Conformational versatility of N-terminal regions of neurotrophins: a molecular dynamics study

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Motivation

Neurotrophins (NTs) play a major role in the differentiation, survival, and maintenance of nervous cells. This protein family includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), neurotrophin 4/5 (NT-4/5) and neurotrophin 6 (NT-6). A particular attention is currently devoted to the development of mimetics endowed with NT-like actions since their potential as therapeutic has been demonstrated by several independent investigations. Therefore, a deep understanding of the structural determinants of their function and specificity is required. Over the years, extensive crystallographic investigations have provided a detailed picture of NTs structures in their unliganded states (McDonald N.Q., Lapatto R., Murray-Rust J., Gunning J., Wlodawer A., Blundell T.L. (1991) Nature, 354, 411-414) and in complex with receptors (Trk and p75) (Wiesmann, C., Ultsch, M.H., Bass, S.H. and de Vos, A.M. (1999) Nature, 401, 184-188; He, X.L. and Garcia, K.C.
(2004) Science, 304, 870-875). On the other hand, limited data are available on the dynamic properties of these proteins. Particularly remarkable is the behavior of their N-terminal regions. There is clear evidence that this fragment becomes ordered only upon binding to tyrosine kinase receptors, exhibiting different structures.

Indeed, the N-terminus of NGF and NT4/5 mediate the interactions with TrkA and TrkB by adopting an alpha-helical and 3-10 helical structure, respectively (Banfield, M.J., Naylor, R.L., Robertson, A.G., Allen, S.J., Dawbarn, D. and Brady, R.L. (2001) Structure, 9, 1191-1199; Wehrman, T., He, X., Raab, B., Dukipatti, A., Blau, H. and Garcia, K.C. (2007) Neuron, 53, 25-38). This region is disordered in NT unliganded states and upon its binding to the receptor p75. Here we present a detailed molecular dynamics investigation aimed at identifying the intrinsic conformational preferences of the N-terminal regions of NGF and NT4/5.

Methods

The starting coordinates for the MD simulations were derived from the crystal structures of NT4/5 (PDB code 1HCF) and NGF (code 1WWW). To avoid any structural bias during the MD simulation, the N-terminus of the starting models was unfolded in an extended-like conformation. To increase the timescale of the MD sampling, simulations were also carried out on the peptides corresponding to the N-termini of NT4/5 and NGF.

All simulations were performed by using GROMACS software package version 3.3. The models were immersed in triclinic boxes filled with water molecules (SPC water model). The simulations were run with periodic boundary conditions using the all-atom OPLS-AA force field.

Results

The analysis conducted on NT4/5 and NGF clearly indicates that the systems are stable in the timescale of the simulations (10 ns). The most flexible regions correspond to the loops whereas residues embedded in secondary structure elements are rather rigid.

In both cases, the N-terminus is highly fluctuating and does not assume any type of secondary structure, even as transient states. Although this result provided an indication of the intrinsic flexibility of this region, it could be surmised that the limited timescale of the simulation was not sufficient for the folding process. Thus, simulations were also conducted over a larger timescale (100 ns) on isolated peptides corresponding to the first sixteen residues of NT4/5 and NGF sequences.

In both cases, this region is highly flexible. However, in the simulations carried out on NT4/5, the residues involved in the complex NT4/5-TrkB are able to form a 3-10 helix in the trajectory. Although transient, this helix is occasionally stable for hundredths of picoseconds. Only sporadic and non-continuous states in alpha-helical conformations are detected. On the other hand, no secondary structure element was found in the simulation of NGF peptide. On the bases of a comparative analysis of NGF and NT4/5 sequences, a putative role for the distinct behavior of NGF and NT4/5 peptides was assigned to the residue NGF Ile6 (Ala in NT4/5 sequences). Indeed, it is well-known that Cbeta branched residues present a low propensity for alpha-helices.

Their propensity for 3-10 helices is even lower. Consequently, MD simulations were also carried out on the NGF N-terminal peptide containing the mutation Ile6Ala. This peptide formed helical structures throughout the trajectory. This finding may suggest that an Ile residue, which prevents the formation of 3-10 helices, is important for the specificity of neutrotrophin-receptor recognition. Along this line, the analysis of 47 NGF sequences indicates that Ile6 is replaced only by Val residue, which exhibits similar secondary structure preferences. Collectively, these findings indicate that some intrinsic conformational preferences of neurotrophin N-terminus may play a key role in the binding of NTs to their high-affinity receptors.
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