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Hormone-Induced Repression of a Peroxidase Isozyme in Plant Tissue

Abstract. *Young stem sections of dwarf peas (Progress No. 9) grown in light contain at least seven peroxidase isozymes separable by electrophoresis on starch gel. An eighth isozyme appears as the tissue elongates and ages, on or off the plant. The appearance of this isozyme in excised sections is repressed by application of the plant growth hormone, indole-3-acetic acid.*

Peroxidase catalyzes the oxidation of the plant growth hormone, indole-3-acetic acid (IAA) (*1*). Conversely, the fact that IAA alters the activity of peroxidase in plant tissues (*2*) suggests that this mutual interaction between hormone and enzyme may be important in the regulation of growth. Although the molecular heterogeneity of plant peroxidases has been known for some time (*3*), no effect of IAA on this heterogeneity has yet been described. We now report one specific peroxidase band whose appearance is repressed by treatment of the tissue with IAA.

Excised 5-mm sections from the sixth internode of light-grown (24-hour photoperiod) *Pisum sativum* 'Progress No. 9' cv. were used. The following basic experiment was conducted four times. Ten sections were incubated in a basal medium consisting of 0.025*M* potassium phosphate, *p*H 6.1, and 1 percent sucrose with or without $5 \times 10^{-5}M$ IAA. Such a concentration is on the ascending limb of the growth versus IAA concentration curve for this tissue (*4*). The sections were treated for 2-, 6-, 12-, 18-, or 24-hour periods in either basal medium or medium supplemented with IAA. The design of the experiment was such that all sections were harvested immediately before an electrophoretic separation of the plant proteins (see *5*).

Prior to electrophoresis the sections were rinsed in distilled water, weighed, and measured. Three sections from each treatment were then macerated onto a rectangle of Whatman No. 1 filter paper (8 by 5 mm). The cell debris was removed with forceps, and the paper was inserted immediately into a starch gel (15.5 by 11.5 by 0.5 cm). The gel was prepared according to the method of Smithies (*6*), with 15 g of hydrolyzed starch (Connaught) per 150 ml of buffer. The gel buffer was made by diluting 46 ml of borate buffer concentrate (Fisher), *p*H 9.0, to 1 liter. The bridge buffer, was composed of 0.3*M* H_3BO_3 and 0.05*M* NaOH, *p*H 8.3. A constant voltage of 400 volts with a variation of 40 ± 5 ma was delivered (Beckman Duostat) giving 10 volt/cm through the starch matrix. The matrix was chilled during the 90-minute run, and after electrophoresis the gel was slieed transversely. The interior surfaces were flooded with $5 \times 10^{-3}M$ guaiacol and $5 \times 10^{-3}M$ H_2O_2 in 0.2*M* phosphate buffer, *p*H 5.8. The zymogram patterns, which appeared within a few minutes, were photographed on Polaroid film with transmitted light within 15 minutes.

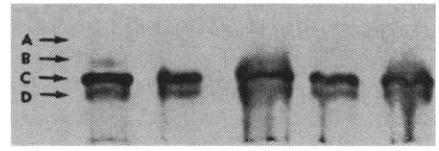

Fig. 2. Starch gel stained with guaiacol-H_2O_2, showing resolution of peroxidases. The bands correspond with the cathodic peroxidases of the first five columns beginning at the extreme left in Fig. 1.

The freshly excised young sections have seven visible peroxidase isozymes (A, C, D, E, F, G, and H, Fig. 1). Between 2 and 6 hours after the sections were removed from the plant, an additional actively migrating cationic peroxidase (band B) appeared in those sections incubated in basal medium alone. The intensity of this band increases progressively with time. The 12- and 18-hour-treated sections possess enough of this isozyme to be clearly photographed, and by 24 hours it is a major peroxidase band (Fig. 2). Sections that have been incubated in medium containing IAA show no signs of this band even after 24 hours. Tissue that has aged normally on the plant (left column, Figs. 1 and 2), shows detectable amounts of band B, whereas freshly cut sections from young sixth intermodes do not have this band.

