Biochimica et Biophysica Acta, 689 (1982) 571–574
Elsevier Biomedical Press

BBA Report

BBA 70053

CRYSTALLIZATION PATTERNS OF MEMBRANE-BOUND ($Na^+ + K^+$)-ATPase

HANS HEBERT [a,*], PETER L. JØRGENSEN [b], ELISABETH SKRIVER [c] and ARVID B. MAUNSBACH [c]

[a] *Max-Planck-Institut für Biochemie, Abteilung für Strukturforschung I, 8033 Martinsried bei München (F.R.G.),* [b] *Institute of Physiology, University of Aarhus and* [c] *Department of Cell Biology at the Institute of Anatomy, University of Aarhus, 8000 Aarhus C (Denmark)*

(Received March 11th, 1982)

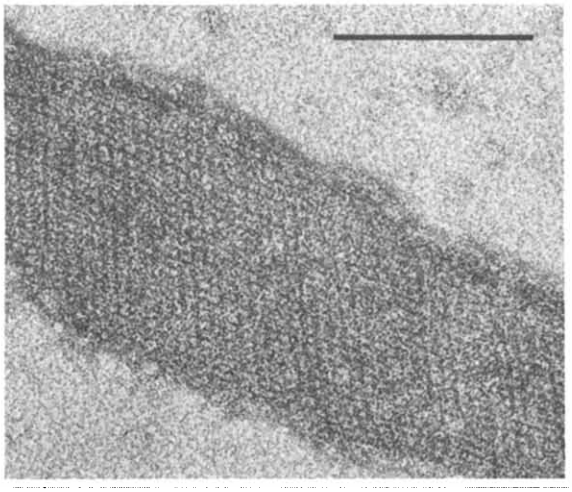
Key words: ($Na^+ + K^+$)-ATPase; Enzyme crystallization

Extensive formation of two-dimensional crystals of the proteins of the pure membrane-bound ($Na^+ + K^+$)-ATPase is induced during prolonged incubation with vanadate and magnesium. Some membrane crystals are formed in medium containing magnesium and phosphate. Computer-averaged images of the two-dimensional crystals show that the unit cell in vanadate-induced crystals contains a protomeric $\alpha\beta$-unit of the enzyme protein. In phosphate-induced crystals an $(\alpha\beta)_2$-unit occupies one unit cell suggesting that interactions between $\alpha\beta$-units can be of importance in the function of the Na^+, K^+ pump.

The membrane-bound ($Na^+ + K^+$)-ATPase can be purified from the outer renal medulla without breaking native lipoprotein associations [1]. The enzyme is pure with respect to the proteins that constitute the ($Na^+ + K^+$)-ATPase, the catalytic α-subunit with M_r 104000, and the β-subunit, which is a glycoprotein, but it is uncertain if an $\alpha\beta$-unit forms the minimum active protein unit or if an oligomeric $(\alpha\beta)_2$ structure is required [2,3]. Recently we demonstrated extensive formation of two-dimensional crystalline arrays of the proteins of the pure ($Na^+ + K^+$)-ATPase after prolonged incubation with vanadate and magnesium and some arrays in media containing phosphate and magnesium [4]. Crystal formation opens new possibilities for studying the structure of the ($Na^+ + K^+$)-ATPase proteins by high resolution electron microscopy, and image reconstruction [5,6]. Even at a limited resolution, this approach may provide important information about molecular sizes and subunit structure of the membrane-bound Na^+, K^+ pump from mammalian kidney [7].

Two-dimensional crystals of membrane-bound ($Na^+ + K^+$)-ATPase are limited in size by the dimensions (100–500 nm) of the membrane fragments in which they grow (Fig. 1 and 2). Comparison of Figs. 1 and 2 and diffraction analysis of electron micrographs of such crystalline membrane fragments show that ($Na^+ + K^+$)-ATPase can crystallize in different forms. The two-dimensional vanadate-induced crystal in Fig. 1 is distinctly different from that illustrated in Fig. 2. Lattice lines are observed in two directions both in Fig. 1 and in Fig. 2, but in the phosphate induced crystal in Fig. 2 the distance between the vertically oriented lattice lines is considerably enlarged. Three crystal forms, A, B, and C are illustrated in Fig. 3 and their unit cell parameters given in the text to Fig. 3. The diffraction patterns for all crystals extend to about 25 Å and they show the lowest possible symmetry (Fig. 3b). The calculated phases of the Fourier components can have any value between 0° and 360° for crystals A and B. For C they are restricted to 0° and 180° with a residual phase error typically between 10° and 20°. This is consistent with assigning two-sided plane group symmetry p1 for crystals A and B and

* Permanent address: Department of Medical Biophysics, Karolinska Institutet, S-10401, Stockholm 60, Sweden.



