S-Glutathiolation Analysis of Yeast 20S Proteasome by Two-Dimensional Electrophoresis

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The so called 20S proteasome is a multicatalytic protease responsible for the breakdown of intracellular proteins. The complex consists of a barrel-shaped core comprised of two inner heptameric β-rings harboring the active sites, whereas the two outer heptameric α-rings play a structural role controlling the substrate access to the active sites. We have previously described *in vitro* and *in vivo* S-glutathiolation of 20S proteasome from yeast *Saccharomyces cerevisiae* and the consequent modulation of its proteolytic activities. The main goal of present work was to identify among the 14 subunits of the 20S proteasome, the S-glutathiolated subunits. To accomplish this goal we first isolated the proteasomal subunits by two-dimensional electrophoresis (2-DE) followed by mass spectrometry fingerprinting. The first dimension was carried out on gel strips at a 3 - 10 and 4 - 7 pH gradients and the second dimension was performed in 12.5% SDS-PAGE. Next, we performed the trypsin in-gel digestion of the spots and the tryptic peptides were analyzed in a MALDI-TOF mass spectrometer in order to generate a reliable protein profile. Afterwards we detected the S-glutathiolated subunits by immunoblotting the 2-DE samples using monoclonal antibodies against glutathione. In this study we identified both α and β S-glutathiolated subunits that might be either modulating the entrance of substrate to the catalytic chamber or modifying the proteasomal active sites.