Structural and functional properties of mouse proNGF

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Abstract

The unprocessed pro-form of the NGF (nerve growth factor), proNGF (NGF precursor, without signal peptide), has been suggested to have additional functions distinct from its role as a promoter of protein folding, i.e. apoptosis and/or neurotrophic activity. Aiming to gain insights into the specific molecular interactions that mediate proNGF biological activity and into the structural determinants stabilizing its pro-region, rm-proNGF (recombinant mouse proNGF) was expressed in Escherichia coli, refolded in vitro and characterized by physicochemical methods. X-ray solution scattering measurements (small angle X-ray scattering) revealed that rm-proNGF is dimeric in solution and appears to be anisometric when compared with the compact structure of the NGF dimer. Two structural models, a globular crab-like shape and an elongated rod-like shape, equally fit to the experimental results, pointing to an intrinsically structural disordered pro-region of NGF. The models obtained allowed the interpretation of TrkA (tropomyosin receptor kinase A) binding and activation assays in cell cultures, shedding new light on the key role of proNGF in neuronal survival and neurodegeneration.

Introduction

The neurotrophin NGF (nerve growth factor) is translated as a pre-pro-protein [pre-proNGF (precursor of NGF with signal peptide)] of 27 kDa, containing a signal peptide for protein secretion (pre-peptide) and the precursor protein [proNGF (NGF precursor, without signal peptide)]. proNGF is cleaved by the convertase furin to mature NGF.

NGF is involved in the maintenance and growth of neurons, whereas the pro-peptide facilitates folding of NGF [1]. proNGF is the predominant form of NGF in brain [2] and was found to be a high-affinity ligand for p75NTR (pan-neurotrophin receptor) and to induce p75NTR-dependent apoptosis [3]. The specific receptor for the proNGF is sortilin [4]. proNGF has also been found to bind to the high-affinity NGF receptor TrkA (tropomyosin receptor kinase A) and to induce the survival-signalling pathway, although it is less efficient than the mature NGF [5].

We focused on the biophysical and biochemical characterization of rm-proNGF (recombinant mouse proNGF), which was expressed in Escherichia coli, refolded and purified. The homogeneity of the protein preparation was assessed by dynamic light scattering [Rg (radius of gyration) = 3.5 nm, Pd (polydispersity) = 26.0%].

A high-throughput crystallization screen was started and at present no positive scores have been detected, suggesting that the high flexibility of the pro-part of proNGF strongly influences its crystallization propensity. Therefore we turned to SAXS (small angle X-ray scattering) as the technique of choice in order to gain information on its three-dimensional structure and dynamics.

Results

rm-proNGF was expressed in E. coli, refolded and purified from inclusion bodies as described in [6]. The protein was successfully purified and, after in vitro cleavage with recombinant furin, mature recombinant mouse NGF of 13 kDa was obtained.

The native structure of the rm-proNGF was proven by fluorescence and CD spectroscopy. Both spectra agree well with those previously published for human proNGF [6]. The proNGF has mainly a β-sheet secondary structure, like NGF, except for an increase in random coil content due to the presence of the very flexible pro-part.

The SAXS data have been collected on the X33 synchrotron beamline of the EMBL at DESY (Deutsches Elektronen Synchrotron), Hamburg, Germany. From the experimental X-ray scattering data, the model for the shape of the protein in solution was obtained ab initio, using the program DAMMIN [7]; a reconstruction of the Ca trace of the mouse proNGF was obtained by addition of the missing N-terminal region to the crystallographic structure of mature NGF, using the program BUNCH [8]. The models were restored using P2 symmetry.

Two possible models were obtained with both DAMMIN and BUNCH programs, both well fitting the experimental
Figure 1 | *Ab initio* models for the shape determination of rm-proNGF

Represented are the models obtained by DAMMIN (grey spheres), superimposed on the Cα traces obtained with BUNCH for the pro-peptide region (light grey); in black, the Cα traces of the crystallographic structure of mouse NGF (PDB code 1BET) are shown. (A) Crab-like shape; (B) rod-like shape.

Data. The first one shows a crab-like shape (Figure 1A), built up by the compact core of the NGF dimer and two arms that represent the two pro-parts of the protein. The second one is more elongated and shows a rod-like shape (Figure 1B), with the two pro-parts extending along the same direction of the long vertical axis of mature NGF. Figure 1 shows the superimposition of the DAMMIN and BUNCH models for both the crab-like and the elongated shapes and confirms that the models obtained with the two approaches fit well one to the other.

**Discussion**

The models obtained by SAXS for rm-proNGF in solution assist in the interpretation of the available biological data for proNGF. In the crab-like model, the flexible arms of rm-proNGF may partially hinder the binding region of NGF to its receptor TrkA [9], supporting the most significant biological data for proNGF, i.e. a lower level of activation of the signalling pathway in comparison with NGF [3,5]. Moreover, the model also supports a possible co-interaction with p75NTR, according to the crystal structure and to the solution X-ray scattering studies of the NGF–p75NTR complex [10,11].

The same is valid for the elongated model, in which the wings might represent an extended surface for the interaction with p75NTR, supporting the reported increased affinity of proNGF for p75NTR [3]. In the same way, the NGF moiety is again partially shielded in the interaction with the TrkA receptor.

Overall, these SAXS results indicate an intrinsic structural disorder of the pro-region of NGF and a consequent high flexibility of the pro-peptide that could help in the interpretation of the biological role of proNGF, especially as far as the interaction with different partners is concerned. Indeed, the pro-peptide of proNGF might be flexible in order to assume different conformations depending on the interacting partner and so act as a thermodynamic tether.

**References**


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