Abstract

Cysteine cathepsins participate in many pathological processes such as cancer and neurodegenerative, cardiovascular, or autoimmune diseases. This work is focused on cathepsins B, L, and V (CatB, CatL, CatV), which represent attractive targets for the development of inhibitors as potential chemotherapeutics and diagnostic tools. The aim of this study was to prepare these cathepsins and structurally characterize their complexes with selected synthetic peptidomimetic inhibitors. Recombinant CatB and CatL were prepared in the yeast *Pichia pastoris,* and the expression conditions were optimized for the production of cathepsin zymogens. A chromatographic purification protocol was designed for CatB and CatL, while CatV was purified using a previously developed procedure. The obtained enzymes were used to prepare complexes with six peptidomimetic inhibitors equipped with a carbamate, vinyl sulfone, or azanitrile warhead, which selectively target CatB, CatL, and CatV, respectively. Their inhibition parameters were determined in a kinetic assay and initial crystallization conditions were identified. After optimizing the crystallization conditions for CatB with three carbamate inhibitors, crystals suitable for X-ray crystallography were obtained. Based on the crystal structures of these complexes, the binding mode of carbamate inhibitors in the active site of CatB and their unique inhibition mechanism were analysed. It revealed that the covalent reaction with the catalytic cysteine is accompanied by cleaving off a part of the inhibitor. The obtained results will facilitate rational design of a new generation of CatB inhibitors with potential use in biomedicine.

Keywords: cysteine cathepsins, recombinant expression, peptidomimetic inhibitors, protein crystallization, 3D protein structure, inhibition mechanism