Seminario

Università degli Studi di Padova Dipartimento di Scienze Chimiche

FRONTIERS IN CHEMISTRY Seminar Series

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Spontaneous Post-Translational Succinimide Formation in Proteins: The Case of a Stable Aminosuccinyl Residue in a Hyperthermophilic Archaeal Glutaminase

> Friday June 20th, 2025, 10:30AM ROOM A - NASINI

Department of Chemical Sciences, via Marzolo 1, I-35131 Padova

Asn-Xxx sequences in proteins often undergo deamidation. with formation of spontaneous а succinimide (SNN) intermediate, which undergoes epimerisation and subsequent hydrolysis to introduce L and D-aspartyl residues along with L and D- β -aspartyl residues into proteins. This presentation summarises work on the characterisation of an unusually stable aminosuccinyl residue in the enzyme glutamine amidotransferase (GATase) the archaeal from hyperthermophile Methanocaldococcus jannaschii.



Electrospray ionisation mass spectrometry permits establishment of the succinimide at Asn 109, with participation of the backbone NH group of D110. Crystal structures confirm the presence of the succinimide ring. Surprisingly, SNN formation enhances the thermostability of the protein which does not unfold even at 100°C. Site-specific mutagenesis suggests a key role for the sidechain of D110 in stabilising the succinimide against hydrolysis. Molecular dynamics simulations support local loop conformational features as a potential source of stabilisation. Site specific mutagenesis is further used to probe the nature of the residues that catalyse succinimide formation, establishing a key role for residues Y158 and D110 in facilitating this spontaneous post-translational modification.

¹ Kumar S., Prakash S., Gupta K., Dongre A., Balaram P. and Balaram H. Unexpected functional implication of a stable succinimide in the structural stability of Methanocaldococcus jannaschii glutaminase. *Nat. Commun.* **2016**, *7*, 12798.



2 Dongre A. V., Das S., Bellur A., Kumar S., Chandrashekarmath A., Karmakar T., Balaram P., Balasubramanian S. and Balaram H. Structural basis for the hyperthermostability of an archaeal enzyme induced by succinimide formation. Biophys. J. **2021**, *120*, 3732–3746.

Your presence will be very much appreciated

Prof. Stefano Mammi Head, Department of Chemical Sciences



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For selected references, see: