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Ultrastructure of the Na, K-ion Pump

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Na, K-ATPase has been analysed by electron microscopy to obtain information about the structure of the enzyme and its organization within the membrane. Following negative staining the membrane-bound enzyme was observed as surface particles which on the basis of their size and frequency and the enzymatic and chemical composition of the membranes are interpreted as protomers ($\alpha\beta$-units). Freeze-fracture electron microscopy revealed the enzyme as intramembrane particles. Quantitative electron microscope studies suggested that the intramembrane particles are oligomers of the protein unit that forms the surface particles. Following reconstitution of the enzyme into phospholipid vesicles it was demonstrated that similar intramembrane particles represent a protein unit which transports sodium and potassium. Vanadate and magnesium induced the formation of two-dimensional crystals in the membrane fragments of the purified Na, K-ATPase. Further information regarding the shape and dimensions of the protomer was obtained through analysis of electron micrographs of negatively stained crystals with optical diffraction and image reconstruction methods.

(Key Words: Na, K-ATPase, Membrane protein, Electron microscopy, Two-dimensional crystals)

INTRODUCTION

Na, K-ATPase is responsible for the active transport of sodium and potassium across the cell membrane. The concentration of the Na, K-pump is particularly high in the thick ascending limb of the loop of Henle in the kidney and the enzyme can be purified from the outer renal medulla in membrane-bound form (6). The purified enzyme preparation contains only two proteins: a large polypeptide, α-subunit, with M_r 104,000, which carries out all catalytic functions, and a small glycoprotein, β-subunit, with M_r 40,000 for protein.

With the aim of characterizing the ultrastructure of the membrane-bound Na, K-ATPase we have analyzed the purified enzyme by electron microscopy following thin sectioning, negative staining and freeze-fracture (3, 8, 9) and following reconstitution of the enzyme into phospholipid vesicles (10). Recently we have also obtained information regarding the structure of the enzyme protein following the induction of two-dimensional crystals in the membrane fragments of the purified Na, K-ATPase (4, 11). In this paper we discuss the ultrastructural characteristics of membrane-bound Na, K-ATPase and illustrate the possibilities for electron microscope

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analysis of the Na, K-pump protein in combination with optical diffraction and image reconstruction methods.

ULTRASTRUCTURE OF MEMBRANE—BOUND Na, K-ATPase

In thin sections the purified enzyme preparation consists of flat or cup-shaped membrane fragments. Following negative staining with phosphotungstic acid the surfaces of purified Na, K-ATPase membranes reveal a uniform population of surface particles (7). The particles have an estimated diameter of 30-50 Å and are arranged in clusters and strands, which are separated by empty areas interpreted as lipid regions. The frequency of the surface particles is 12,500/μm^2 and both membrane surfaces show a similar distribution of particles. On the basis of the chemical and enzymatic composition of the preparation we have calculated that an $\alpha\beta$-unit (protomer) should have a diameter of about 50 Å and a frequency of 13,700/μm^2 (3). Since these calculated parameters are very close to the observed values for the surface particles we have concluded that one surface particle represents one protomer $\alpha\beta$-unit of the enzyme protein.

The organization of the Na, K-ATPase protein within the membrane was analyzed by freeze-fracture electron microscopy (Fig. 1). The frequency of intramembrane particles, determined as the combined particle frequency of the two fracture faces of the membranes, was 6,100 particles/μm^2. The diameter of unidirectionally shadowed particles was approximately 90 Å. These quantitative results demonstrate that negative staining and freeze-fracture electron microscopy reveal different aspects of the Na, K-ATPase protein. Since the ratio between the frequencies of surface particles and intramembrane particles was close to 2:1 we suggested that the intramembrane particles are oligomers, most likely dimers, of the protein units that constitute the surface particles (3, 9).

Freeze-fracture followed by rotary replication provided further evidence for an oligomeric organization of the intramembrane particles. Symmetric shadowing of fracture-faces with a thin layer of platinum at an angle of 10° revealed that many particles with a total diameter of about 90 Å, appeared to be divided into two approximately equal subunits. Correlations of the qualitative and quantitative electron microscope observations of the Na, K-ATPase membranes with the enzymatic and chemical characteristics of the membranes are consistent with the interpretation that the intramembrane particles observed by freeze-fracture represent Na, K-ATPase molecules with a dimeric composition.

