Purification and characterization of zebrafish proteasomes

（Doctoral qualification by coursework, Form 7)

Abstract of Dissertation

Department：Bioscience　　Name：ALI MD HASAN

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Abstract：The proteasome is a sophisticated large non-lysosomal, polymeric and multi-catalytic protease complex which is responsible for the hydrolysis of client proteins. In this study, the 20S proteasome was purified and identified from cytosol fractions (150,000 g supernatant) from zebrafish (whole body) using five steps of column chromatography such as DEAE-cellulose, Q-Sepharose, Sephacryl S-300 gel, Hydroxylapatite and Phenyl-Sepharose.The native protein complex of zebrafish 20S proteasome was purified and identified its 14 subunits. The cytosol fractions were possessed peptide hydrolyzing activity used a well-known substrate Suc-LLVY-MCA for 20S proteasome. The highest hydrolyzing activity of 20S proteasome at 0.04% SDS and each step of purifications, proteasome activity was examined by using this protease assay. The zebrafish 20S proteasome subunits were appeared molecular mass ranging from 21 to 33 kDa in electrophoresis and western blot analyses results. In 2-dementional gel electrophoresis, 20S subunits were identified by TOF-MS analysis according to 2D-PAGE separation. In case of zebrafish 20S proteasome, the unique character that encoding gene is a4 subunit and psma7 is absence in genome. a8 subunit was encoded by psma8 gene which was identified in zebrafish. Also, it is suggested that a8 subunit is in 20S complex instead of a4 subunit. As special character of zebrafish, two proteins of a1 subunit was identified and mention their named as subunit a1a and a1b. For the protein modifications some spots were identified as same subunit (a2, a3, a6 and b4). Another interesting and special finding was found in this study, a protein was appeared with proteasome subunit during the purification of five steps of column chromatography. The appeared protein molecular weight around 35 kDa. It seems like made a complex but there was no any report such kind complex. This protein band was identified by TOF-MS analysis. In case of 2-dementional gelelectrophoresis, indicated

this protein spot by asterisk and tropomyosin protein was confirmed by MALADI-TOF/MS analysis. The p35 protein was appeared in 35 kDa by reversed phase FPLC on Superose-6 gel column and silver staining (Ag.). All of experiments provided the positive result to be supported that p35 protein seems like made complex with 20S proteasome but in case of immunoprecipitant analysis did not get positive promising result. So, need to be further analysis about p35 then conform p35 really make complex with 20S proteasome or not

Finally, concluded that purification and identification of zebrafish 20S proteasome in this study provide the basis for detailed analysis