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۵-۶ دی ماه ۱۴۰۳، دانشگاه هرمزگان

Kinetics of Caspase7 Thermal Inactivation

Fatemeh Zaarea*, Jamshid Davoodi

Institute of Biochemistry and Biophysics (IBB), University of Tehran, Tehran, Iran

Abstract

Caspase 7 is a cysteine protease that induces programmed cell death in the internal pathway of apoptosis. The active enzyme consists of a homodimer of a hetero dimer. Thus, we sought to determine the mechanism of inactivation, i.e. aggregation versus the loss of the quaternary structure. For this purpose, recombinant caspase7 protein was expressed and purified by affinity chromatography method. The purified enzyme was subjected to elevated temperatures for a period of 0 to 16 hours. Then the enzymatic activities of aliquates withdrawn at certain time points were measured by the hydrolysis of the chromogenic substrate DEVD-pNA monitored at 405 nm. The enzyme was completely inactive following 10 hours incubation at 37 degrees. SDS-PAGE analysis of the samples revealed no hydrolysis of the proteins due to self cleavage or by proteases that might be present as minor impurities. Analysis of the kinetics of enzyme inactivation by fitting to 1st and 2nd degree equations showed that that enzyme inactivation follows the 1st degree equation. This indicates that either the hetero dimer is being dissociated or homodimer is falling apart leading to loss of activity.

Key words: Caspase7, Inactivation, Denaturation

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References

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- [1] Dicleli M, Bruneau M. Seismic performance of single-span simply supported and continuous slab-on-girder steel highway bridges. Journal of Structural Engineering, ASCE; 121(10): 1497-1506, 1995.
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