

CRYSTALLINE CHOLERA TOXIN SHOWS FIVE-FOLD MOLECULAR SYMMETRY

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ABSTRACT

Cholera toxin is a multisubunit protein that interacts with specific cell surface receptors and signals an internal adenylate cyclase to produce cyclic AMP. It is composed of five B subunits (each 11,400 daltons) which bind the receptors and an A (27,000 daltons) which carries the catalytic component of the toxicity. Crystals of the cholera toxin have been grown which are very suitable for x-ray diffraction analysis. An examination of the rotational symmetry of the molecule, using the intensities to 6Å resolution, indicates that a substantial fraction of the molecule has a high degree of 5-fold rotational symmetry. We conclude that the 5 B subunits are arranged with approximate five-fold symmetry around the A subunit, a situation which resembles the 5-fold vertices of certain icosahedral viruses.

INTRODUCTION

Cholera toxin is a multiple subunit protein of molecular weight 84,000 which causes a massive secretory diarrhea by stimulating a cyclic AMP mediated chloride pump in the crypt cells of the ileum (Field, 1978). It consists of one A subunit (27,000 daltons) and five B subunits (each 11,400 daltons) (Gill, 1976; Sigler *et al.*, 1977). The B subunits attach the toxin to the outside surface of the vertebrate cell membrane by binding the ganglioside G_{M1} (King & Van Heyningen, 1973; Holmgren *et al.*, 1973; Cuatrecasas, 1973). The A subunit consists of two proteolytic fragments of a single polypeptide chain held together by a disulfide bridge; A₁ (~20,000 daltons) which contains all of the toxic activity and A₂ (~7,000 daltons) which stabilizes the interaction of A with the B subunits (Gill, 1977). In a broken cell preparation in which the

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B subunits are not required, the A_1 subunit activates an internal membrane-bound adenylate cyclase by catalyzing the transfer of an adenosyldiphosphoryl ribose from NAD to the guanyl nucleotide binding protein (G-protein) of the adenylate cyclase (Cassel and Pfeuffer, 1978; Gill and Meren, 1978).

We have grown crystals of the cholera toxin which are large, well shaped, radiation resistant and diffract x-rays to at least 2.5 Å resolution (Figures 1 and 2) (Sigler *et al.*, 1977). As a first step in the high resolution structure determination we set out to establish the molecular architecture, in particular the disposition of the five identical B subunits. We report here our effort to establish the type and degree of rotational symmetry within the molecule and the orientation of the molecular symmetry axis in the unit cell.

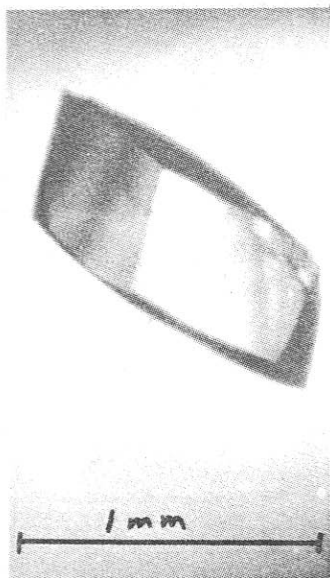


Fig. 1. A typical cholera toxin crystal.

