Three-dimensional structure of renal Na,K-ATPase determined by electron microscopy of membrane crystals

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Received 28 May 1985

The three-dimensional structure of Na,K-ATPase was determined by electron microscopy and image processing. Tilt series of negatively stained membrane crystals were recorded. The projections were analyzed by Fourier methods and the data combined to a 3-D model. The unit cell contains two rod-shaped stain-deficient regions interpreted as $\alpha\beta$-protomers of Na,K-ATPase. The rods are related by dyad axes oriented perpendicular to the membrane. Outside the lipid bilayer the rods contact different protein units on the two sides of the membrane.

Na,K-ATPase *Three-dimensional structure* *Two-dimensional crystal* *Membrane protein* *Electron microscopy* *Image processing*

1. INTRODUCTION

Incubation of membrane-bound Na,K-ATPase with vanadate in the presence of magnesium induces the formation of two-dimensional crystals of the enzyme [1]. Electron micrographs of the crystals have been used to determine the Fourier-filtered projection structure of the protein [2]. Here, a 3-D model of Na,K-ATPase has been determined by means of Fourier analysis of tilted projections of negatively stained membrane crystals.

2. MATERIALS AND METHODS

Na,K-ATPase was isolated from the outer medulla of rabbit kidney in the membrane-bound form [3,4]. The specific activity of the enzyme was 28–34 μmol $P_i \cdot min^{-1} \cdot$ mg protein^{-1}. The purified enzyme was incubated for 2–28 days at 4°C with 3 mM NH_4VO_3 and 3 mM $MgCl_2$ in 10 mM imidazole buffer, pH 7.0. Samples were negatively stained with 1% uracyl acetate on hydrophilic carbon films and examined in a JEOL 100 CX electron microscope at a magnification of 50000 ×. Optical diffraction was used to select one tilt series of electron micrographs showing low astigmatism and drift and slightly underfocused so that the first zero of the contrast transfer functions fall at frequencies higher than 0.05 Å^{-1}. The micrographs were digitized in a diode array scanner [5] into 512 × 512 pixel squares. The spot size and increment was 25 μm, corresponding to 5 Å at the specimen level. The procedure for 3-D reconstruction was essentially that of Henderson and Unwin [6,7]. Fourier transforms were calculated for each projection followed by least-squares fitting of reciprocal lattices. The origin in projection was refined to coincide with the possible two-fold symmetry axis. The 3-D phase origin was fixed by minimizing the sum of phase differences along lattice lines for subsequent projections. The amplitude and phase values along lattice lines were plotted and smooth curves were drawn by hand. The combination of 3-D data was made in two-sided plane groups p1 and p21. Calculations of structures were made by Fourier summation using a standard X-ray crystallographic program running on an Amdahl V/7A computer. All other processing of the data was made by the 'EM' system [8] implemented on a NORD-500 computer.

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

3. RESULTS

Pertinent details about the 3-D reconstruction are summarized in table 1. Analysis was performed on a tilt series of a single specimen rather than on tilted views of several crystalline areas since 2-D crystals of Na,K-ATPase show variations in unit cell parameters [2]. To be able to calculate a 3-D structure without any bias from imposed symmetry, data were recorded within one half of reciprocal space, thus tilting from −60 to +60°C. Fourier transforms and Fourier-filtered images of 0° projections recorded as the 1st, 11th and 22nd exposure in the analyzed series were essentially the same. Thus, the accumulated electron dose on the membrane crystal did not cause observable deterioration of the crystal.

Earlier observations have shown that Na,K-ATPase crystallizes in two principally different forms with projection symmetries p1 and p2, respectively [2,9–11]. The crystalline area analyzed in the present work (fig.1) has p2 symmetry when projected perpendicular to the membrane surface (table 1). The 3-D data, sampled on 10 lattice lines (fig.2) of which two examples are given in fig.3, approximately obey the conditions $F_{hkl} = F_{\overline{h}\overline{k}l}$ and $\alpha_{hkl} = -\alpha_{\overline{h}\overline{k}l}$ (table 1). This is consistent with assigning two-sided plane group symmetry p21. The structure presented here thus corresponds to data combined in p21. As a control a 3-D reconstruction was also performed in p1 giving essentially the same result.

