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Crystallization and preliminary x-ray diffraction study of neurotoxin-I from *Naja naja oxiana* VENOM

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Crystals of the neurotoxin-I (NTX-I) from the venom of the middle Asian cobra *Naja naja oxiana* have been grown by vapour diffusion and dialysis methods. The crystals belong to space group $P2_12_12$ with dimension of $a=25.19$ Å, $b=75.59$ Å, $c=36.09$ Å and diffract to 1.9 Å resolution. The asymmetric unit contains one molecule ($V_m=2.2$ Å/Da). Using the molecule of α-cobratoxin (CTX) as a starting model for NTX-I structure determination coordinates of Cα atoms of the NTX-I molecule were obtained and the position of NTX-I in the unit cell was derived.

1. INTRODUCTION

Neurotoxin-I is one of the main protein components purified from the venom of the middle Asian cobra *Naja naja oxiana*. NTX-I is known to bind specifically to acetylcholine receptors thus preventing the transmission of the neuroconductivity signal from synaps to muscles. Since neurotoxins serve as a very selective tool for the investigation of mechanisms of neuroconductivity, hormone regulation and protein biosynthesis, they are of great interest. High resolution structures of neurotoxins are necessary for the understanding of these processes at the molecular level.

The length of the polypeptide chain (73 residues, molecular mass 7.8 kDa) as well as the number of disulfide bridges means that NTX-I belongs to the 'long'-type neurotoxins [1].

2. MATERIALS AND METHODS

The venom of *N. naja oxiana* was harvested and liophilized at Karakhaly serpentary (Turkmen SSR). Purification of the protein components including NTX-I was carried out according to [2] with modifications [Nickitenko et al. (1990) Kristallografiya (in press)].

NTX-I crystals (Fig. 1) were grown either by vapour diffusion or dialysis methods using specially prepared microdialysis cells. The intensities of reflections from native NTX-I crystals were measured in the range of 38–2.1 Å by ω-scan method on a Syntex $P2_1$ diffractometer, operating in automatic mode [3]. To determine the position and mode of packing of the NTX-I molecule in the unit cell, program packages MERLOT [4] and BLANC (Vagin A.A., Institute of Crystallography Acad. Sci. of the USSR) were applied running on a NORD-500 computer.

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3. RESULTS AND DISCUSSION

For crystallization we used NTX-I which was free of any salt contaminations and was proved to be homogeneous by gel electrophoresis. This preparation was lyophilized and stored until crystallization.

In crystallization probes the initial concentration of NTX-I was 15–25 mg/ml in 10 mM CH_3 COOH. When crystallized by vapour diffusion methods the counter solution contained 50–55% isopropanol and 5% 2-methyl-2,4-pentanediol. Crystals grew in 2–3 weeks at 4°C. Typical crystal size was $2.0\times2.0\times0.2$ mm. The space group was $P2_12_12$ and unit cell dimensions, equal to $a=25.19$ Å, $b=75.59$ Å, $c=36.09$ Å, were determined from x-ray precession photographs with subsequent refinement on a Syntex $P2_1$ diffractometer [3]. There is one NTX-I molecule per asymmetric unit, $v_m=2.2$ Å^3/Da.

The data set in the range of 38–2.1 Å (3600 independent reflections which corresponds to 94% of the complete data, contain only reflections for which $[F[>3\sigma$ and $R=\Sigma\sigma/\Sigma[F[=5.8\%$) was measured from six crystals. The crystals were exposed to the x-ray beam until the intensities of control reflections decreased by 10%.

The 3D-structure of NTX-I was determined by molecular replacement methods with a molecule of CTX from *Naja naja siamensis* [5] serving as a starting model. Atomic coordinates of this toxin at 2.8 Å resolution were taken from the Brookhaven Protein Data Bank. The polypeptide chain of NTX-I contains 73 amino acid residues, two residues more than CTX. The homology between the primary structures of the two toxins is high, 63% (see Fig. 3). In both cases molecules are stiffened by 5 disulfite bridges [1].

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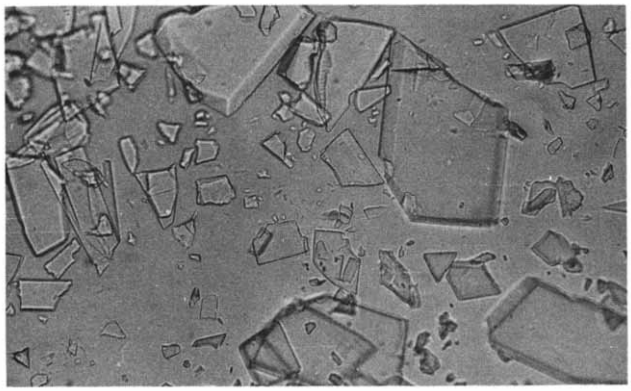

Fig. 1. Crystals of neurotoxin-I from *N. naja oxiana* venom.