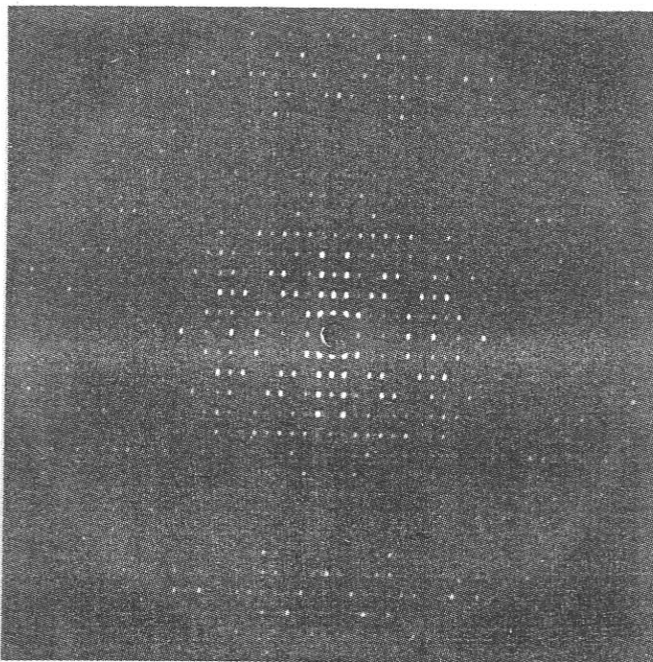


Fig. 2. Twelve degree ($d \geq 3.7^\circ\text{A}$) precession photograph of the $(hk0)$ reciprocal lattice plane, taken for 40 hours with graphite monochromatized $\text{CuK}\alpha$ radiation from an Elliott GX-6 rotating anode generator running at 40 kilovolts and 40 milliamperes.

EXPERIMENTAL PROCEDURE

Pure cholera toxin was prepared by the method of Finkelstein *et al.* (1970, 1971). Monoclinic crystals were obtained by vapor diffusion at room temperature from polyethylene glycol (Sigler *et al.*, 1977).

A unique and nearly complete set of 2272 measurable intensities from 28 Å to 6 Å resolution were recorded with Ni-filtered CuK_α radiation using a Syntex P2₁ automated diffractometer, fitted with a 70-cm helium-filled tube between the crystal and the counter. Measurements were made with an ω -scan technique in which the background was measured once on each side of the reflection, so that the ratio of scan/background times was 0.73. An empirical absorption correction was applied as described by North *et al.* (1968). Radiation damage and crystal misalignment were monitored by remeasuring five standard reflections after every 100 intensity measurements. An empirical correction for radiation damage, based on the decay of the monitor reflections, was applied up to a maximum intensity loss of 15%.

In order to search for intramolecular rotational symmetry, a rotation function (Rossmann and Blow, 1962) was computed using Crowther's (1973) fast rotation function as modified by Tanaka (1977) to express the orientation of the axis of rotational symmetry in the spherical polar coordinates (ϕ and ψ). The output subroutine was modified so as to map the rotation function in planes of constant κ , the degree of rotational symmetry. The rotation search was performed in intervals of 5° between 0° to 360° in κ , and hence the maps of the 2, 3, 4, 6 and 9-fold axes were precisely determined. Maps having rotational symmetry not equal to $360^\circ/5n$ (n , integral; i.e., 5, 7, 8 and 10) were interpolated between the closest grid points. The search was, in general, restricted to Patterson vectors no longer than 25 Å, although modest variations in vector length did not have a strong qualitative effect on the results.

As a "bench mark" similar analyses were carried out on the intensities of α -chymotrypsin*, a molecule of 25,000 molecular weight which crystallizes in the same space group as the cholera toxin (P2₁) and has two molecules in the asymmetric unit related by a local dyad (Blow *et al.*, 1964; Matthews *et al.*, 1967; Tulinsky *et al.*, 1973).

In an effort to understand the emergence of unexpected local symmetry, simulation studies are currently being carried out on intensities calculated from a model unit cell having the same dimensions as that of the cholera toxin crystals and containing 5 pancreatic ribonuclease molecules in each

*A complete list of intensities was generously provided by Professor A. Tulinsky of Michigan State University.

asymmetric unit symmetrically disposed about a five-fold axis oriented similarly to that observed in the cholera toxin crystal as described below. Atomic coordinates for the ribonuclease molecule (Wyckoff, *et al.*, 1970) were obtained from the Brookhaven Data Bank.

RESULTS AND DISCUSSION

The rotation function showed significant maxima for 2, 5 and 10-fold rotational symmetry (Figure 3b). Specifically, no peaks exceeded the noise level (20% of the crystallographic 2-fold Patterson symmetry) in the maps of 3, 4, 6, 7, 8, and 9-fold rotational symmetry.