The calculated 3-D structure was obtained as sections parallel to the membrane at 5-Å intervals

Table 1

Parameters of the 3-D reconstruction of Na,K-ATPase

Tilt range: −60 to +60° with 6° increments
Unit cell dimensions in projection: a = 126 Å, b = 76 Å, γ = 96.5°
Average deviation of phases from 0 or 180° for ($hk0$) reflections: 13°
Plane group symmetry of projection: p2
Interval for measurements along lattice lines < 0.005 $Å^{-1}$
Mean of difference $F_{hkz^*} - F_{\overline{h}\overline{k}z^*}$ relative to maximum amplitude: 0.17
Mean of difference $\alpha_{hkz^*} + \alpha_{\overline{h}\overline{k}z^*}$: 63°
Two-sided plane group symmetry: p21
Number of sampled lattice lines: 10
Resolution in the plane of the crystal: 30 Å
Sampling interval along lattice lines: 0.002 Å

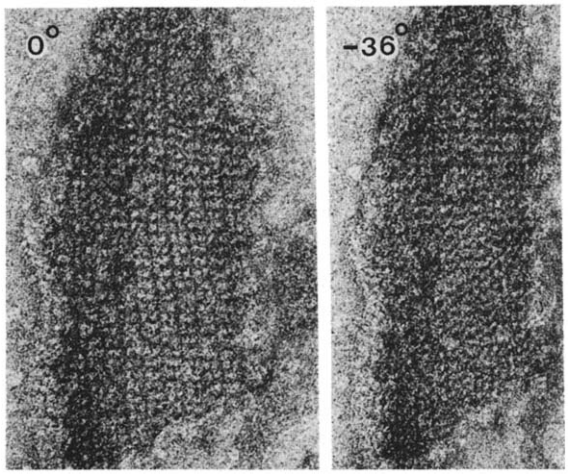

Fig.1. Electron micrographs of membrane crystal of Na,K-ATPase recorded at tilt angles 0 and −36°. Magnification 200000 ×.

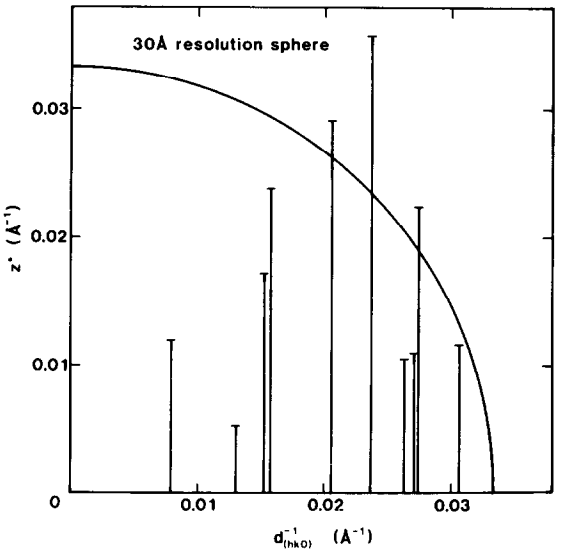

Fig.2. Distribution of experimental data in reciprocal space used for 3-D reconstruction.

through the unit cell. Sections showing significant contrast variation above the noise level are located between approx. +50 and −50 Å relative to the middle of the structure (fig.4). The 2nd positive contour line on these sections (fig.5) was arbitrarily chosen as the protein boundary for construction of a balsa wood model (fig.6). The model shows two symmetrically related rod-like protein regions in the unit cell. The rods are slightly flattened and inclined relatively to the *c*-axis, which runs perpendicular to the membrane. Bridge-like, stain-deficient regions are formed between adjacent rods on both sides of the crystals. On one side the contact is between rods within a unit cell and on the other side it is between rods in neighbouring unit cells located in the direction of the *b*-axis of the crystal.

