ACADEMIC PRESS

Biochemical and Biophysical Research Communications 297 (2002) 1008–1010

BBRC

www.academicpress.com

Crystallization and preliminary X-ray diffraction studies of cobra venom β-nerve growth factor

Lichuan Gu,[a] Yuequan Shen,[a] Sujuan Xu,[a] Yuyan Shu,[b] Tao Jiang,[a] and Zhengjiong Lin[a,*]

[a] *National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China*
[b] *Guangxi Medical University, Institute of Snake Venom, Nanning 530021, China*

Received 15 August 2002

Abstract

β-Nerve growth factor (β-NGF) is a 118 amino acid residue polypeptide with an important role in the survival and development of certain neuronal populations. A β-NGF purified from cobra venom was crystallized by the hanging-drop vapor diffusion method. The crystals belong to the tetragonal space group $P4_12_12$ (or its enantiomorph $P4_32_12$) with unit-cell parameters $a = b = 61.75$, $c = 154.40$ Å. X-ray data from these crystals were collected to 3.2 Å resolution. Analysis of the packing density shows that the asymmetric unit probably contains two molecules. The self-rotation calculation implied that a β-NGF dimer might exist in the asymmetric unit.

Keywords: Nerve growth factor; Cobra venom; Crystallization

β-Nerve growth factor (β-NGF), i.e., 2.5 s NGF is a 118 amino acid residue polypeptide. It is the most important subunit of the 7 s NGF which is an inactive multisubunit complex of two α subunits, two β subunits, and two γ subunits [1]. The 7 s NGF is stabilized by Zn^{2+} cations can be autocatalytically dissociated in the absence of this cation. Then the three different subunits are released. Being active in the absence of the other subunits, β-NGF plays an important role in the survival, development, and differentiation of sympathetic and embryonic sensory ganglia [2]. It belongs to a family of structurally and functionally related molecules referred to as the neurotrophins. Besides NGF there are four other family numbers: Brain-derived neurotrophic factor (BDNF), Neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), and neurotrophin-5 (NT-5). Being similar with its four other family members, β-NGF carries out physiological activity through binding to its two cell surface receptors, TrkA and p75 [3,4].

The NGF structure was first determined for the protein extracted from submaxillary glands of mice [5]. The crystal structure of the murine NGF consists of three antiparallel pairs of β strands, together forming a flat surface. Two subunits associate through this surface forming a stable dimer. Four loop regions may determine the different receptor specificities. Subsequently, the structures of the bis-desocta$_{1-8}$ form of murine NGF and human NGF in complex with human TrkA-d5 were reported [6,7] and they revealed similar structural features. Snake venom is an abundant, economics and humane source for NGF. In fact, NGF was originally isolated from snake venom [8]. Snake venom NGF shows the same physiological function as does the submaxillary glands NGF and has sequence identity of 63% compared with murine NGF. Some residues presumably important for physiological function or binding receptor for murine β-NGF vary in the snake β-NGF [9,10]. The crystallographic studies of venom NGF become attractive. However, no crystal structure of snake venom NGF was reported yet. In this paper, we report the crystallization and preliminary X-ray crystallographic analysis of a β-NGF from venom of Chinese cobra, *Naja kaouthia*.

Materials and methods

The β-NGF provided for crystallization was extracted from the venom of *Naja kaouthia* (collected from Guangxi Province, China)

* Corresponding author. Fax: +86-10-6487-7837.
E-mail address: lin@sun5.ibp.ac.cn (Z. Lin).

0006-291X/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
PII: S0006-291X(02)02323-9

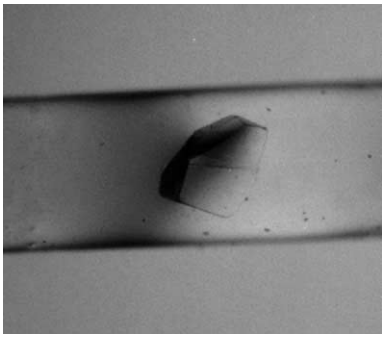

Fig. 1. Crystal of cobra venom β-NGF with dimensions of $0.6 \times 0.5 \times 0.4$ mm.

