X-ray Crystallographic Study of Laticotoxin a*

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SUMMARY

Single crystals of the neurotoxin laticotoxin a have been grown to a suitable size for x-ray analysis. The space group is determined to be P4₃2₁ (or its enantiomorph, P4₁2₁2). The cell dimensions are a = b = 39.78 ± 0.07 Å and c = 72.74 ± 0.17 Å; there is 1 protein molecule per asymmetric unit (8 molecules per unit cell). The cell dimensions are similar to those of cobrotoxin, and the same space group ambiguity exists.

Laticotoxin a is a neurotoxic protein found in the venom of two sea snakes, Laticauda laticaudata and Laticauda colubrina (1). It is a member of a large family of neurotoxins which are all highly basic, small, single chain polypeptides (60 to 75 residues) with three of the amino acids. Recent studies have shown this toxin to be a mixture of two similar proteins with at least four to five invariant disulfide bridges and which require at least one invariant tryptophan residue for toxicity. In L. laticauda, laticotoxin comprises 72% of the protein and 100% of the toxicity.

METHODS AND RESULTS

Laticotoxin a from L. laticaudata was first crystallized from a 0.15 M sodium chloride solution by the addition of saturated ammonium sulfate to 60% saturation (1). The crystals obtained were elongated lathes approximately 50 to 80 µm long. A different procedure was used to obtain crystals large enough for x-ray analysis. Laticotoxin a powder (1.6 mg) was dissolved at room temperature in 50 µl of 0.01 M sodium phosphate buffer (pH 6.56) and saturated ammonium sulfate added to 60% saturation (final ammonium sulfate saturation was about 29%). Well-formed crystals (300 × 80 × 50 µm) were present after 2 days; they gave a 3° hkl zone identical with the same zone for the room-temperature crystals.

A laticotoxin a crystal grown at 2°C reached 200 µm in length after 16 days and appeared identical with those grown at room temperature.

The density of the crystals was measured using the gradient tube method (2). A water-saturated xylene-bromobenzene gradient column was employed (subsequently calibrated with drops of aqueous potassium bromide solution). Two crystals (about 200 µm long) of laticotoxin a gave an average density of 1.26 ± 0.01 g per cm³. The density of the protein-free crystallization medium was measured pycnometrically, and a value of 1.104 g per cm³ was established. The space group and lattice dimensions of the laticotoxin a crystals grown at room temperature were determined using CuKα (λ = 1.5418 Å) radiation from an unfiltered Jarrell-Ash (Waltham, Mass.) microfocus unit (39 kv, 7 ma) at 20°C. A Supper precession camera with crystal-to-film distance of 45 mm and 0.15-mm collimator was used. Films of the hkl, h0l, and o0l zones show 4/mmm lattice symmetry in a primitive array. The o0l zone exhibits 00l reflections only for k = 2n and 00l reflections only for l = 4n. The space group consistent with these diffraction records is P4₁2₁2 (or its enantiomorph, P4₁2₁2) with eight asymmetric units per unit cell. An 11° precession photograph of an o0l zone is shown in Fig. 2. The cell dimensions are a = b = 39.78 ± 0.07 Å and c = 72.74 ± 0.17 Å. The minimum spacing observed on the photographs taken was 2.5 Å. The crystals appear to be unusually stable to x-radiation.

A laticotoxin a crystal grown at 2°C and stable to x-rays at 20°C gave a 3° hkl zone identical with the same zone for the room-temperature crystals.

DISCUSSION

The molecular weight of laticotoxin a is 6970, the crystal density has been determined, and the density of the liquid of crystallization may be assumed to be approximately that of the protein-free crystallization medium. The volume fraction of the protein in the unit cell may be assumed to be approximately that of the asymmetric unit. The volume fraction of protein occupied by 8 molecules of laticotoxin a is 58.8% of the total cell volume. The partial specific volume of the protein thus calculated is 0.73 cm³ per g, which is in reasonable agreement with the calculated value of 0.71 cm³ per g (1). Thus the presence of 1 molecule in the asymmetric unit is established.

It is probable that the two component proteins of laticotoxin a may be incorporated in the crystal lattice in the same molar ratio as in the original crystallization medium. The differences between the 2 component molecules may be too small to lead to the separation of two different homogeneous crystalline forms.

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The cell dimensions reported here for laticotoxin a are in very close agreement with those reported for cobrotoxin (3), which also occurs in the space group P4_2_2_2 (or P4_12_2_2). Comparison between the amino acid compositions of laticotoxin a (1) and cobrotoxin (4) indicates that there are a significant number of residue differences (at least 16) between the two proteins. A close three-dimensional structural similarity is to be expected in the whole class of neurotoxins. The erabutoxins a and b from the sea snake, Laticauda semifasciata, with 1 residue difference between them, both crystallize in the same space group—the crystals are orthorhombic space group P2_12_12_1. Both the near identity of the axial lengths and of the intensity distributions demonstrate their isomorphism (5).

Laticotoxin a should support x-ray crystal structure analysis either by utilizing rigid-body search techniques based on an erabutoxin molecular structure as the search group or by the heavy atom isomorphous replacement technique as an independent study.

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REFERENCES
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