Volume 47(1-4):85, 2003
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

DISSERTATION SUMMARY

Structural and fuctional study of the Drosophila *melanogaster* 19S regulatory complex

Máté Sümegi

Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

In addition to transcription and translation, regulated degradation plays also an important role in the determination of the halflife of proteins. In eukariotic organisms some of the cytosolic regulator proteins are degraded by the ubiquitin-proteosome pathway. This pathway consists of the ubiquitin ligase system and the 26S proteasome. The components of the ubiquitin ligase system are the ubiqutin polypeptide (Ub), the ubiqitin activatig enzyme (E1), the ubiquitin conjugating enzymes (E2) and the ubiquitin ligase enzymes (E3). This system attaches a multiubiquitin chain covalently to the proteins before degradation. The 26S proteasome is a large multiprotein complex made of two different subcomplexes called 20S core particulum and 19S regulatory complex.The 20S core particulum is a barrelshaped ATP independent aspecific protease. The main role of the 19S regulatory complex is to ensure specificity to the aspecific 20S core protease complex towards multiubiquitinated proteins. The 19S regulatory complex can recognize the substrate proteins multiubiqitinated by the ubiquitin ligase system, unfold and feed them into the 20S core particulum's central cave, where the hydrolysis occurs.

Comparing the immunoblots of one dimensional denaturing polyacrylamide gel electrophoresis (1D-SDS-PAGE) with the immonoblots of the isoelectric focusing-denaturating polyacrylamide gel electrophoresis (2D-IEF-SDS-PAGE), we noticed that some of the antibodies recognising a distinct band on the 1D-SDS-PAGE blots, did not recognise any spot on the 2D-IEF-SDS-PAGE blots. This finding suggested that the former determinations of the 19S regulatory complex subunit composition based on 2D-IEF-SDS-PAGE systems were incomplete. To fully complement this subunit composition we adopted a principaly different gel electrophoresis system called two dimensional 16-BAC- SDS denaturating polyacrylamide gel electrophoresis (2D-16BAC-SDS-PAGE), which reveals all the subunits of the 19S regulatory complex (Hölzl et al. 2000). Using immunoblotting and MALDI aminoacid sequencing, we determined the full subunit composition of the 19S regulatory complex.

We investigated the structural heterogeneity of the 19S regulatory complex by crosslinking experiments (Kurucz et al. 2002). Some of our antibodies, that recognise the same subunit, give different crosslinking pattern, suggesting that the regulatory complex may contain these subunits in two different steric conformations. Such a conformational heterogeneity may be the consequence of posttranslational modification.Therefore we investigated the posttranslational modifications of the 26S proteosome subunits with wheat germ agglutinin (WGA) and monoclonal antibodies specific for N-acetylglucosamine modification(MA-072,MA-076). We managed to show that some subunits of the 20S core particulum and the 19S regulatory complex are O-glycosilated.

For fuctional experiments we are setting up an in vitro ubiquitination-protein degradation assay. We want to combine chromatographically purified enzymes (E1) with recombinant proteins (E2, Vihar), and imunoprecipitated enyimes (E3,APC) preactivated with recombinant protein factors (Fizzy, Fizzzrelated). Using chromatographycally purified active 26S proteosome we would like to investigate in detail the multiubiquitination and the degradation steps of the ubiqutin-proteasome pathway. We have already produced most of the componets of this system, and now we are working on the activation of the E3 APC component.

References

Holzl H, Kapelari B, Kellermann J, Seemuller E, Sumegi M, Udvardy A, Medalia O, Sperling J, Muller SA, Engel A, Baumeister W (2000) The regulatory complex of Drosophila melanogaster 26S proteasomes. Subunit composition and localization of a deubiquitylating enzyme. J Cell Biol 150:119-30.

Kurucz E, Ando I, Sumegi M, Holzl H, Kapelari B, Baumeister W, Udvardy A (2002) Assembly of the Drosophila 26 S proteasome is accompanied by extensive subunit rearrangements. Biochem J 365:527-36.

Supervisor: Andor Udvardy
E-mail: sumegi@nucleus.szbk.u-szeged.hu