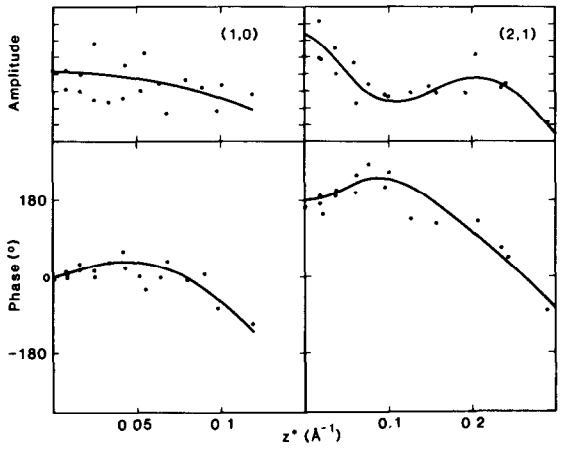

Fig.3. Amplitudes and phases along two lattice lines.

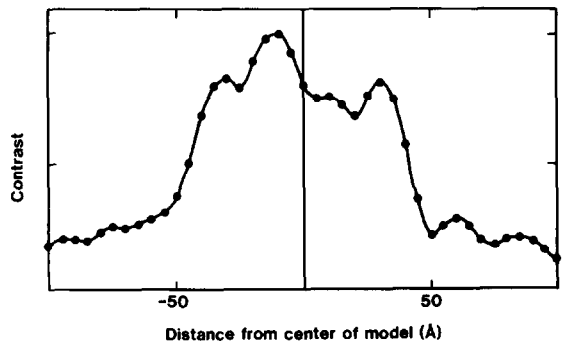

Fig.4. Contrast variation along the *c*-axis determined as the difference between maximum and minimum density on each section through the 3-D map.

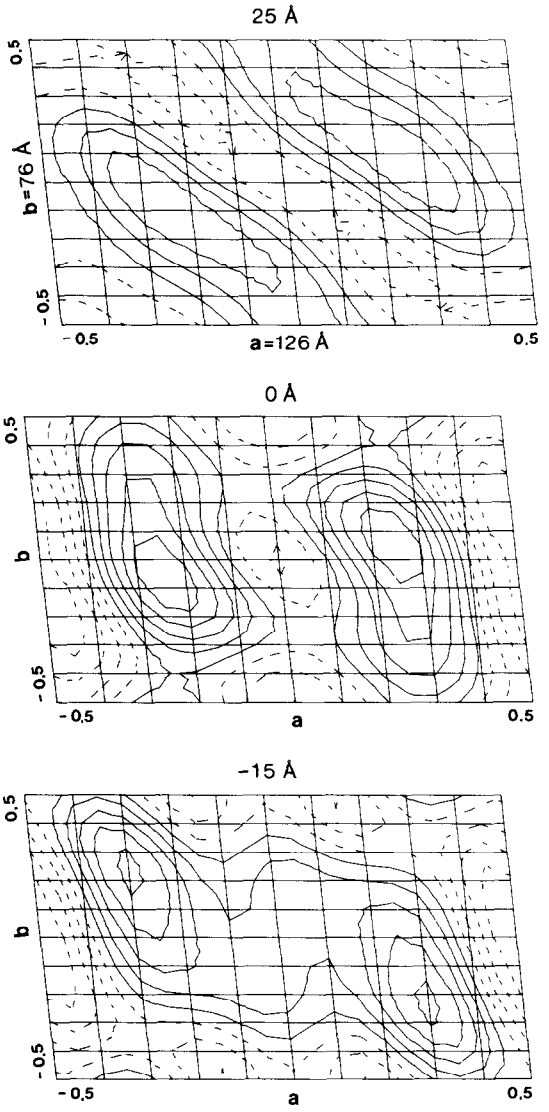

Fig.5. Sections through the 3-D map at 25, 0 and −15 Å relative to the center of the structure as indicated in fig.6. Solid lines correspond to protein-rich regions while negatively stained areas have dashed contours.

