Modulation of the 20S proteasome activity by the interplay between ATP and Mg2+ ions.

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A. Morozov[I], T. Astakhova[II], A. Burov[I], P. Erokhov[II], V. Karpov[I]

[I]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia, [II]Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia

The proteasomes represent elements of the ubiquitin-proteasome system (UPS) directly responsible for the degradation of cellular proteins. There are several mechanisms that regulate activity of proteasomes including association with regulators, cofactors and via posttranslational modifications of proteasome subunits. Interestingly, the activity of proteasomes was shown to be influenced by divalent ions Ca2+ and Mg2+. Magnesium forms a key complex with intracellular ATP and most intracellular ATP is bound to Mg2+, however in certain conditions including stress, when Mg2+ concentrations are decreased the amount of free ATP may rise. Herein, using purified preparations of 20S constitutive and immunoproteasomes and a set of short fluorogenic proteasome substrates we demonstrated that chymotrypsin and caspase-like proteasome activities were gradually decreased from 90 to 50% with the rise of free ATP concentration from 0,25 to 10 mM. In contrast Mg2+ concentrations in range from 0,5 mM to 20 mM dose dependently increased the chymotrypsin-like activity of purified 20S proteasomes from 17% to 67% correspondingly. Concordantly, when proteasomes were incubated with combinations of ATP and Mg2+ the chymotrypsin-like activity was directly proportional to the molecule ratio, being equal to control values when identical concentrations of Mg2+ and ATP were used. At the same time, when casein labeled with Cy5, or Rhodamine was used as a proteasome substrate instead of short peptides minimal effect of free ATP on the substrate turnover was demonstrated.
Taken together, obtained results indicate that free ATP and Mg2+ differently influence the degradation of different substrates likely relevant to their size and charge. Furthermore, proteasome regulatory mechanism based on the balance between free ATP and Mg2+may exist in cells.
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