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Three-Dimensional Reconstruction of Protein Kinase Cδ And Its Regulatory Domain By Electron Microscopy of Two-Dimensional Crystals

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Protein Kinases C (PKC) are a family of eleven signal-transducing isozymes that are activated by their interactions with membrane lipid components following stimulation of transmembrane receptors by hormones, neurotransmitters, and growth factors. Since different isozymes are implicated in different signal transduction pathways or diseases, drug design must be targeted to individual isozymes. Progress in development of isozyme-selective drugs has been slow in part because a three-dimensional structure of intact PKC has yet to be determined. A few 3D crystal structures of portions of PKC - the Cys2 activator-binding domain of PKCδ and, very recently, the catalytic domain of PKCθ - have been reported [1-2]. However, all attempts to grow three-dimensional crystals of intact PKC have failed, probably because it is difficult to concentrate PKC beyond 10 μg/ml. High resolution electron microscopy is very attractive for PKC structure determination because 1) it requires much lower enzyme concentrations and 2) PKC crystallized from aqueous solutions would be in an inactive form, whereas 2D crystals grown on lipid monolayers probably reflect an active form. Here we present the first 3D structural reconstructions of calcium-independent PKCδ and its regulatory domain (RDδ) based on processing of high-resolution electron micrographs of tilted series of 2D crystals.

Two-dimensional crystals of PKCδ and RDδ have been grown on lipid monolayers composed of dioleoylphosphatidylcholine: dioleoylphosphatidylserine: diolein (45:50:5, molar ratio). Images have been taken with a TECNAI-12 electron microscope at acceleration voltage of 100 keV, magnification of 67000^{x}, and at tilt angles of +55° to -50°. Analysis of projection maps of untilted images revealed that the unit cells of both PKCδ and RDδ have sides $a \approx b$ and inter-edge angle $\gamma = 120^{o}$. RDδ shows an edge length of 33 ± 1 Å, whereas intact PKCδ has an edge length of 46 ± 1 Å [3]. FIG.1 shows the density maps of one PKCδ (a) and RDδ (b) unit cell. The unit cell parameters suggest P3 symmetry. Calculations with programs (e.g. CRISP) designed to discriminate between P1 and higher order plane groups provided phase residuals for P3 symmetry less than 25^{o} and showed a clear minimum in comparison with other groups [4]. However, 2D reconstruction does not indicate three-fold rotational symmetry nor are the areas of the cells large enough to accommodate three molecules. It is inferred that one unit cell contains only one molecule and thus, only P1 symmetry was applied for further processing.

Data from processed projection maps averaged over 18 (PKCδ) and 14 (RDδ) crystalline areas were used to create untilted core data sets. The results of this procedure are shown in the FIG.2a. Data from tilted films were then added using low tilt angles first, high tilt angles last. Crystals rotated by 180° with respect to the core data set were identified by phase residual comparisons after merging rotated or unrotated data with the core data set in reciprocal space. FIG. 2b shows a typical

distribution of intensities of the Fourier components of PKCδ along the z* axis, that is perpendicular to the a*b* plane, after merging of tilted images. Data presented in FIG.2 allow us to estimate the resolution of the reconstruction as ~3 Å in the a*b* plane and ~10 Å along the z* direction. The problem of determining the correct phase origin in the 2D projection as well in three-dimensions will be discussed. Because the regulatory domain confers membrane attachment, strong inferences can be made about the orientation of the intact PKCδ molecule relative to the lipid monolayer.

References

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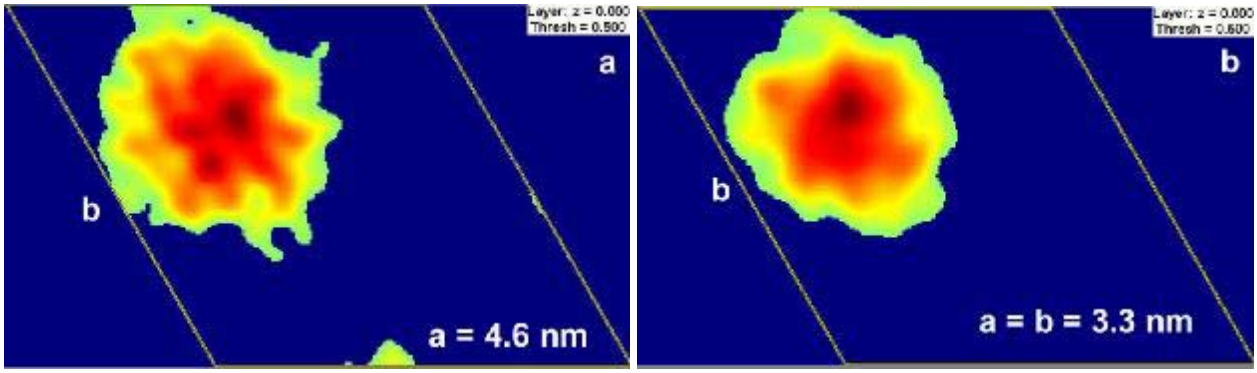

FIG.1. The projection density map of one PKCδ (a) and RDδ (b) unit cell. Color scheme: Red is closer to an observer.

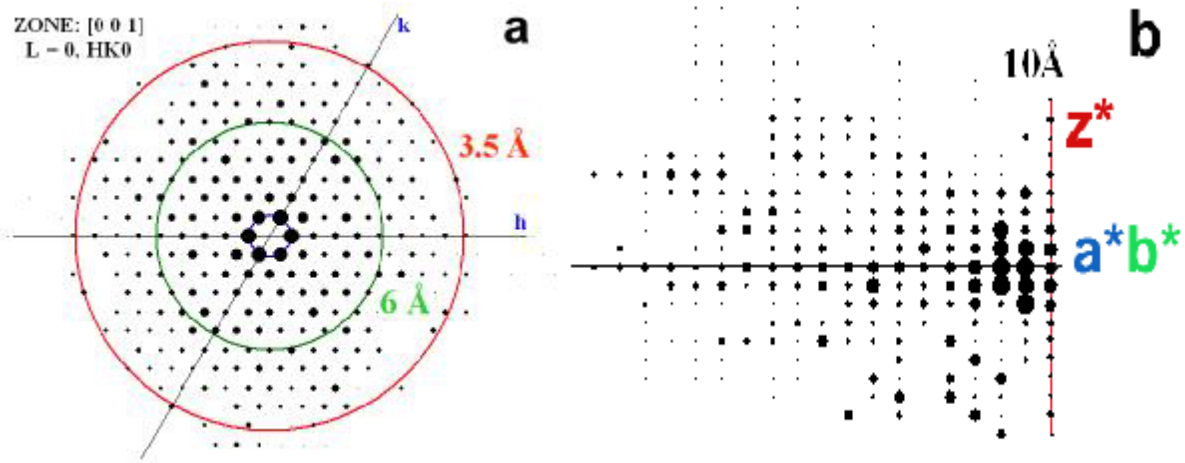

FIG. 2. Plot of intensities of the Fourier components of PKCδ: a – in the a*b* plane; b – along the z* direction (fragment). The circle sizes of individual reflections indicate their IQ values. The large circles depict the different resolution levels. Similar data, not shown, were obtained for RDδ.

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