The hepatopancreas protein, therefore, probably represents the membranous proton channel of the vacuolar ATPase and electron microscopy has given us the clearest impression yet of its organization; an organization clearly consistent with the functional characteristics of the chloroplast ATPase–CF_{1} complex [22]. It is hoped that techniques such as electron diffraction may improve the structural resolution obtained.

The intriguing question which now arises is the mechanism by which specific unidirectional proton transport is achieved. Clearly, the central channel does not possess a sufficient degree of discrimination. This will most probably be conferred on the system by the additional water-soluble subunits which make up the whole molecular assembly. By analogy with the mitochondrial ATPase, where DCCD effectively blocks proton transport, it may be that initial uptake by the membrane involves the region between the four helices which constitute the individual subunit. Whatever process occurs, it is clear that the 16 kDa protein presents us with an advantageous system through which the principles of membrane protein structure and assembly can be investigated.

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Optical methods for measuring protein–protein interactions

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Optical techniques for measuring protein–protein interactions in membranes were developed some 10 or more years ago, and there is now a wide literature covering their applications. The techniques fall into two categories, both dependant on the fact that interaction of a protein with another (or others) will slow down the protein’s Brownian diffusion. This diffusion may be rotational, in which case it is necessary to use a time-resolved decay of anisotropy technique. Rotational diffusion of integral membrane proteins is slow, with correlation times in the micro- to millisecond time domain. With the exception of photosensitising CO-complexes of cytochromes, it is necessary to label the protein of interest with a so-called triplet probe. Rotational diffusion is affected by the viscosity of the immediate environment and is strongly affected by the radius of the protein molecule normal to its axis of rotation in the membrane, and therefore by protein–protein interactions. Apart from size effects, rotational diffusion can be greatly slowed if a membrane protein is attached to a large extramembrane structure, such as the cytoskeleton. For reviews, see [1, 2].

The lateral Brownian diffusion of membrane proteins is also much slower in membranes than in aqueous solutions. It is relatively insensitive to size, but greatly slowed if the protein is tethered to an immobile structure, embedded in a gel-phase lipid domain, or obstructed along the measurable diffusion path length by a barrier to diffusion. The minimal measurable diffusion path for optical methods is related to the optical resolution set by the operating wavelength and imperfections of the optical equipment, irrespective of whether a fluorescence photobleaching method or video-recorded observation of colloidal-gold-labelled membrane proteins is used, and is about 1–2 μm in practice. (However, for nanometre resolution, see [3]). For reviews, see [2, 4].

Apart from the diffusional dynamics of membrane proteins, the ability of membrane proteins to bind other molecules external to the membrane is of considerable interest. I wish to emphasize the potential of certain biosensor technologies for studying such binding. Much of the commercial drive for developing biosensors has come from the wish to develop cheap disposable devices for immunoassay or DNA hybridization. Of greatest interest are methods which depend on the binding from bulk phase of one partner of a binding pair to the other partner previously attached to the surface of a transducer, and where the signal
Comparison of frozen-hydrated and negatively stained crystals of Ca-ATPase suggests a shape for the intramembranous domain

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The Ca\(^{2+}\) pump from sarcoplasmic reticulum (SR) (Ca-ATPase) has been intensively studied over a long period and now represents an archetype for a broad class of ion pumps, which also includes the Na\(^{+}/K^{+}\)-ATPase from mammalian cell membranes, the H\(^{+}\)-ATPase from yeast, and the K\(^{+}\)-ATPase from bacteria. Like most membrane proteins, however, the structure of Ca-ATPase has been difficult to study and the physical basis for the vast amount of biochemical and biophysical data, therefore, remains speculative. Low resolution structures have been obtained from SR membranes both by X-ray diffraction of partially oriented pellets [1] and by electron microscopy of two-dimensional arrays [2, 3]. These structures reveal a pear-shaped, cytoplasmic head (roughly 65 Å×45 Å×45 Å) that is connected to the membrane by a relatively narrow stalk (25 Å diameter and 25 Å high). Very little protein protrudes from the luminal surface of the SR membrane, indicating not only that Ca-ATPase has a highly asymmetric structure, but also that all molecules protrude from the same side of the SR, as is required for vectorial Ca\(^{2+}\) transport. The shape of the intramembranous domain is uncertain owing, in the case of electron microscopy, to the use of negative stain and, in the case of X-ray diffraction, to cylindrical averaging that results from the lack of crystallinity in pellets. However, scrutiny of the amino acid sequence has led to the prediction of 8–10 transmembrane helices [4]. Several polar residues buried in the middle of four of these helices appear to form the Ca\(^{2+}\)-binding sites, according to recent studies of site-directed mutants [5]. Sites of ATP-binding and phosphorylation have also been identified in the sequence, but there is no real consensus regarding the physical location of these sites.

Recently, small three-dimensional crystals were grown from detergent-solubilized SR [6, 7]. By purifying Ca-ATPase and then reconstituting it with precise amounts of lipid, we obtained larger, better ordered crystals than those shown in the first reports. With the use of negative stain, the crystals were shown to comprise stacks of disc-like layers with Ca-ATPase molecules protruding from both surfaces of each layer (Fig. 1). Because considerable amounts of both lipid and detergent are present, the layers were presumed to be mixed bilayers of lipid and detergent with detergent also forming an annulus at the edges of each layer. As molecules protrude from both surfaces of the crystalline bilayer, the packing density was estimated to be approximately double that of the SR membrane. Contacts between neighbouring


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Fig. 1. Packing of Ca-ATPase molecules in the three-dimensional crystals

Each Ca-ATPase molecule is depicted as an ellipsoid on a narrow cylinder placed asymmetrically into the planar, hydrophobic layer; this shape was previously deduced from negatively stained, two-dimensional arrays within SR membranes [2, 3]. Molecules at the front and on the right margins have been sectioned to reveal the portion crossing the hydrophobic layer, which in reality is much larger than depicted here. Proposed molecular contacts occur along the axis of stacking (c) between the tops of Ca-ATPase heads (ellipsoids), and along the axis of ribbons (b) between the ends of Ca-ATPase heads. The contact between ribbons (along a) has not been clearly observed and is therefore probably within the hydrophobic layer. The unit cell dimensions are those for negatively stained crystals, while dimensions for frozen hydrated crystals are 166.5 Å×55.7 Å×164 Å. From [8] with permission of the Biophysical Journal.