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Proteolytic Enzymes from the Latex of *Ficus pumila* L. *(Moraceae)*

Mario PERELLÓ, María Cecilia ARRIBÉRE, Néstor O. CAFFINI & Nora S. PRIOLO *

Laboratorio de Investigación de Proteínas Vegetales (LIPROVE), Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CC 711, 1900 La Plata, Argentina.

SUMMARY. The presence of proteolytic activity was detected in fruits of *Ficus pumila* L. *(Ficus repens* Hort.). The crude extract was obtained by clarification of the latex through centrifugation at 16,000 x g for 30 min. Subsequently the supernatant was ultracentrifuged at 100,000 x g for one hour. The crude enzyme preparation showed high proteolytic activity on casein in the presence of 12 mM cysteine, but the activity was inhibited by thiol-specific inhibitors like $HgCl_2$ and E-64, suggesting the proteases belong to the cysteine family. This crude enzyme extract showed maximum caseinolytic activity within an alkaline range of pH (7.0-9.0) and a remarkable thermal stability. The purification was carried out by a simple procedure involving ultracentrifugation, acetone precipitation and cationic exchange chromatography. The main fraction obtained was partially characterized: its optimum pH range is 7.0-9.0, is very stable at high temperatures, showed a Mr = 28.6 kDa (SDS-PAGE) and an isoelectric point higher than 9.3.

RESUMEN. "Enzimas proteolíticas presentes en el látex de *Ficus pumila* L. *(Moraceae).* Se detectó la presencia de actividad proteolítica en frutos de *Ficus pumila* L. *(Ficus repens* Hort.). El extracto crudo fue obtenido por clarificación del látex centrifugado a 16.000 x g durante 30 min y ultracentifugando luego el sobrenadante a 100.000 x g durante una hora. La preparación enzimática cruda mostró elevada actividad proteolítica sobre caseína en presencia de cisteína 12 mM, pero la actividad fue inhibida por inhibidores tiol-específicos tales como $HgCl_2$ y E-64, sugiriendo que las proteasas pertenecen a la familia de las peptidasas cisteínicas. El extracto crudo mostró un rango de pH óptimo en la zona alcalina (pH 7,0-9,0) y una notable estabilidad térmica. La purificación fue llevada a cabo por un simple procedimiento que incluye ultracentrifugación, precipitación acetónica y cromatografía de intercambio iónico. La principal fracción proteolítica ha sido parcialmente caracterizada: su pH óptimo es igual al de la preparación cruda, es termoestable, su Mr (SDS-PAGE) es de 28,6 kDa y tiene un pI mayor que 9,3.

INTRODUCTION

Proteases play a prominent role in plant physiology, being the catalysts of important processes like hydrolysis of storage proteins during seed germination, activation of proenzymes, degradation of defective proteins, etc. [1], but the presence of high concentration of proteolytic enzymes in some tissues is more difficult to explain [2].

Many plants exude a latex containing a high amount of digestive enzymes, mainly cysteine and serine proteinases [2]. It has been known for many years that the milky latex flowing from cuts of the stem, leaves and unripe fruits of several species belonging to the genus *Ficus* contains proteolytic enzymes. The name ficin was coined by Robbins [3] for the purified white powder with antihelminthic activity obtained from any member of the genus. A crystalline preparation from an unnamed species was obtained by Walti [4] and also named ficin. There is more than 1,300 species of *Ficus*, many of which show proteolytic activity [5], sometimes due to the presence of more than one proteinase [6]. The term ficin must therefore be regarded as generic. In 1992 the International Union of Biochemistry

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* Author to whom correspondence should be addressed. *E-mail:* priolo@biol.unlp.edu.ar