, 3.63; lower stems: 0.027, 0.204; and leaves: 0.276, 1.52. Metal extractabilities for plant uptake were established for the soil samples adhering directly to the root system of the corn plants studied; these extractabilities were then compared to metal concentrations in the leaf and root tissues.

The use of anaerobically digested sewage sludge as a product to enhance the agricultural productivity of marginal soils currently is receiving wide attention. Studies with marginal agricultural land, which is naturally deficient in nutrients and organic matter, have evaluated sludge application methods and rates involving field corn and pasture plots (Hyde, 1976). However, the reclamation of strip-mined soils (Boesch, 1974) represents a major effort in this area, primarily because extensive strip-mined lands exist now, and it is expected that more areas will be strip mined as the development of coal resources accelerates. Definitive studies are needed to establish if sludge can be used effectively and safely as a soil conditioner and fertilizer for growing crops. The benefits of land application of sewage sludge for growing crops and the potential problems relating to the uptake of metals by these plants, as well as the subsequent incorporation of these metals into the food chain, have been reviewed by Hinesly et al. (1972), Leeper (1972), Chaney (1973), and Page (1974).

The present study involves corn grown in Fulton County in central Illinois on strip-mined soil amended with anaerobically digested sludge by the Metropolitan Sanitary District of Greater Chicago. Sludge had been applied topically to the soil as a liquid at the rate of 25 dry tons/acre (56 metric tons/ha). In a former study (Garcia et al., 1974a), the quality of the harvested grain was assessed and the heavy metal content was established for the three contiguous corn plant tissues—kernels, cobs, and husks—to determine if any hazard might be expected from the grain itself or the plant tissues directly contacting the grain. This study examines the translocation of Zn, Mn, Cu, Pb, Cr, Cd, and Hg from the soil to corn tissues of the total corn plants to establish where each metal may accumulate. The study also examines possible effects of these metals as they enter the food chain.

To make strip-mine soils agriculturally productive by treatment with sludge, crop quality and yield must improve as a direct result of the treatment. The levels of heavy metals translocated from the amended soil to the plant must not be extensive enough (a) to be phytotoxic or (b) to cause the plant to accumulate metals at levels high enough to be hazardous for consumption by animals or humans.

The uptake of metals by plants is complex and is governed not only by the metal content of the soil, but also is influenced greatly by such factors as: metal availability to plants, soil pH, soil organic matter content, and competitive metal interactions. For definitive translocation studies, it is thus important to assess the availability to the plant of each metal from both unamended and sludge-treated soils. Metal availability usually is approximated by various treatments of the soil, either by mild chemical extractions or by metal complexation techniques. The total metal content of the soil must first be established for each metal studied. This preferably is done on the soil adhering to the root system of the selected plants and should include the rhizosphere, where plant–soil interactions occur (Nicholas, 1965). The root zone depth will vary with plant size; larger plants with larger root systems will encompass soil samples at greater depths.

The uptake of the metals can then be demonstrated by analysis of the total plant, where metal concentrations are determined for the major plant tissues. This serves to define metal distribution patterns within the plant and will show where concentrations of toxic elements may have accumulated in the tissues. Such information is useful in determining if specific plant tissues can be utilized se-

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