PURIFICATION AND CHARACTERIZATION OF A FISH GRANZYME A INVOLVED IN CELL-MEDIATED IMMUNITY

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ABSTRACT

Cell-mediated immunity is crucial immune response against non-self antigen such as allograft, tumor and virus infected cells. Non-self antigens could be eliminated by effector cells (e.g. cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells) by secretory and/or non-secretory cell-death pathways in mammal. Cytotoxic effector cells in fish have also been characterized in lymphocyte subsets level; however, biochemical mechanisms of the killing remain unknown. Here we tried to identify the protease involved in the killing adopting ginbuna crucian carp which has been used as a nice model for the study on cell-mediated immunity in fish.

In order to identify the protease involved in the killing of allogeneic target cells, we performed cytotoxicity assays in the presence of serine or cysteine protease inhibitors using leukocytes of ginbuna crucian carp. Cytotoxicity was significantly inhibited by serine protease inhibitor ‘‘3,4-dichloroisocoumarin’’ (DCI). Then, we tried to explore a substrate of the serine protease involved in killing activity. We found that granzymeA-like activity was inhibited by ‘‘DCI’’ and significantly enhanced by allo-antigen stimulation in vivo. These results suggest that granzymeA-like protease was involved in killing against allogeneic cells in ginbuna.

We tried to purify the granzymeA-like protease for enzymatic characterization. Purification was sequentially performed on two step of benzamidine-sepharose and SP-sepharose chromatography. The purified enzyme was visualized as a single protein band and a molecular mass was estimated to be approximately 27,000 Da by using SDS-PAGE. The protease was totally inhibited by serine protease inhibitors and showed granzymeA-like substrate specificity. Therefore, we conclude that the purified enzyme belongs to mammalian granzymeA (EC 3.4.21.78).

We succeeded to characterize the granzymeA in fish involved in the killing against allogeneic cells. Now we initiate proceeding to examine a function and to find endogenous substrate of the protease with the purified protease.

KEYWORDS

Granzyme; Serine protease; Purification; Cytotoxicity; Cell-mediated immunity; ginbuna crucian carp

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