Thus, isoperoxidase B appears to be a normal component of stem tissue developing *in situ*. Once this peroxidase has appeared in the sections, treatment with IAA directly before and during maceration and electrophoresis does not lead to its disappearance. Band A becomes more intensely staining within the first 2 hours, but is unaffected by IAA treatment.

Analysis of the medium in which the sections were incubated revealed that leakage of peroxidase from the tissue (*7*) was much greater with con-

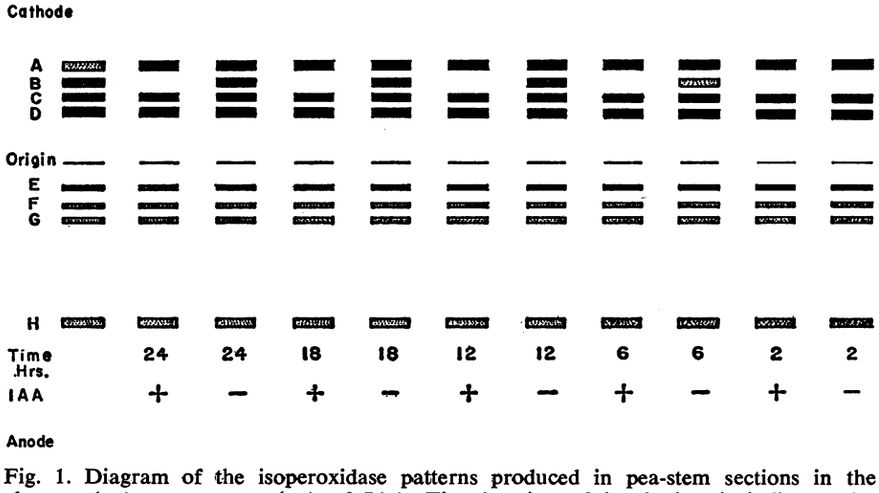

Fig. 1. Diagram of the isoperoxidase patterns produced in pea-stem sections in the absence (−) or presence (+) of IAA. The duration of incubation is indicated for each treatment. The degree of stippling approximates the staining intensity. The column at the extreme left refers to mature stem tissue homogenized directly after excision, without incubation.

trol than with IAA-treated sections. Also, there was no difference in the isozymal composition of the extracellular enzyme from control and IAA-treated tissue. Therefore, the differences in the tissue cannot be accounted for by differential leakage. Although high concentrations of IAA inhibit peroxidase action (*8*), the concentration used is too low to produce this effect.

Gibberellic acid (GA_3), which induces growth and whose effects on the peroxidase isozymes of plant tissue may be measured quantitatively (*9*), appears to act like IAA in this system, but much less effectively and rapidly. When added with IAA, it enhances the effect of the latter.

Therefore, it appears that peroxidase B develops as a consequence of aging, perhaps in response to a diminution in the supply of endogenous auxin. The application of exogenous IAA to stem sections prevents the depletion of growth hormone and concomitantly represses the formation of this specific peroxidase.

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Radium Isotope Accumulation in Animal Thyroids

Abstract. *Uranium and thorium daughters are ten times more concentrated in thyroids from some bovine animals than in the teeth of the same animals. These radioactive isotopes are believed to be from natural sources, but their resulting annual dosage of thyroid radiation has exceeded that from iodine-131 fallout.*