Further evidence that intramembrane particles represent the protein of the Na, K-pump was obtained by correlating the ultrastructure and the transport characteristics of phospholipid vesicles reconstituted with different amounts of pure Na, K-ATPase (10). The vesicles were reconstituted with purified Na, K-ATPase using a cholate dialysis method similar to that of Anner *et al.* (1). The transport properties of the vesicles were determined with ^{22}Na and ^{42}K. The experiments demonstrated that the capacity for active cation transport increased in proportion to the amount of Na, K-ATPase used in the reconstitution of the vesicles. Freeze-fracture electron microscopy showed that the preparations consisted of vesicles approximately

900 Å in diameter and that the vesicle membrane contained 90 Å intramembrane particles. The frequency of intramembrane particles in the vesicle membrane increased in proportion to the amount of Na, K-ATPase added to the reconstitution medium. The observations therefore show that the intramembrane particles in the reconstituted phospholipid vesicles represent a protein unit which transports sodium and potassium. Furthermore, the diameter (90 Å) of the intramembrane particles in the phospholipid vesicles was similar to the diameter of intramembrane particles in purified Na, K-ATPase membranes which indicates that they represent the same protein unit.

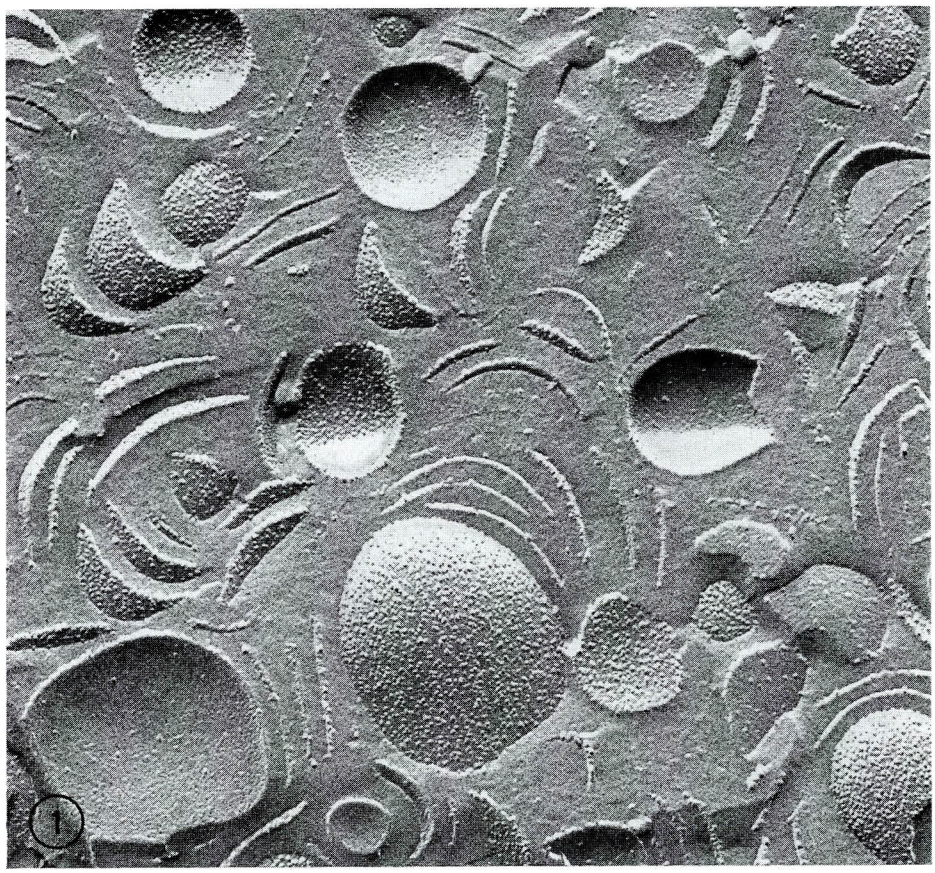
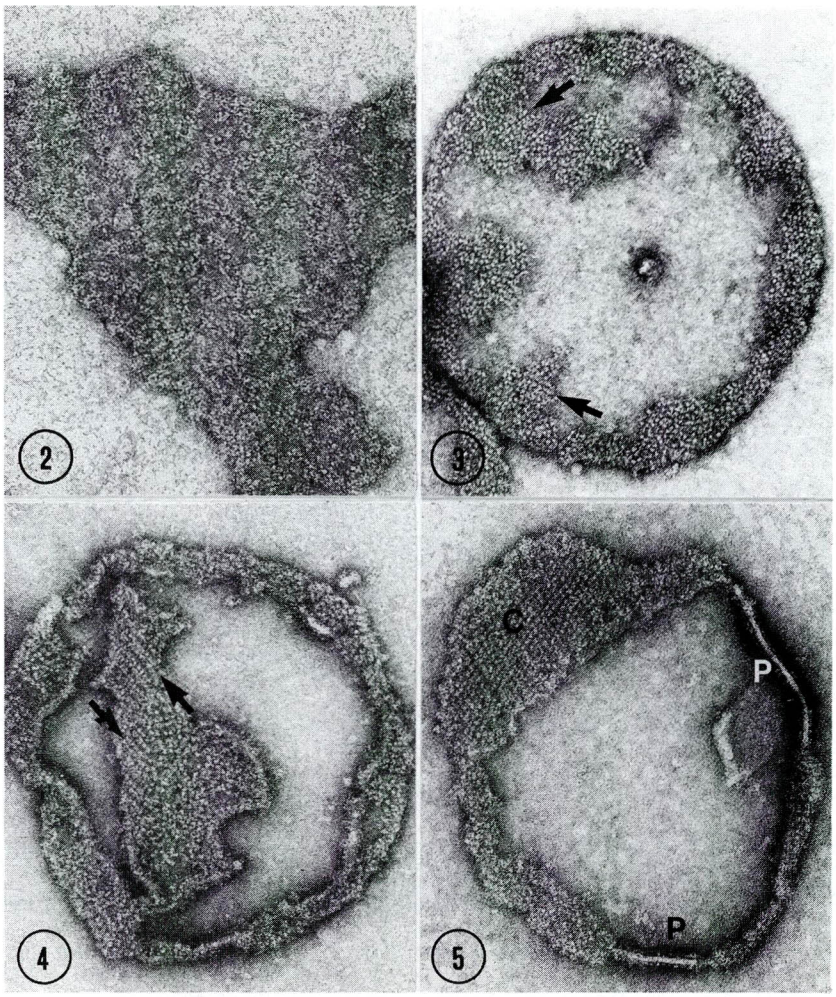


