

INTERMEDIATE SPECIES ARE PRESENT IN THE UNFOLDING PATHWAY OF CRAMOLL 1, THE MAJOR ISOLECTIN FROM *CRATYLIA MOLLIS* SEEDS

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Cramoll 1 is the major isolectin isolated from *Cratylia mollis* seeds. In this study, fluorescence spectroscopy and circular dichroism (CD) were used to obtain new insights about Cramoll 1 unfolding process induced by urea and/or High Hydrostatic Pressure (HHP). At pH 7.0 and 8M urea, the tryptophan fluorescence spectra shifted completely to the red suggesting its full exposition, the protein underwent denaturation. The secondary structure of the protein, as measured by the CD spectra, also changed upon urea addition. On the other hand, an enhanced capacity to bind bis-ANS was observed around 3M urea, suggesting that the Cramoll 1 unfolding process occurs with the accumulation of an intermediate species, called I_{3M} . At 37 or 1°C under 3.1 kbar, there was almost no change in the tryptophan spectra indicating that the protein is pressure-resistant. After addition of 3M urea to the buffer, the tryptophan were already totally exposed the protein presented an enhanced binding to bis-ANS, suggesting that Cramoll 1 is not completely unfolded under HHP, presenting another intermediate species (I_P) which is different from the I_{3M} , since the later species maintains its tryptophan in the native environment. These data suggest that the unfolding pathway of Cramoll 1 can be summarized as: $N \rightarrow I_{3M} \rightarrow I_P \rightarrow U$.
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