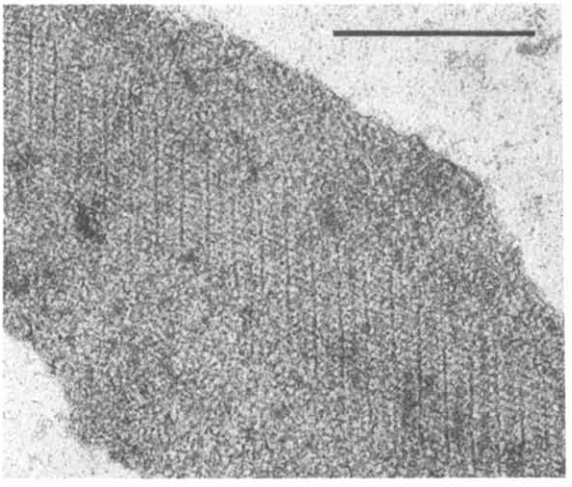


Fig. 1. Two-dimensional crystals of $(Na^+ + K^+)$-ATPase formed during incubation for four weeks at 4°C in 0.25 mM sodium monovanadate, 1 mM magnesium chloride and 10 mM Tris-HCl (pH 7.5) at 20°C. The enzyme was purified in membrane-bound form from pig kidney outer medulla by selective extraction of plasma membranes with SDS in presence of ATP, followed by isopycnic zonal centrifugation in a Ti-14 Beckman zonal rotor [1]. The membranes were negatively stained with uranyl acetate and micrographs were obtained with a Jeol 100 CX electron microscope. Magnification 265000×. Bar 0.1 μm.

Fig. 2. Two-dimensional crystals in membrane fragment of pure $(Na^+ + K^+)$-ATPase purified as in Fig. 1 but incubated in 12.5 mM phosphate, 3 mM magnesium chloride and 10 mM Tris-HCl (pH 7.5). Magnification 265000×. Bar 0.1 μm.

p21 for C (nomenclature according to Ref. 9). The results of the image reconstructions are shown in Fig. 3c.

The size of an $\alpha\beta$-unit (protomer) of $(Na^+ + K^+)$-ATPase has been estimated earlier from electron microscopy of negatively stained and freeze-fracture specimens [10,11]. According to these studies the diameter of an $\alpha\beta$-unit when viewed along an axis perpendicular to the membrane surface is about 50 Å. In the crystalline specimens, the areas of the two-dimensional unit cells for crystals A and B are 3540 Å^2 and 2300 Å^2, respectively. Consequently, it is reasonable to conclude that each unit cell only contains one $\alpha\beta$-unit. The reconstructed images also show one distinct positive peak per unit cell that corresponds to the $\alpha\beta$ protomer. If the outermost unbroken contourline in the maps in Fig. 3c, which corresponds to the average contrast in the original electron micrograph, is defined as border of the protein, the diameter of an $\alpha\beta$-unit is between 40 Å and 60 Å. This is in good agreement with the value of about 50 Å given above. In the C crystals on the other hand, two $\alpha\beta$-units can easily occupy one unit cell. From the projection map, it is also evident that the unit cell contains two strong positive regions. These regions are subdivided into one large and one smaller peak possibly corresponding to the α- and β-subunits, respectively. The dimensions and shape of the large peak are similar to the positive peaks in A and B. The protein-rich regions in the p21 crystals are bounded by rows of negative stain along the *b*-direction. At an angle of about 120° to these rows, adjacent protein-units are connected by stain deficient regions. Thus, two-fold rotationally related protein-units tend to form pairs in the $[1\bar{1}]$-direction of this crystal.

Although the packing arrangements of the enzyme molecules vary considerably in the crystal systems studied, some common features can be observed. The *b*-axis in A and the *a*-axis in B are both 53 Å. Moreover, the angle between *b* and the $[\bar{1}\bar{1}]$-direction (diagonal d_A) in A is close to 120° corresponding to γ in B. Possibly B is a result of shrinkage of A along d_A, which, in turn, may be related to a decrease in the amount of lipid within the membrane crystal during the maturation of the crystal. Crystal systems A and B may thus represent different developmental stages of the crystal. The angle between *b* and the $[1\bar{1}]$-direction (diago-