Fig. 1 Freeze-fractured membrane-bound Na, K-ATPase from pig kidney. Tangentially oriented fracture-faces are convex or concave and show either a very high or a low frequency of intramembrane particles. 60,000x.

ANALYSIS OF TWO—DIMENSIONAL CRYSTALS OF Na, K-ATPase

Negative staining of membrane-bound Na, K-ATPase with uranyl acetate following incubation in 10 mM Tris-HCl without ligands invariably showed the surface particles to be arranged randomly in clusters and strands (Fig. 2). Incubation of the enzyme with 1 mM $MgCl_2$ alone or with 1 mM

$MgCl_2$ and 2mM P_i-Tris caused an increased packing of the surface particles in larger clusters separated by areas devoid of enzyme. The packing of the surface particles was even more pronounced when the ligand concentrations were raised to 3mM $MgCl_2$ and 12.5mM P_i-Tris (Fig. 3). In this medium linear arrays of particles were observed but regular two-dimensional crystals were rare. However, following incubation of the membrane-bound Na, K-ATPase with sodium monovanadate in the presence of magnesium linear arrays and later two-dimensional crystals formed in a reproducible way in almost all membrane fragments (Figs. 4, 5, 6). The crystals were fully devel-



Figs 2-5 show membrane fragments of purified Na, K-ATPase following incubation in 10mM Tris-HCl with different ligands and negative staining with uranyl acetate. Fig. 2: Incubation in 10mM Tris-HCl without ligands for 4 weeks. 200,000x. Fig. 3: Incubation in 3mM $MgCl_2$ and 12.5mM P_i-Tris for 4 weeks. Arrows indicate linear arrays of particles. 145,000x. Fig. 4. Incubation in 0.25mM $NaVO_3$ and 1mM $MgCl_2$ for 2 hours. Arrows show linear polymers composed of paired protein units. 130,000x. Fig. 5: Incubation in 0.25mM $NaVO_3$ and 1mM $MgCl_2$ for 4 weeks. C, two-dimensional Na, K-ATPase crystal; P, elongated membrane projections. 185,000x.

oped after about 4 weeks of incubation. Since the preparations show a high purity with respect to ultrastructure, enzymatic assays and protein composition (3, 6) there is no doubt that the crystals are composed of the proteins of Na, K-ATPase.

