

This Week's Citation Classic

CC/NUMBER 17
APRIL 27, 1981

Ryan C A. Proteolytic enzymes and their inhibitors in plants. *Annu. Rev. Plant Physiol.* **24**:173-96, 1973. [Dept. Agricultural Chemistry, Washington State Univ., Pullman, WA]

This paper reviewed the status of the types, occurrence, and functions of proteinases and proteinase inhibitors in plant tissues with an emphasis on their physiological roles. [The SCI® indicates that this paper has been cited over 120 times since 1973.]

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March 27, 1981

"As suggested by ISI®, I thought about the background that prompted the writing of this review, and why I happened to do it. The basis for it goes back to my postdoctoral days in 1961 when my interests in proteinases and their inhibitors began.

"I was a postdoctoral fellow in the laboratory of A.K. Balls at the US Department of Agriculture's Western Regional Research Laboratory in Berkeley, California, studying the effects of various chemical modifications on the activity of chymotrypsin. One day, within only a month or so after I began to work with Balls, Floyd DeEds, the group leader of the pharmacy section, mentioned to us that he had read in an obscure midwestern research report that there was a compound in potato peels that inhibited cholinesterase. To Balls that was significant since it was a similar observation, that DFP inhibited cholinesterase, that contributed to the discovery by him and Gene Jansen several years before of the inactivation by

DFP of the active sites of trypsin and chymotrypsin.¹ So we decided to have a look at the possible inhibition of chymotrypsin by extracts of potato peel. I walked across the street to a little grocery store and bought a couple of potatoes. We carefully scraped off the outer skin, homogenized it in water, and assayed it for inhibition of chymotrypsin. Nothing! We wondered about the tissue underneath. I cut out a little chunk of cortical tissue, homogenized it, and tested it. It completely inhibited the chymotrypsin activity. Even after several dilutions, the extract still had potent inhibitory activity. Within a few months I was able to crystallize 'Chymotrypsin Inhibitor I' from the potato proteins, the first of several inhibitor types we have now identified in Russet Burbank potato tubers.

"By 1973 my program was at Washington State University and was deeply involved in studying the functions of proteinase inhibitors in plants. A considerable interest in the properties and functions of both the plant proteinases and proteinase inhibitors had developed among a number of plant scientists and someone on the editorial board of *Annual Review of Plant Physiology* thought the subject was important enough to review. I was simply fortunate enough to be asked to write about it. The review was the only comprehensive coverage of the field (actually two fields) for several years. The reasons for the many citations of this article appear to be the increased research activities concerning the roles of proteinases in plants during seed embryogenesis, germination, and growth; and the roles of proteinase inhibitors as antinutrients and as possible regulators of the plant proteinases. More recently, Richardson² and Birk³ have reviewed the plant proteinase inhibitor area and soon two new reviews on plant proteinases and plant proteinase inhibitors will appear.^{4,5}

1. Balls A K & Jansen E F. Stoichiometric inhibition of chymotrypsin. *Advan. Enzymol.* **13**:321-43, 1952.
2. Richardson M. The proteinase inhibitors of plants and micro-organisms. *Phytochemistry* **16**:159-69, 1977.
3. Birk Y. Inhibitors from plants. *Methods Enzymol.* **45**:695-739, 1976.
4. Walker-Simmons M W & Ryan C A. Plant proteinases. (Marcus A, ed.) *Proteins and nucleic acids*. New York: Academic Press. In press, 1981.
5. Ryan C A. Proteinase inhibitors. (Marcus A, ed.) *Proteins and nucleic acids*. New York: Academic Press. In press, 1981.