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Landscape of the ATP binding to neurotrophins: effects on conformation and dynamic modulation by divalent cations

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Neurotrophic factors are involved in the maintenance and growth of neuronal populations. The first discovered member of neurotrophin (NT) family, Nerve Growth Factor (NGF), is essential for the development and maintenance of neurons and has a crucial role in activation of immune and endocrine systems and in the pain pathway. Mature NGF is expressed as precursor, proNGF, whose pro-peptide is an intrinsically unstructured domain (IUD). NGF and proNGF show different biological properties, the latter being involved in neuronal apoptosis. Additionally, the relative ratio of the two proteins is linked to neurodegeneration.
The signaling pathway triggered by NGF involves the binding to TrkA and p75NTR receptors. Besides these receptors, proNGF also binds to sortilin receptor. Much is known about NGF's role in neuronal physiology. However few reports have explored the role of essential endogenous ligands as modulators of NGF biological activity. Recent reports described the binding of ATP to NGF. The formed complex was proven protective versus death in neural cells. Various cellular studies investigated the ATP neurotrophic role in synergy with NGF and highlighted a crosstalk between NGF and ATP signaling systems.
To fill the gap of understanding about the structural and mechanistic determinants of this binding, we used integrative structural biology to unveil for the first time the binding cartography of ATP to recombinant human NGF (rhNGF) [1]. Isothermal Titration Calorimetry (ITC) allowed us to measure a millimolar binding constant for ATP to rhNGF, and this was supported by 1H Saturation Transfer Difference NMR (1H STD-NMR) measurements. The 3D solution NMR structure of rhNGF was determined and exploited for a comprehensive binding study by ATP titration on rhNGF followed by 1H15N HSQC with related Chemical Shift Perturbation (CSP) analysis. The 15N NOESY collected at titration endpoint enabled us to identify new NOEs upon ATP binding. These were incorporated in MD simulations to identify the likely binding mode (position and orientation) of ATP on rhNGF. Surface Plasmon Resonance (SPR) allowed the effect of ATP on NGF binding to its receptors to be investigated.
We also undertook a complementary biophysical study using Differential Scanning Fluorimetry (DSF), ITC, 1H STD-NMR and transferred NOESY NMR (trNOESY) measurements on the binding of ATP to recombinant human proNGF (rh-proNGF). Our results reveal a different binding profile for mature and precursor proteins. ITC suggested ATP binding to rh-proNGF with a micromolar dissociation constant. Integrative structural biology approach by Small Angle X-ray Scattering (SAXS), Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) and limited proteolysis showed that ATP binding induces a change in the conformation and/or dynamics of rh-proNGF, predominantly in the IUD pro-peptide. Interestingly, we have uncovered that the strength of ATP binding to rh-proNGF is modulated by Mg2+ and by their relative stoichiometric ratios.
Combined, these results suggest a functional role for ATP in modulating the biological role of proNGF/NGF in health and disease states.

[1] F. Paoletti, F. Merzel, A. Cassetta, I. Ogris, S. Covaceuszach, J. Grdadolnik, D. Lamba, S. Golič Grdadolnik 1., *Comput. Struct. Biotechnol. J.*, **2021**, *19*, 2938.

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