An investigation of cysteine proteinases and substrate interactions in the presence of increasing salt concentrations

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The effects of increasing ionic strength on the kinetic factors, Km and Vmax, for the cysteine proteinases: cathepsin L (from sheep liver), cathepsin B (from rabbit liver) and fruit bromelain were analysed. Cathepsin L showed decreasing Vmax and increasing Km with increasing salt concentration: surprisingly, this is consistent with the disruption of an ionic bond between substrate and enzyme. In turn, this suggests that the P1 site might play a predominant role in binding the substrate, Z-Phe-Arg-NHMec, by an ionic mechanism, although the P2 site Phe is required for specificity. Cathepsin B, assayed with Z-Arg-Arg-NHMec, showed decreasing Vmax and Km values with increasing ionic strength, consistent with an increased affinity for the substrate but a decreased turnover. Both Phe and Arg are accepted in the P2 position but there is a 7-fold preference for the hydrophobic Phe group. With Phe in the P2 position (i.e. using Z-Phe-Arg-NHMec), increasing the ionic strength resulted in increasing activity, which can be attributed largely to the decrease in Km (increasing affinity between enzyme and substrate), which is consistent with a hydrophobic interaction between E and S. Turnover decreased with increasing ionic strength but the ratio of Vmax/Km increased. Bromelain showed an increasing activity with increasing salt concentration, when assayed with Z-Phe-Arg-NHMec. Affinity for substrate increased with ionic strength. The increase in activity is limited by decreasing turnover above a certain salt concentration. Overall, the results suggest that ionic strength effects are specific for particular enzymes within a related group.