
1 Introduction

Protein protease inhibitors have been found in numerous animal tissues and fluids, in many plant tissues – particularly in legume seeds and other storage organs – and in micro-organisms. Their existence in nature was first reported at the end of the nineteenth century when Fermi and Pernossi (1894) noted “anti-trypsin activity” in serum. However, the isolation and characterization of protein protease inhibitors, as well as the introduction of many of the fundamental concepts associated with protease – inhibitor interactions, are marked by the pioneering work of M. Kunitz during the 1930s and 1940s (Kunitz and Northrop 1936; Kunitz 1945, 1946, 1947a, b; summarized in Birk 1987, 1989). Since then, the inhibitors have been the object of research in many disciplines. Inhibitor proteins have been studied as model systems for elucidation of the mechanism of inhibition of proteases, as well as for studies of protein–protein association. They attracted the attention of nutritionists due to their presence in valuable plant foods and their subsequent, possible, involvement in nutritive properties. Findings on the involvement of plant protease inhibitors in prevention of tumorigenesis may contribute to the nutritional utilization of valuable plant protein sources, such as legume seeds. The inhibitors are also being used as valuable tools in medical research because of unique pharmacological properties that suggest clinical application.

During the past decades, increasing attention has been given to protein protease inhibitors. The driving force behind

this current interest is the awareness that proteases exercise many control functions in the organism. Proteolytic processes are involved in the processing of protein precursors and polypeptides, which are biologically and pharmacologically active, such as proenzymes, hormones and kinins. The proteolytic processes are also involved in the mobilization of tissue proteins, in the regulation of intracellular protein metabolism and in neuropeptide processing and neural functions. Protease inhibitors, in their turn, control the proteases and may be essential for physiological regulation. Publications dealing with the biological, chemical and physical properties of the inhibitors, their roles and significance, continue to appear in ever-increasing numbers. Although their overall physiological function is in the prevention of unwanted proteolysis, many facets of the subject are still unexplained and they may still be classified as naturally occurring, biologically active proteins in search for functions.

The structural basis of the interaction between endoproteinases and protein protease inhibitors has been recently presented in a comprehensive review by Bode and Huber (2000): "Most of the inhibitors for which three-dimensional structures are available are directed towards serine proteinases, interacting with the active sites in a 'canonical', i.e. substrate-like manner via an exposed reactive site loop of conserved conformation. More recently, some non-canonically binding serine proteinase inhibitors directed against coagulation factors, in particular thrombin, a few cysteine proteinase inhibitors and zinc endopeptidase inhibitors have been characterized in the free and complexed state, displaying novel mechanisms of inhibition with their target proteinases. These different interaction modes are presented and briefly discussed with respect to the different strategies applied by nature."

The plant protease inhibitors are diverse in number and in specificity toward various proteolytic enzymes. Several different kinds of inhibitors can be present in a single tissue, as exemplified in soybeans, barley grains, and potato tubers. The

multiplicity of plant protease inhibitors may partly be ascribed to the self- and mixed-association of a few monomers in each plant, as well as to partial proteolysis of the inhibitors during purification, especially when the inhibitors are purified by affinity chromatography. The frequent presence of several protease inhibitors in the same source tissue, and the finding that the same inhibitor often inhibits more than one enzyme, are partly responsible for the difficulty in establishing their nomenclature.

The knowledge of the distribution of protein protease inhibitors of plant origin reveals hundreds of inhibitors dispersed among different botanical families (summarized and reviewed, Liener and Kakade 1980). The molecular weights of plant protease inhibitors are mainly in the range of 3,000–25,000 Da. Many of them have been isolated in the pure form and characterized with respect to amino acid composition, partial or full amino acid sequence, chemistry of the reactive sites, three-dimensional structure and the nature of the complexes formed with the respective proteases. Some of the inhibitors are multiheaded as a consequence of gene elongation via gene duplication or multiplication.

The physiological significance of plant protease inhibitors has been questioned for a long time. Only few inhibitors are known to inhibit the endogenous proteases of seeds. The fact that certain seeds, such as soybeans and wheat grains, contain inhibitors of growth and of larval gut proteases of several stored-product insects, suggest the possibility that these inhibitors may have evolved as a defense mechanism against predatory insects. Although the most frequent source of the inhibitors is the seed, an “immune response” of the plant to the attack of insects has been established for leaves. The extensive studies of C.A. Rayn and associates revealed the expression and rapid accumulation in potato and tomato leaves of protein protease inhibitors in response to a systemic signal. The signal – protease inhibitor-inducing factor (PIIF) – appears in increased levels as a direct result of the wounding of plant leaves, either mechanically or following attack

by insects. The latter, in turn, become starved as a result of the inhibition of their own digestive proteases by the ingested plant protease inhibitors (reviewed Ryan and Jagendorf 1995).