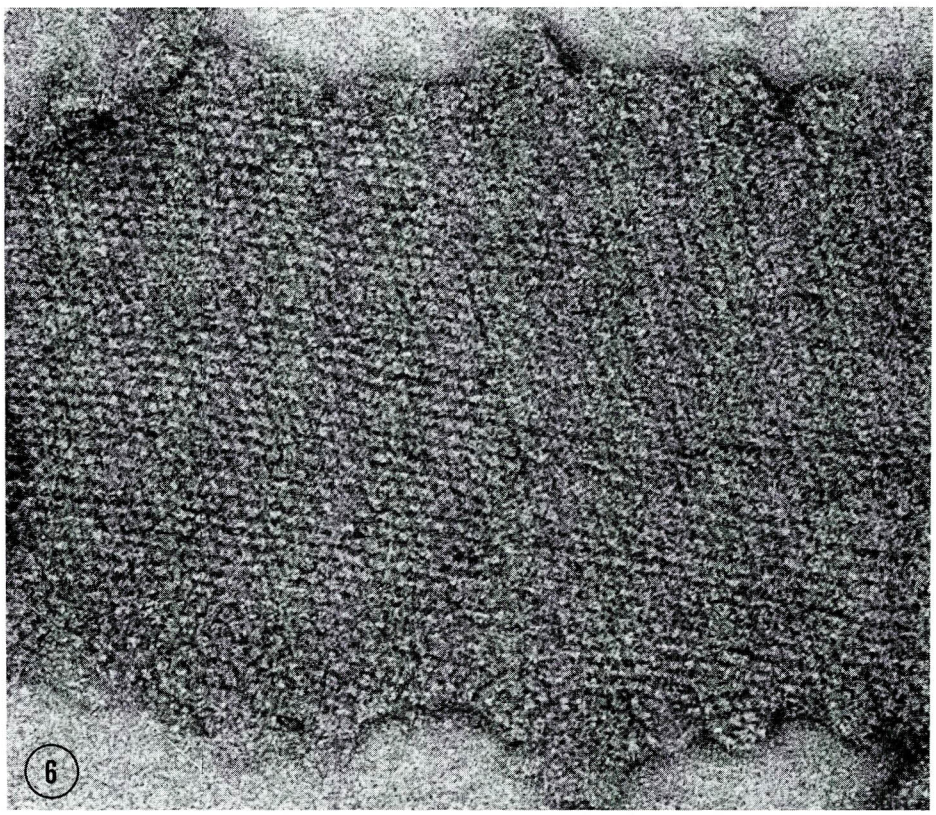


Fig 6 Two-dimensional crystals of membrane-bound Na, K-ATPase induced by incubation in 0.25 mM $NaV0_3$ and 1 mM $MgCl_2$ for 4 weeks at 4°C. 475,000x.

The first observable step in the assembly of the crystals was the formation of linear, ladder-shaped polymers, which consisted of paired protein units (Fig. 4). The linear polymers could be observed already 2 hours after start of incubation and seemed to associate laterally to form two-dimensional crystals. The formation of crystals was associated with a separation of protein and lipid within the membrane. In many membrane fragments this separation coincided with the appearance of elongated projections with particles projecting on both sides. The projections extended from membrane regions with a high density of surface particles (Fig. 5).

