ABSENCE OF A PROTEINACEOUS INVERTASE-REGULATORY SYSTEM IN SUGARCANE STEM TISSUE[1]

Two concepts of plant invertase regulation are currently present in the literature: (a) an auxin-metabolite mediated-control system which regulates invertase synthesis, and (b) endogenous proteinaceous inhibitors which regulate invertase activity.[2, 3, 4, 5, 6, 7] Schwimmer et al.[8, 9] found evidence of an invertase inhibitor in crude extracts of potato tubers. Pressey[10, 11] confirmed the inhibitor's presence and achieved its separation, purification, and characterization. More recently, Pressey[12] isolated and partially purified invertase inhibitors from roots of red beet, sugarbeet, and sweet potato. Engard and Nakata,[13] and Van Overbeek et al.[14] found growth-inhibiting substances in sugarcane, but no attempts at purification or characterization were made. Maretzki and Alexander[15] found tacit evidence of endogenous inhibitors during gel filtration studies of acid invertase from sugarcane meristem tissue.

[1] Manuscript submitted to Editorial Board July 7, 1971.

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[7] Glasziou, K. T., and Waldron, J. C., Regulation of acid invertase levels in sugarcane stalks by auxin- and metabolite-mediated control systems, Nature 203: 541–42, 1964; Aust J. Biol. Sci. 17(3): 609–18, 1964.

[8] Schwimer, S., Makower, R. U., and Rorem, E. S., Invertase and invertase ininhibitor in potato, Plant Physiol., loc. cit.

[9] Rorem, E. S., and Schwimmer, S., Double pH optima of potato invertase, Experientia 19(3): 150–51, 1963.

[10] Pressey R., Separation and properties of potato invertase and invertase inhibitor, Arch. Biochem. Biophys., loc. cit.

[11] ____, Invertase inhibitor from potatoes: Purification, characterization, and reactivity with plant invertases, Plant Physiol., loc. cit.

[12] ____, Invertase inhibitors from red beet, sugar beet, and sweet potato roots, Plant Physiol., loc. cit.

[13] Engard, C. J., and Nakata, A. H., A growth inhibitor and a growth promotor in sugarcane, Science 105: 577–80, 1947.

[14] Van Overbeek, J., Dávila Olivo, G., and Santiago de Vázquez, E. M., A rapid extraction method for free auxin and its application in geotrophic reactions of bean seedlings and sugarcane nodes, Bot. Gaz. 106–440–51, 1945.

[15] Maretzki, A., and Alexander, A. G., Gel filtration studies of invertase from sugarcane meristem, Enzymologia 30: 299, 1967.

More recent studies attempted to establish the operation of a cane invertase regulatory system based on proteinaceous inhibitors of the type reported in potato tubers. Immature storage tissue from plants grown in sand culture was frozen, lyophilized, ground to pass a 60-mesh screen, and stored at −2° C., in accordance with the procedures described by Alexander.[16] Several isolation methods were attempted using clarified water extracts of the powdered tissue: (a) Use of a Waring blendor for the purpose of destroying either invertase or the inhibitor, following the method of Pressey;[17] (b) differential precipitation by the use of ammonium sulfate in increments of 10-percent saturation; and (c) gel filtration methods with variations in column and particle size, flow rates, and fraction volumes. Protein preparations were incubated with a standardized invertase-sucrose reaction digest, i.e., 5-percent sucrose, pH 5.5, for 1 hour. Invertase activity values corrected for the protein dilution factor were computed as specific activity (activity units per mg. of protein). Other activity estimates were based on total reducing sugar liberated from sucrose under standard conditions.

None of the initial experiments showed appreciable invertase repression by any of the protein preparations. Low levels of inhibition in the order of 30 percent were obtained with protein effluent fractions from Sephadex columns. Attempts to further purify the suspected factor(s) were performed with recombined effluent fractions, but the results were negative. Several modifications were made in the standard invertase assay to facilitate a potential inhibitor's action against the enzyme: (a) Preincubation of invertase with protein solution for 1 hour at 37° C.; (b) examination of reaction rates over shortened time courses (5, 10, 15, and 30 minutes); (c) increased protein volumes, from 0.5 to 2.0 ml.; and (d) further purification of inhibitor preparations on alumina and calcium phosphate gels. None of the treatments effected further inhibition of sugarcane invertase; that is, most of the repression could be traced to dilution by the protein volumes. In this connection, early workers found that dilution of enzyme preparations will produce activity losses of a magnitude greater than the diminution of catalytic protein.

Further study may be justified. Cane tissues were currently sampled during the late-morning hours when invertase activity is exceptionally

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high.[18] Greater amounts of inhibitor might be found in the late afternoon or evening. Moreover, the neutral (pH 7.0) invertase of mature stalk tissue might show a greater sensitivity to proteinaceous inhibitors than the acid invertase actually examined. However, present evidence is consistent with the view that the metabolite-auxin control system expounded by Glasziou and Waldron[19] constitutes the main control system for sugarcane acid invertase.

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