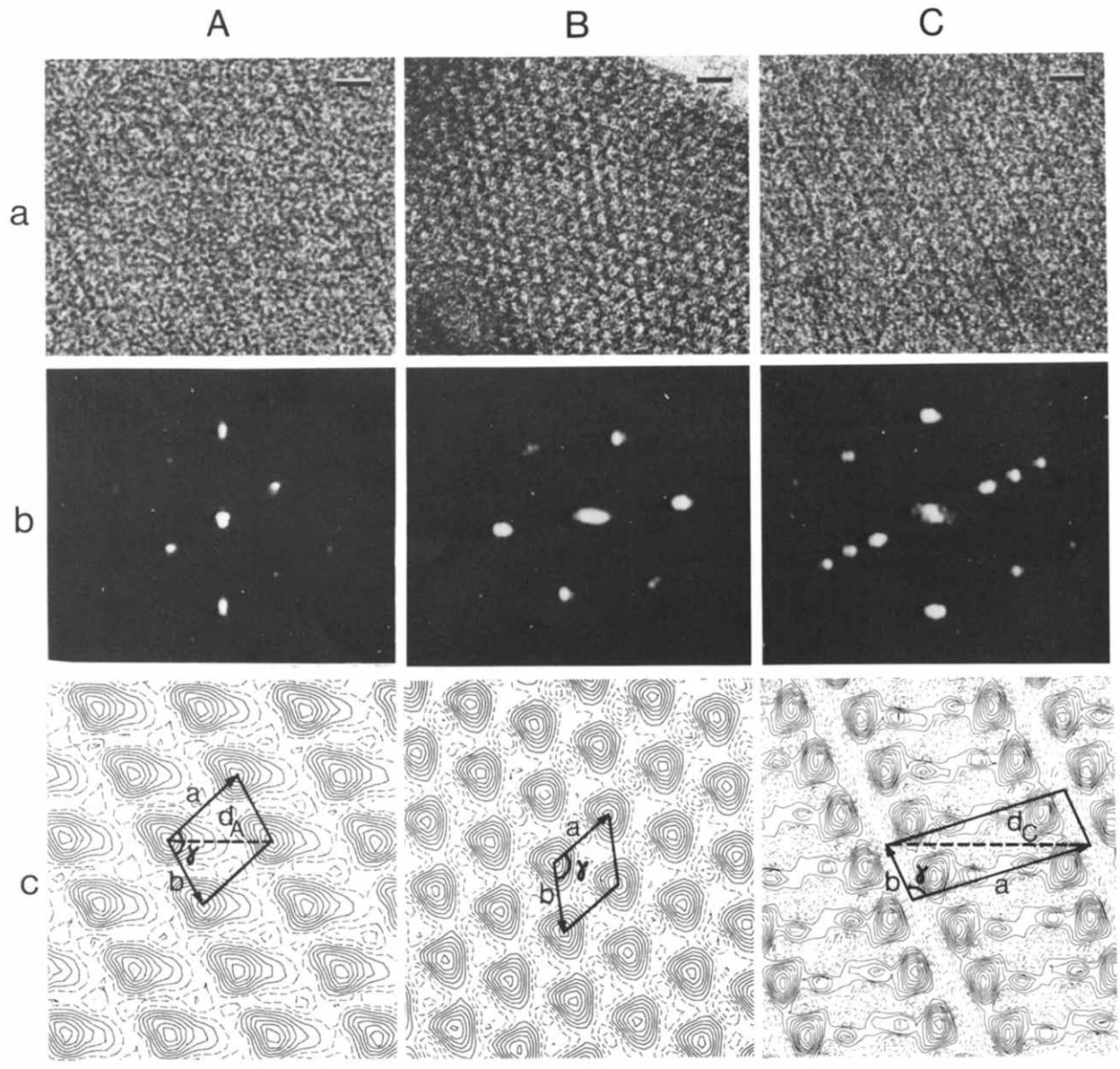


Fig. 3. Two-dimensional crystals of $(Na^+ + K^+)$-ATPase. Each crystal form (A, B, C) is illustrated with an electron micrograph (a), the corresponding diffraction pattern (b), and the computer-reconstructed image from the same same crystal (c). Crystal A is part of the crystal shown in Fig. 1 and Crystal C is part of the crystal shown in Fig. 2 while crystal B was formed in the same way as crystal A. Selection of well-ordered crystalline arrays was made by optical diffraction. Images suitable for further analysis were densitometered at 20-μm intervals. The scanned area was 512×512 steps corresponding to 1872×1872 Å^2 (54700×) and 1575×1575 Å^2 (65000×) at the specimen level. Projection maps were calculated using the Fourier transform amplitudes and phases collected at the reciprocal lattice points. No defocus correction was made since the images were taken sufficiently close to focus that the contrast transfer function was uniform over the range of object spacings of interest. The processing of the electron micrographs was made on a VAX 11/780 using the image analysis system EM [8]. The protein-rich regions (positive regions) were drawn with unbroken contour lines while negative stain regions have dashed lines. The unit cell dimensions are: (A) $a = 69$ Å, $b = 53$ Å, $\gamma = 105°$; (B) $a = 53$ Å, $b = 51$ Å, $\gamma = 120°$; (C) $a = 135$ Å, $b = 44$ Å, $\gamma = 101°$. The diagonals d_A and d_C are referred to in the text. Magnification of electron micrographs (a) 520000×. Bar 100 Å. In the diffraction patterns (b) 1 mm corresponds to $1.6 \cdot 10^{-3}$ Å^{-1}. In the reconstructed images (c) 1 mm corresponds to 4.9 Å.

