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Insight into the functional and structural transition of garlic phytocystatin induced by urea and guanidine hydrochloride: A comparative biophysical study

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Abstract

Cysteine proteinase inhibitors play an essential role in maintaining the proper functioning of all living cell by virtue of its thiol protease regulatory properties. Chemical denaturation of a new variant of cystatin super family has been studied by various biophysical techniques in order to characterize the unfolded and denatured state. Denaturation of garlic phytocystatin has been investigated using urea and guanidine hydrochloride (GdnHCl). Different biophysical techniques such as intrinsic fluorescence, circular dichroism and FTIR exhibited an altered structure of garlic phytocystatin with increasing concentration of denaturant. The inhibitory activity of GPC decreases with increasing concentration of denaturant. Increased fluorescence intensity along with red shift reflects the unfolding of GPC at higher concentration of denaturant. GdnHCl induced unfolding showed presence of indiscernible intermediate as followed by ANS binding studies. However, denaturation by urea and 2.32±0.1 M GdnHCl. Circular dichroism and FTIR results indicate the 50% loss of secondary structure at 5 M urea and 2.5 M GdnHCl. This study provides intriguing insight into the possible alteration of structure, stability and function of GPC induced by urea and GdnHCl.

Keywords: Cystatin; protein unfolding; circular dichroism

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