

AaCI, a Cysteine Proteinase Inhibitor from Brazilian Pine (*Araucaria angustifolia*)
Seeds

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Proteinaceous inhibitors of cysteine proteinases of papain family, phytocystatins are involved in the regulation of activity of endogenous cysteine proteinases, during seed development, and due to their ability to inhibit exogenous proteinases such as those present in the digestive tracts of insects, also play a defence role. Despite their wide distribution in Angiosperms, inhibitors of cysteine proteinases have not been characterized from Gymnosperms. A cysteine proteinase inhibitor (AaCI) was purified from the seeds of the native Brazilian pine *Araucaria angustifolia* (Bert.) O. Kuntze (Araucariaceae: Gymnospermae) by ion exchange chromatography followed by Superdex 30 gel filtration chromatography and reversed phase HPLC on a C-18 column. SDS-PAGE showed a single protein band of approximately 20 kDa while under reducing conditions two bands were observed, indicating that AaCI consists of two polypeptide chains. Besides papain ($K_i = 0,35$ nM), the inhibitor acts on cruzain and cathepsin L but not on cathepsin B. AaCI was stable over a wide range of pH and temperature. Following incubation at 100°C, the inhibitor lost 50% of their activity within 2 hours. Its stability was confirmed by CD spectroscopy since no modifications of secondary structure were observed in the pH range 2 to 10. The partial loss of its structure was observed by treatment at 100°C. The cluster analysis showed that AaCI belongs to the $\alpha+\beta$ class of proteins whose a larger intensity band is at 208 nm, not at 222 nm. Support: FAPESP, FADA/ FAP, CAPES, MCT/CNPq