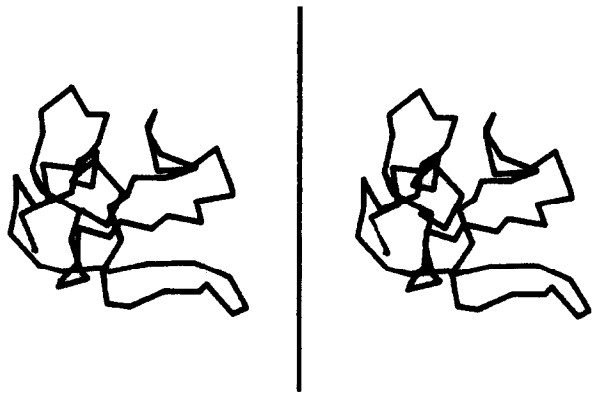

Fig. 3. Stereo drawing of the Cα atoms of neurotoxin-I.

To establish the orientation of the CTX molecule in the NTX-I unit cell the former was rotated to an angle $\alpha = 0°$, $\beta = 125°$, $\gamma = 90°$ with respect to the position given in the Protein Data Bank. Calculations, carried out with MERLOT, have revealed the most probable orientation of the model molecule, characterized by the angles $\alpha = 100.0°$, $\beta = 78.0°$, $\gamma = 111.0°$.

Native data between 4–2.7 Å ([F[)3σ) were used for these calculations while the integration radius was varied from 12.5 to 23.9 Å. Then the translation of the model in the unit cell was calculated, using programs from the BLANC package, giving coordinates of the center of mass: $x = 5.6$ Å, $y = 32.0$ Å, $z = 16.2$ Å. The correctness of our preliminary data concerning the position of the CTX molecule in the unit cell might be estimated by values:

$$R = \Sigma[F_0 - F_c[/\Sigma \sim F_0[= 0.54$$

$C = \Sigma((F_0 - <F_0>)(F_C - <F_C>))/ \quad (\Sigma(F_0 - <F_0>)^2 \Sigma (F_C - <F_C>)^2) = 0.37$, calculated with native data. [F[)3σ, between 6.0–2.3 Å. The position of the model in the unit cell was then refined with the program CORELS [6], considering the CTX molecule as a rigid body. The deviation of the position from that initially obtained was: $\Delta\alpha = 1.8°$, $\Delta\beta = -2.2°$, $\Delta\gamma = -3.3°$, $x = 0.33$ Å, $y = 0.40$ Å, $z = 0.49$ Å.

Prior to refinement of every amino acid residue, the refinement of the distinct parts, 'domains', of the molecule was performed. At the final stage the number of 'domains', used as rigid bodies, was 10. This procedure increased the accuracy in determination of the position of the model in the unit cell.

A starting model of the NTX-I Cα-backbone was then generated. Refinement was carried out with Hendrikson-Connert program [7], using native data, [F[2σ, between 6.0–2.7 Å. An initial model was constructed from a CTX molecule (see Fig. 2) by replacing all 25 amino acids which are not identical in NTX-I and CTX molecules with Ala residues. Fig. 3 shows the Cα-backbone of the NTX-I molecule after the refinement. The accuracy of the model is characterized by $R = 27.7\%$ and $C = 80.7\%$ for data between 6.0–2.7 Å, [F[)2σ, pointed above.

So, using a molecule of CTX as a starting model for NTX-I structure determination, coordinates of Cα atoms of the NTX-I molecule (Fig. 3) were obtained and the exact position of NTX-I in the unit cell was derived. The work to refine the structure further as well as to collect data to higher resolution is in progress now.

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NTX  1   ITCYKTPIPITSETCAPGQNLCYTKTWCDAWCGSRGKVIELGCAATCPTV
CTX  1   IRCFITPD-ITSKDCPNGH-VCYTKTWCDAFCSIRGKRVDLGCAATCPTV
         * *   **   ***   *   *    ********* *   ***    **********
                                  |----|           |---|
                                    β-I             β-II
NTX 51   ESYQDIKCCSTDDCNPHPKQKRP
CTX 49   KTGVDIQCCSTDNCNPFPTRKRP
              ** ***** *** *   ***
              |---|
              β-III
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Fig. 2. Sequence alignment of neurotoxin-I and α-cobratoxin.

REFERENCES

[1] Grishin, E.V., Sukhikh, A.P., Slobodyan, L.N., Ovchinnikov, Y.A. et al. (1974) FEBS Lett. 45, 118–121.
[2] Apsalon, U.R., Shamborant, O.G. and Miroshnikov, A.I. (1977) Bioorganicheskaya Khimiya 3, 1553–1559.
[3] Nekrasov, Y.V. (1988) Kristallografiya. 33, 795–797.
[4] Fitzgerald, P.M.D. (1988) J. Appl. Crystallogr. 21, 273–278.
[5] Walkinshaw, M.D., Saenger, W. and Maelicke, A. (1980) Proc. Natl. Acad. Sci. USA 77, 2400–2404.
[6] Susman, J.L., Holbrook, S.R., Church, G.M. and Kim, S.-H. (1977) Acta Cryst. A33, 800.
[7] Hendrickson, W.A. and Konnert, J.H. In: Biomolecular structure, function, conformation and evolution/Ed. Srinivasan R. (1981) Oxford, Pergamon, 1, 43.