During continuous observation of animal thyroids for I^{131} from radioactive fallout, a small percentage of the glands contained long-lived radioactivity, which was interpreted to indicate the presence of radium (*1*). Within the past 2 years we have observed, in 28 of 275 bovine thyroids from Cali, Colombia, measurable quantities of gamma radiation at 0.24, 0.29, 0.35, and 0.61 Mev; these energies are the energies of radium daughters. The possibility that the radiation was due to surface contamination was disproved because we found a slightly greater concentration of radioactivity in the center of three glands than in their peripheries. Two of the thyroids were radioactive in 1961, and they showed no measurable loss of radioactivity by 1965. It may be relevant that 97 percent of 1700 supposedly unselected cattle thyroids sent from Colombia have been abnormally large, and the country has extensive areas of endemic goiter. We found radium daughters in only 8 of 750 cattle thyroids from the United States; however, the common presence of I^{131} in North American thyroids may have masked the presence of radium-daughter radiation in some samples. Teeth of 12 Colombian animals with the largest concentrations of radioactivity in their thyroids were also tested; the long-lived radioactivity per gram (wet weight) of thyroid was more than four to ten times greater than the radioactivity per gram (wet weight) of teeth. In four cases, however, the teeth from a Colombian animal contained more long-lived radioactivity than the thyroids, but in these animals both tissues contained minimally detectable quantities of radium.

Five typically radioactive thyroids were selected for detailed quantitative study to identify the long-lived radiation as originating from short-lived daughters of the uranium-radium and thorium series. Table 1 summarizes the results of the analyses.

Method A utilized coincidence-counting techniques (*2*) to identify and quantitate Ra^{226} and Th^{228}, while method B used gas-emanation techniques in which radon and thoron were separated and quantitated. In method A, the coincident 0.609 and 1.12-Mev gamma rays of Bi^{214} served to identify the Ra^{226} daughters. The samples, which had been preserved for more than 2 months, were sealed in plastic containers to prevent loss of Rn^{222} (half-life 3.82 days) and to allow the Bi^{214} daughter to build up to equilibrium with its parent Ra^{226}. Because the daughters of Ra^{226} (half-life 1620 years) which occur between its first daughter Rn^{222} (3.82 days) and Bi^{214} (19.7 minutes) are very short-lived, the measurement of Bi^{214} provided a precise measurement of the Ra^{226} in the preserved samples.

Studies of the uptake of natural radionuclides by plants in areas of high natural radioactivity have been interpreted to indicate that Ra^{226} and Ra^{228} are taken up directly from the soil (*3*). These isotopes do not appear to be produced in the plants in significant amounts by decay of their precursors; however, the major source of Th^{228} in plants appears to be from the decay of Ra^{228}, which is taken up directly. The uptake of these radionuclides by animals may be similar to that in plants. The Ra^{226} was probably concentrated by the thyroid as Ra^{226}. Even though Ra^{226} could be formed in the thyroid

Table 1. Radium-226 and Th^{228} in thyroid and teeth of cattle. Analyses were made by nondestructive coincidence gamma spectroscopy (A) and performed by Perkins (*2*), except on the sample for which the slaughter date was 14 November 1964: this sample was analyzed by radon and thoron emission (B), with the assay performed on the ashed tissue by A. T. Keane. The weights used as a basis for the figures were wet weights of trimmed tissues preserved with paraformaldehyde powder.

Slaughter date	In thyroid			In teeth	
	Ra^{226} (pc/g)	Th^{228} (pc/g)	Calculated dose (mrem/day)*	Ra^{226} (pc/g)	Th^{228} (pc/g)
United States					
14 Oct. 64	2.6	2.3	53		
28 Apr. 65	1.6	0.84	24		
Colombia					
26 May 65	2.0	3.0	61	0.17	0.32
26 May 65	1.8	2.9	58	0.09	0.37
14 Nov. 64	3.0	4.0	83		

* See reference *4*: μrad/day = 51 [(pc Ra^{226}/g) $\times$ (weighted average Mev/disintegration) + (pc Th^{228}/g) $\times$ (weighted average Mev/disintegration)] or mrem/day = $10^{-3} \times (10 \times 51)$ [(pc Ra^{226}/g) $\times$ (4.8 + 30 percent of 24.4) + (pc Th^{228}/g) $\times$ 31.9].