according to the procedure outlined previously [11]. The sample (10 mg ml^{-1}) was screened at 293 K against Hampton Crystal Screen and Crystal Screen 2 using hanging-drop vapor diffusion by mixing 2 μl protein solution with 2 μl reservoir solution [12]. Microcrystal emerged in Hampton Crystal Screen condition # 21 (0.1 M KH_2PO_4, 0.1 M NaH_2PO_4, 2 M NaCl, and 0.1 M MES, pH 6.5). Crystals were optimized through adjusting the concentration of precipitants. The optimal crystallization conditions for growing crystals suitable for X-ray analysis were as follows: the droplet containing 3 mg ml^{-1} protein, 0.2 M KH_2PO_4, 0.2 M NaH_2PO_4, 0.5 M NaCl, and 0.05 M MES as buffer (pH 6.5) was equilibrated over the reservoir solution containing 0.5 M KH_2PO_4 and 0.5 M NaH_2PO_4. Single crystals appeared within one week with a maximum size of $0.6 \times 0.5 \times 0.4$ mm (Fig. 1). The crystals diffract to 3.2 Å resolution.

Diffraction data were collected on Mar 345 Research Image Plate area detector with Cu Kα radiation (1.5418 Å) at 293 K. A total of 180 frames of data to 3.2 Å were collected from a single crystal (1° oscillations and 720 s exposure time per frame). During the collection no obvious radiation damage was observed. The data were processed using programs DENZO and SCALEPACK [13].

Results and discussion

Auto-indexing revealed that the crystal belongs to tetragonal space group $P4_12_12$ or its enantiomeric $P4_32_12$ with $a = b = 61.75$, $c = 154.40$ Å. The data set in the range 20.0–3.2 Å contains 140,761 observations of 5386 unique reflections and has an R_{merge} of 16% (41.4% for the last shell) and completeness of 100%. I/σ (I) for this data set was 20.3 (8.4 for the last shell). Details of the data-collection statistics are summarized in Table 1. Analysis of the packing density shows that 2–3 molecules in the asymmetric unit would yield a reasonable solvent content, with two molecules being most likely ($V_m = 2.83$ Å^3 Da^{-1}; $V_{solv} = 56.6\%$) [14].

A self-rotation function calculated using the POLARRFN program (CCP4 suit) revealed a prominent peak (3.5σ) appearing at 38.4% of the height of the crystallographic peaks (Fig. 2) for section $\kappa = 180°$. This indicated the presence of a non-crystallographic twofold symmetry axis lying in the plane orthogonal to the crystallographic fourfold axis and deviates 23° from the a axis. The self-rotation function calculation implied that being similar to the previously reported β-NGF structures the active dimer might be still preserved in the crystal of β-NGF from cobra venom. It was reported that the β-NGF structures display structural flexibility [6]. The crystallization conditions and space group of the crystal reported here differ from those of other β-NGF crystals. Thus the structure determination of β-NGF in this new crystal form may also increase our understanding of the structural flexibility of the protein. The crystal structure determination of β-NGF from cobra venom by X-ray crystallography is underway now.

Table 1
Data-collection statistics for cobra venom β-NGF

X-ray source	Cu Kα
Wavelength (Å)	1.5418
Temperature (K)	293
Space group	$P4_12_12$ or $P4_32_12$
Unit-cell parameters (Å)	$a = b = 61.75$, $c = 154.40$
Resolution (Å)	3.2
Completeness (%)	100 (100)
R_{merge} (%)	16.0 (41.4)
$I/\sigma(I)$	20.3 (8.4)
Total observations	140,761
Independent reflections	5386

Values in parentheses are for the highest resolution shell (3.31–3.20 Å). $R_{merge} = \Sigma_h \Sigma_i |I(h,i) - \langle I(h) \rangle| / \Sigma_h \Sigma_i I(h,i)$, where $I(h,i)$ is the intensity of ith measurement of the reflection h and $\langle I(h) \rangle$ is the mean value of the $I(h,i)$ for all i measurements.