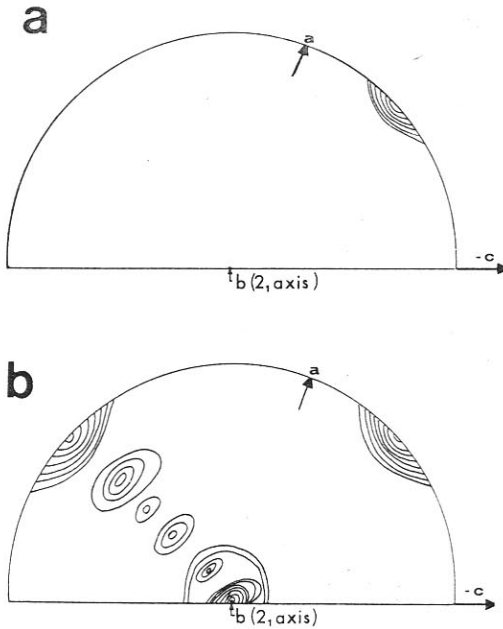


Fig. 3. Stereographic projections of the rotation function, computed with the crystalline cholera toxin intensities between 28 and 6 Å resolution. a) The 5-fold map ($\kappa = 70^\circ$). b) The 2, 5 and 10 folds rotation map superimposed ($\kappa = 180^\circ, 70^\circ, 35^\circ$).

Figure 3a shows a five-fold symmetry axis (52% of the space group symmetry peak) perpendicular to the crystallographic screw dyad and 135° from the c axis. There are 11 local dyads, one of which is the strongest peak in the entire analysis (72% of the crystallographic 2-fold Patterson symmetry).

The strongest dyad is coincident with the 5-fold axis. The remaining 10 dyads are normal to the 5-fold and represent the 5-fold rotation of a line segment through the origin perpendicular to both the crystallographic dyad and the 5-fold axis. The noncrystallographic dyads may well represent elements of Patterson symmetry generated by the crystallographic dyad plus the true local 5-fold symmetry and therefore will have no counterparts in real space. The net result is planar 10-fold symmetric array of dyads through the origin of the Patterson and normal to the 5-fold axis.

It is clear that point symmetries often arise in Patterson functions from a combination of local and crystallographic rotations. These apparent symmetries are considered "false" in that they have no counterpart in the real cell (Litvin, D. B., 1975; Johnson *et al.*, 1975; Eventoff and Gurskaya, 1975). For example, the local dyad in α -chymotrypsin intersects the crystallographic dyad in the Patterson map at right angles, necessitating the existence in the Patterson map of a third mutually orthogonal intersecting dyad that has no corresponding symmetry element in the structure. The emergence of such "false" symmetries in the rotational analysis of the cholera toxin structure amplitudes are currently under study by simulating various molecular assemblies (J. A. Zelano, work in progress). Preliminary results of a systematic survey show that position and apparent magnitude of rotation function maxima (whether real or "false") may be sensitive to the crystallographic resolution and Patterson vector lengths (A. Yonath, work in progress). In any case, it can be stated that a reasonably large fraction of the cholera toxin molecule has 5-fold rotational symmetry at 6 \AA resolution and it is likely that no further molecular symmetry need be invoked to explain the impressive array of extra symmetry elements.

DISCUSSION

The symmetric or quasi-symmetric arrangement of 5 B subunits which interact with a single A subunit poses an interesting problem in molecular assembly; namely, how do five protomers (here the B subunits) dispose themselves symmetrically about a protein or molecular assembly which does not have 5-fold symmetry. A similar problem arises in the architecture of the 5-fold quasi-symmetric vertices of certain viral capsids such as $\phi\chi$ -174 and

adenovirus which contain a central fiber or spike. Assuming that the A subunit in the crystalline molecule is involved in intermolecular crystal packing contacts and therefore is not crystallographically disordered, the cholera toxin structure may provide a detailed description of the intersubunit contacts. It has not escaped our attention that there are also rough functional parallels between the vertices of certain spherical viruses and cholera toxin in that both have 5-fold quasi-symmetric arrays which mediate the transfer of an alien, functional and deleterious molecule across the cell membrane. In addition to answering questions regarding five-fold symmetric assembly, the local symmetry once established should also clarify the interpretation of heavy atom distributions in isomorphous derivatives used for phase analysis and help understand general features of ligand binding responsible for specific interactions and ultimately transmembrane signalling.

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