HUMAN CATHEPSIN X
A novel cysteine protease with unique specificity

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1. INTRODUCTION

Cysteine proteases constitute attractive targets for development of inhibitors as potential therapeutic agents due to their involvement in several pathological conditions. As is the case with many other gene families, the mining of databases from genome-sequencing projects has lead to the discovery of several new cysteine proteases of the papain family e.g. cathepsin V (Adachi et al. 1998, Santamaría et al. 1998a), cathepsin X (Nägler and Ménard 1998, Santamaría et al. 1998b) and cathepsin F (Wang et al. 1998, Nägler et al. 1999) have all been cloned in the last two years. Although restricted expression patterns might exist, the increasing number of cysteine proteases is likely to render more challenging the task of designing inhibitors specific for a given enzyme. This is particularly important considering that the information available to date indicates relatively broad, overlapping specificities for these enzymes (Storer and Ménard 1996).

The primary structure of cathepsin X contains several unique features that clearly distinguish it from other human cysteine proteases. One of these distinctive features is a three amino acid residue insertion in a highly conserved region between the glutamine (Gln22) of the putative oxyanion hole and the active site cysteine (Cys31). This highly unusual insertion for a papain-like cysteine protease might confer special properties to the enzyme.
We have used molecular modeling to gain insights into the possible functional consequences of this insertion observed in the primary sequence, and to assist in the design of a good substrate for this protease. Kinetic characterization of substrate hydrolysis has been used to describe the specificity profile of this novel enzyme. The combination of functional characterization with structural model building has enabled us to propose the fine molecular details defining the specificity of cathepsin X.

2. EXPERIMENTAL

2.1 Materials

The vector (pPIC9) and *Pichia pastoris* strain GS115 were purchased from Invitrogen Corporation (San Diego, CA). The substrate Cbz-FR-MCA was purchased from IAF Biochem International Inc. (Laval, Qc). The substrates Abz-FRF(4NO₂) and Abz-FRF(4NO₂)A were from Enzyme Systems Products (Livermore, CA), and Abz-AFRSAQQ-EDDnp was obtained from Luiz Juliano (São Paolo, Brazil). Human cathepsins B and L were prepared as described previously (Nägler et al. 1997, Carmona et al. 1996). Human cystatin C was prepared as described earlier (Ekiel et al. 1997).

2.2 Expression and Purification of Recombinant Cathepsin X

The human cathepsin X cDNA was cloned into the vector pPIC9, sequenced and expressed in the yeast *Pichia pastoris* as a prepro-α-factor fusion construct using the culture conditions recommended by Invitrogen. Procathepsin X was expressed and secreted at levels of approximately 5 mg/L of initial culture medium. After dialfiltration against sodium acetate pH 5.0, and purification on a CM-sephadex column, mature cathepsin X was obtained by incubating the partially purified procathepsin X with a small amount of human cathepsin L. The sample was then dialyzed against phosphate buffer pH 7.0, and further purified on a DEAE column. Cathepsin X was stored at 4 °C in the elution buffer containing 100 μM of MMTS.

2.3 Kinetic Measurements

Kinetic experiments were performed as previously described (Nägler et al. 1997, Ménard et al. 1990). Inhibition studies with cystatin C have been