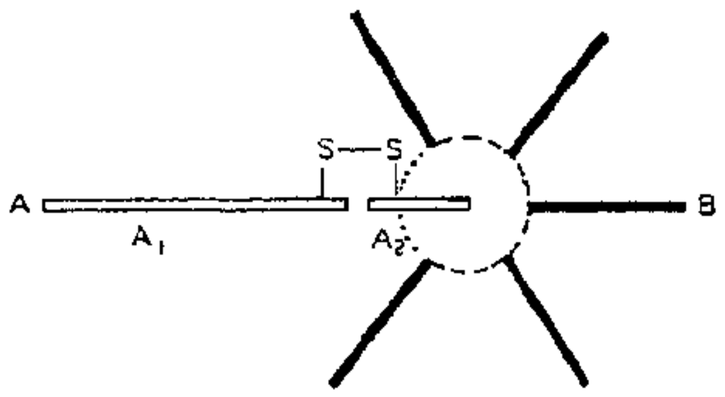
Special Issue on S. N. De and
Cholera Enterotoxin

Towards the Molecular Structure of Cholera Toxin

Cholera toxin was purified to homogeneity by Finkelstein[1], a decade after De's demonstration of the enterotoxicity of the culture filtrates of the vibrio[2]. In 1977, the first report on the crystallisation of the toxin appeared[3]. However, no detailed three-dimensional structure, is as yet, available. Chemical studies have established that cholera enterotoxin is an oligomer of a single A-subunit (M_r 27234) and five B-subunits (M_r 11677 each). The B-subunits are arranged with apparent 5-fold symmetry and the intact pentamer (choleragenoid) interacts with a ganglioside G_{M1} membrane receptor. Subunit A is proteolytically nicked during secretion giving rise to two disulfide linked polypeptides A_1 (M_r 21826) and A_2 (M_r 5407). The entire oligomeric protein (Figure 1 shows a schematic picture) is necessary for toxic activity [4, 5].



Schematic model of the cholera toxin structure (taken from ref. 5).

Now, almost thirty years after De's paper comes a report on the crystallisation of isoelectrically pure cholera toxin. B. D. Spangler and E. M. Westbrook of the Argonne National Laboratories, U.S.A., describe crystals of the oligomeric protein (one A- and 5 B-subunits) in the space group $P2_1$ ($a = 73.0$ Å, $b = 92.2$ Å, $c = 60.6$ Å, $\beta = 106.4°$, one molecule in the asymmetric unit), which diffract to better than 2.8Å resolution[6]. Using data recorded at 3.0Å, Spangler and Westbrook have determined the orientation of the 5-fold molecular symmetry axis, with respect to the crystallographic screw axis. Rotation function calculations are consistent with 'the apparent 5-fold rotational symmetry of the B subunits, with the A subunit sitting on the 5-fold axis, displaced slightly above the plane of the B subunits'. Similar models have indeed been also derived earlier from low resolution electron microscopy of the toxin bound to lipid layers forming two dimensional crystalline arrays [7,8].

The successful crystallisation of cholera toxin appears to have been the consequence of careful purification of a single isoelectric variant by ion exchange chromatography. While early reports using isoelectric focussing suggest that cholera toxin appears as a single band focussing at pH 6.6[9] or pH 6.65[10], Spangler and Westbrook observed multiple bands in the region pH 6.7–7.0 for a wide range of toxin samples. Deamidation has been suggested as a possible cause[6].

A detailed three-dimensional structure of cholera toxin appears to be around the corner. This should provide a new impetus for developing means of counteracting the effects of the toxin by immunological or pharmacological methods[5]. Success in these areas would have surely pleased S. N. De.

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