Vanadate-induced two-dimensional crystals showed a non-orthogonal lattice. Many crystals exhibited a high degere of order and the diffraction patterns of the membrane crystals extended to 25 Å. Two-dimensional computer-reconstructed images were calculated for vanadate-induced crystals using the image analysis system EM (5) to obtain the averaged structure of the Na, K-ATPase protein in projections perpendicular to the

membrane (4). The unit cell dimensions of the computer-averaged image seen in Fig. 7 are a = 69 Å, b = 53 Å, and $\gamma = 105°$. The unit cell contains only one protein particle which is interpreted as an $\alpha\beta$-unit (protomer) since the area of the unit cell is 3,540 $Å^2$ and since there is no evidence for oligomerization. The reconstructed images suggest that the $\alpha\beta$-unit in this projection varies in diameter between 40 and 60 Å, which is in good agreement with our observation on particles in membrane-bound Na, K-ATPase (3, 9) and with estimates from chemical and enzymatic composition (6). Although the vanadate-induced crystals show no evidence of oligomerization, the existence of oligomers in the membranes outside the crystals cannot be excluded. In fact, the initial formation of linear polymers consisting of paired protein units (Fig. 4) indicates that oligomers may occur as well. Furthermore, an $(\alpha\beta)_2$-unit occupied one unit cell in some crystals induced by phosphate and magnesium (4), which also indicates that interactions between $\alpha\beta$-units can be of importance in the function of the Na, K-ATPase.

The nature of the bonds between the protein units in the crystals remains to be determined but the present observations suggest that the interaction between protein units in the membrane is enhanced when the protein is in the E_2-conformation which is stabilized by vanadate (2, 7).

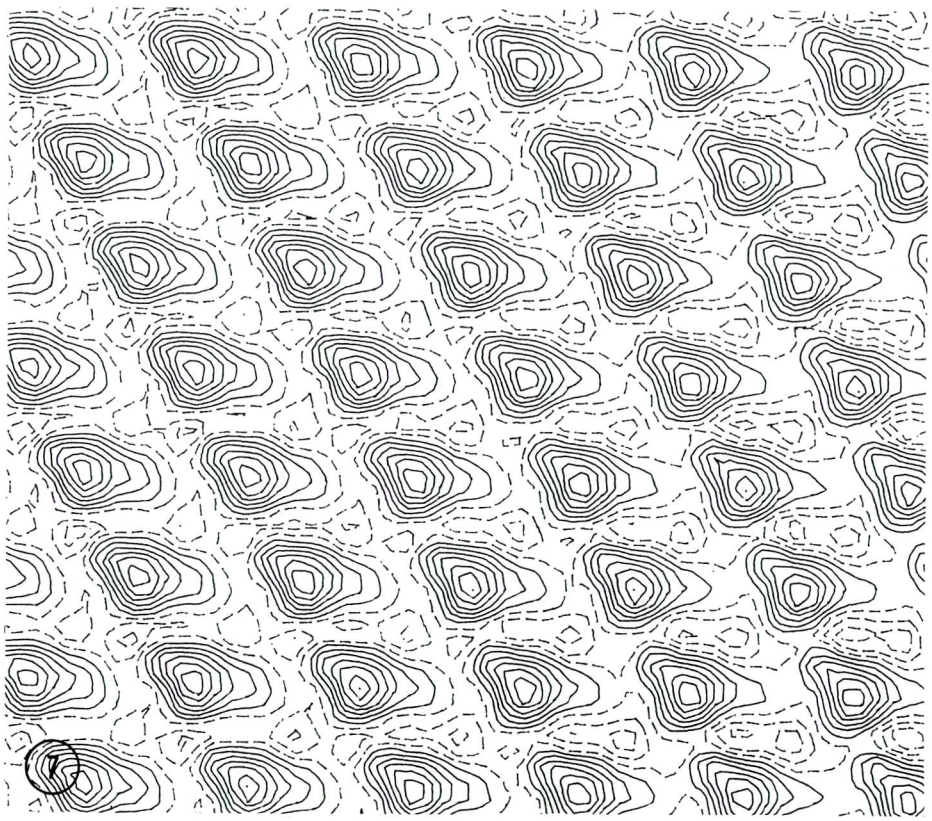


Fig. 7 Computer-reconstructed image from vanadate-induced two-dimensional crystal of Na, K-ATPase. The repeating units drawn with unbroken contour lines represent enzyme protein. The dashed lines outline uranyl acetate-rich regions which may represent depressions in the membrane surface. Scale: 1 mm corresponds to 3.45 Å.

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