

oval channel is about $14 \times 25 \text{ \AA}$ and it varies somewhat among the 6 quasi-equivalent rows of channels. At the resolution of this reconstruction, the substructure or exact size of the channel cannot be determined. Studies of ion and molecular permeability through these channels⁹ indicate a size of about 20 \AA . The outside of the tube is dominated by loops or ridges of protein that extend from the surface. The outside openings of the vesicle channels are at the base between the ridges.

Each channel arises from the interaction of protein densities from opposite sides of the channel. Using our contour cutoff, which correctly represented the phospholipid thickness, we calculated the total volume of the map exceeding the threshold. When we subtracted the volume occupied by a cylindrical annulus at $240\text{--}280 \text{ \AA}$ radius (the phospholipid), the remaining protein volume was $1.04 \times 10^8 \text{ \AA}^3$ per helical repeat. Dividing by 27 units, and by 6 for the sixfold repeat of the channels, we arrive at about $640,000 \text{ \AA}^3$ of protein associated with each channel. This corresponds to 544K, about 4 times the M_r of each NT molecule. We thus interpret that the mass on either side of the channel is a dimer, so every channel is surrounded by four molecules. This indicates that a portion (10%) of the NT is embedded in the lipid, though our estimated error is of the same order (7%). It was suggested¹⁰ that for botulinum type C, aggregation into dimers or higher oligomers occurred during channel formation. Recently, 23 amino-acid tetanus toxin peptide has been shown to form channels in phospholipid bilayers¹¹. Molecular modelling produced a plausible 4-helix bundle structure whose α -helices have a centre-to-centre distance of $10\text{--}25 \text{ \AA}$ and surround a 5 \AA pore. All NT studied so far contain at least one homologous amphipathic stretch in the N-terminal region of their heavy chain¹². Thus the channel may not be internal to each molecule; it could be formed cooperatively from several molecules after membrane insertion.

The bridge of density over the channel (arrowhead, Fig. 3) may be involved in the control of channel accessibility. Electrophysiological experiments¹⁰ implicated two alternating states, one of high and one of low conductance. The partial obstruction of the channel by the protein that we observe here could indeed be the time-averaged structural expression of this dynamic fluctuation. Alternatively, a channel with such an obstruction may influence the order of events that lead to channel formation. For example, *in vivo* the channel may form soon after the NT molecules bind to the cell surface, but in a partially blocked state (low conductance). Following endocytosis, the change in pH from near neutral to acidic could affect the uncovering of this channel, rather than its creation in the membrane. To decide between these mechanisms, this specimen will be studied under other conditions of external pH to see if other structural states can be observed. This is, to our knowledge, the first direct structural observation of a channel in a membrane formed from a soluble protein. □

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