nal d_C) in C is also close to 120°. Thus, in all crystal systems, an angle close to 120° is found either between two unit cell axes or between an axis and a diagonal. It is not possible to draw final conclusions about the shape of the protein at this resolution level and angle of projection. None the less it can be observed that the strong positive peaks in the reconstructed images of crystal A and the larger peak in C have almost 3-fold symmetry. Moreover, in B, the unit cell parameters are very close to those of a hexagonal or trigonal system although the diffraction pattern does not reveal a perfect 6- or 3-fold symmetry. It is thus possible that one part of the $\alpha\beta$-unit has three structurally similar regions inducing a quasi 3-fold symmetry. Such a quarternary arrangement of domains in the $(Na^+ + K^+)$-ATPase molecule may also explain the commonly observed 120° angle between appropriate directions in the membrane crystals. The small peak in the p21 crystal (C) is not visible in the p1 crystals (A and B). Possibly this part of the molecules is covered by negative stain in those more packed structures. Crystal system C, in contrast to systems A and B, has a unit cell corresponding to an $(\alpha\beta)_2$-unit. This demonstrates that $(Na^+ + K^+)$-ATPase crystallizes in two principally different forms but we cannot exclude the possibility that the phosphate-induced system C represents a transitory stage in the formation of crystals of type A and B. This possibility is consistent with the presence of linear polymers consisting of paired protein units during the formation of vanadate-induced crystals [4].

The identification of the $\alpha\beta$-unit as the minimum asymmetric unit in the vanadate-induced crystals is not the first observation consistent with the possibility that this is the functional unit of $(Na^+ + K^+)$-ATPase. The $\alpha\beta$-unit may possess one binding site for each of the ligands ATP, P_i, vanadate, and ouabain [3,12,13] and the soluble complex of renal $(Na^+ + K^+)$-ATPase consists predominantly of protomeric $\alpha\beta$-units [2]. There is also previous evidence for association between $\alpha\beta$-units both in solution [2,14] and in the membrane-bound state [7,10,11]. Our demonstration that interactions in the two dimensional crystals may occur either directly between protomeric $\alpha\beta$-units of the Na^+, K^+ pump or between dimers of the protomers may in part explain the ambiguity of the previous results of examinations on the subunit structure of the $(Na^+ + K^+)$-ATPase.

Acknowledgements

We thank Lene Jacobsen, Janne Petersen, Inger Nielsen and Einar Hansen for technical assistance. The study was supported by the Danish Medical Research Council.

References

1 Jørgensen, P.L. (1974) Biochim. Biophys. Acta 356, 53–67
2 Brotherus, J.R., Møller, J.V. and Jørgensen, P.L. (1981) Biochem. Biophys. Res. Commun. 100, 146–154
3 Kyte, J. (1981) Nature 292, 201–204
4 Skriver, E., Maunsbach, A.B. and Jørgensen, P.L. (1981) FEBS Lett. 131, 219–222
5 Unwin, P.N.T. and Henderson, R. (1975) J. Mol. Biol. 94, 425–440
6 Fuller, S.D. (1981) in Methods in Cell Biology (Turner, J.N., ed.), Vol. 22, pp. 251–296, Academic Press, New York
7 Jørgensen, P.L. (1980) Physiol. Rev. 60, 864–917
8 Hegerl, R. (1980) in Proceedings of the Seventh European Congress on Electron Microscopy, The Hague (Brederoo, P. and De Priester, W., eds.), Vol. 2, pp. 700–701
9 Holser, W.T. (1958) Z. Kristallogr. 110, 266–281
10 Deguchi, N., Jørgensen, P.L. and Maunsbach, A.B. (1977) J. Cell. Biol. 75, 619–634
11 Maunsbach, A.B., Skriver, E. and Jørgensen, P.L. (1979) in $(Na^+ + K^+)$-ATPase: Structure and Kinetics, (Skou, J.C. and Nørby, J.G., eds.), pp. 3–13, Academic Press, London
12 Moczydlowski, E.G. and Fortes, P.A.G. (1981) J. Biol. Chem. 256, 2346–2356
13 Peters, W.H.M., Swartz, H.G.P., De Pont, J.J.H.H.M., Schuurmans-Stekhoven, F.M.A.H. and Bonting, S.L. (1981) Nature 290, 338–339
14 Hastings, D.F. and Reynolds, J.A. (1979) Biochemistry 18, 817–821