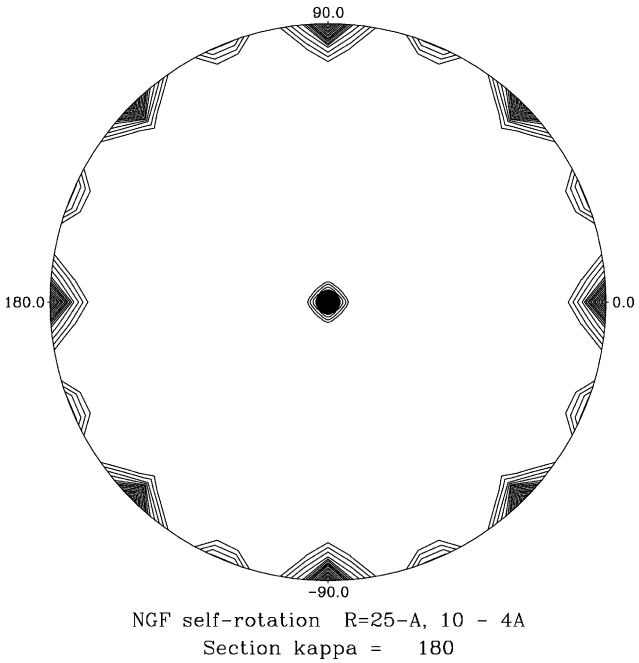

Fig. 2. Self-rotation function ($\kappa = 180°$ section) for NGF crystals reveals a peak corresponding to a non-crystallographic twofold axis at ω of 90° and φ of 23°. An integration radius of 25 Å and a resolution range of 10–4 Å were used in the calculation.

Acknowledgments

We thank Dr. Xudong Zhao for his help during data collection and processing. This work was supported by National Natural Science Foundation of China.

References

[1] M. Young, M.J. Koroly, Nerve growth factor zymogen. Stoichiometry of the active-site serine and role of zinc(*II) in controlling autocatalytic self-activation, Biochemistry 19 (1980) 5316–5321.
[2] R. Levi-Montalcini, The nerve growth factor: thirty-five years later, EMBO J. 6 (1987) 1145–1154.
[3] D. Johnson, A. Lanahan, C.R. Buck, A. Sehgal, C. Morgan, E. Mercer, M. Bothwell, M. Chao, Expression and structure of the human NGF receptor, Cell 47 (1986) 545–554.
[4] S.O. Meakin, E.M. Shooter, Molecular investigations on the high-affinity nerve growth factor receptor, Neuron 6 (1991) 153–163.
[5] N.Q. McDonald, R. Lapatto, J. Murray-Rust, J. Gunning, A. Wlodawer, T.L. Blundell, New protein fold revealed by a 2.3-Å resolution crystal structure of nerve growth factor, Nature 354 (1991) 411–414.
[6] D. Holland, L. Cousens, W. Meng, B. Matthews, Nerve growth factor in different crystal forms displays structural flexibility and reveals zinc binding sites, J. Mol. Biol. 239 (1994) 385–400.
[7] C. Wiesmann, M.H. Ultsch, S.H. Bass, A.M. de Vos, Crystal structure of nerve growth factor in complex with the ligand-binding domain of the TrkA receptor, Nature 401 (1999) 184–188.
[8] A. Wlodawer, K. Hodgson, E. Shooter, Crystallization of nerve growth factor from mouse submaxillary glands, Proc. Nat. Acad. Sci. USA 46 (1975) 777–779.
[9] T. Oda, M. Ohta, S. Inoue, K. Ikeda, S. Furukawa, K. Hayashi, Amino acid sequence of nerve growth factor purified from the venom of the Formosan cobra *Naja naja atra*, Biochem Int. 19 (1989) 909–917.
[10] S. Inoue, T. Oda, J. Koyama, K. Ikeda, K. Hayashi, Amino acid sequences of nerve growth factors derived from cobra venoms, FEBS Lett. 279 (1991) 38–40.
[11] X.B. Li, M.J. Chen, D.Q. Lei, B. Yang, G.S. Liao, Y.Y. Shu, S.X. Tang, Bioactivities of nerve growth factor from Chinese cobra venom, J. Nat. Toxins 8 (1999) 359–362.
[12] J. Jancarik, S.H. Kim, Sparse matrix sampling: screening method for crystallization of proteins, J. Appl. Crystallogr. 24 (1991) 409–411.
[13] Z. Otwinowski, W. Minor, Processing of X-ray diffraction data collected in Oscillation mode, Methods Enzymol. 276 (1997) 307–326.
[14] B.W. Matthews, Solvent content of protein crystals, J. Mol. Biol. 33 (1968) 491–497.