4. DISCUSSION

This work provides a 3-D model for membrane-bound Na,K-ATPase as delineated by negative staining. The minimum thickness of the model, i.e. the length of the protein perpendicular to the membrane, is about 100 Å, as deduced from the contrast variation curve in fig.4. The position of the

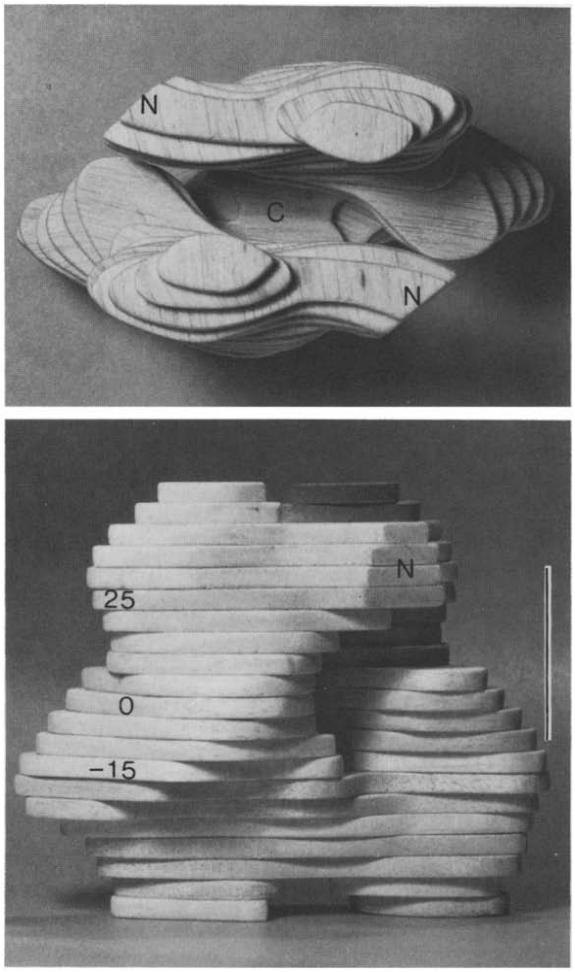

Fig.6. 3-D model of Na,K-ATPase dimer observed in the direction of the *c*-axis (upper panel). There is a deep cleft between symmetry-related units, which are connected at C and relate to neighbouring units at N. Lower figure shows model in a direction parallel to the membrane and about halfway between the *a*- and *b*-axis. The vertical line to the right indicates the level where the contrast variation has a minimum, which may correspond to the level of the lipid bilayer. The levels of the sections shown in fig.5 are indicated to the left.

lipid bilayer is difficult to assign. If the negative stain does not penetrate the bilayer [12] the model should have little contrast in this region. However, at low resolution a sharp discontinuity is smeared out, leaving a local minimum in the contrast variation curve. Such a minimum is seen in the curve in fig.4 between −10 and +30 Å. If this corresponds to the lipid bilayer the protein is asymmetrically distributed in the membrane. It protrudes about 40 Å on one side of the bilayer, which probably corresponds to the cytoplasmic side [10,13] and about 20 Å on the other, probably extracellular side.

The volume of one asymmetric unit, containing one rod-shaped structure, is 475000 Å^3. This is slightly smaller than the value for cytochrome oxidase crystals [12,14,15] accommodating one monomeric protein unit of 165 kDa. Thus, it is reasonable to assume that the continuous stain-deficient region of an asymmetric unit of the Na,K-ATPase crystal corresponds to one $\alpha\beta$-unit of the enzyme since the widely accepted molecular mass of an $\alpha\beta$-protomer is about 140 kDa.

The two asymmetric units of the cell are related by a dyad axis. Therefore, in this crystal form the Na,K-ATPase molecules point in the same direction and form dimers related by 2-fold symmetry. This may reflect the occurrence of dimers in the native membrane. It can be seen from the model that contact regions between monomers are present on both sides of the membrane forming chains of molecules in the *b*-axis direction. Thus, the crystal seems to be held together by strong forces along *b* while weaker interactions take place in the *a*-axis direction. This is consistent with frequent observations of linear arrays of Na,K-ATPase molecules [1]. 3-D reconstruction of Ca-ATPase from sarcoplasmic reticulum also shows contacts between protein units outside the lipid bilayer [16].

ACKNOWLEDGEMENTS

This investigation was supported by the Danish Medical Research Council, the Swedish Medical Research Council (144), Kong Christian den Tiendes Fond and Magnus Bergwalls Stiftelse.

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