Polycystin-2 (PC2) is a Ca\(^{2+}\)-permeable transient receptor potential channel activated and regulated by changes in cytoplasmic Ca\(^{2+}\). PC2 mutations are responsible for ~15% of autosomal dominant polycystic kidney disease. Although the C-terminal cytoplasmic tail of PC2 has been shown to contain a Ca\(^{2+}\)-binding EF-hand domain, the molecular basis of PC2 channel gating by Ca\(^{2+}\) remains unknown. We propose that the PC2 EF-hand is a Ca\(^{2+}\) sensor required for channel gating. Consistent with this, Ca\(^{2+}\) binding causes a dramatic decrease in the radius of gyration (\(R_g\)) of the PC2 EF-hand by small angle x-ray scattering and significant conformational changes by NMR. Furthermore, increasing Ca\(^{2+}\) concentrations cause the C-terminal cytoplasmic tail to transition from a mixture of extended oligomers to a single compact dimer by analytical ultracentrifugation, coupled with a >30 Å decrease in maximum interatomic distance (\(D_{\text{max}}\)) by small angle x-ray scattering. Mutant PC2 channels unable to bind Ca\(^{2+}\) via the EF-hand are inactive in single-channel planar lipid bilayers and inhibit Ca\(^{2+}\) release from ER stores upon overexpression in cells, suggesting dominant negative properties. Our results support a model where PC2 channels are gated by discrete conformational changes in the C-terminal cytoplasmic tail in response to changes in cytoplasmic Ca\(^{2+}\) levels. These properties of PC2 are lost in autosomal dominant polycystic kidney disease, emphasizing the importance of PC2 to kidney cell function. We speculate that PC2 and the Ca\(^{2+}\)-dependent transient receptor potential channels in general are regulated by similar conformational changes in their cytoplasmic domains that are propagated to the channel pore.

Autosomal dominant polycystic kidney disease (ADPKD)\(^3\) is a common systemic disorder that affects between 1 in 400 and 1 in 1000 individuals worldwide. The disease is characterized by renal and hepatic cysts, intracranial or aortic aneurysms, and mitral valve prolapse (1). Most cases of ADPKD (>95%) are caused by genetic mutations in either the \(Pkd1\) or the \(Pkd2\) gene, which encode polycystin-1 (PC1) and polycystin-2 (PC2/TRPP2), respectively (2).

PC2 is a six-transmembrane Ca\(^{2+}\)-permeable TRP channel with cytoplasmic C and N termini (1). After their initial discovery as environmental sensors of chemical and physical stimuli, TRP channels were found to perform a wide range of physiological functions (3). Although all TRPs are thought to be six-transmembrane tetrameric channels, sequence conservation is limited to residues forming the predicted channel pore. Very little is known about the molecular movements needed to activate any TRP channels, including PC2, to open the channel pore to allow ionic fluxes. However, a common theme among TRP channels is the presence of cytoplasmic extensions with various functional domains, such as ankyrin repeats, kinase domains, coiled coil motifs, and EF-hand domains (3). These widely variable cytoplasmic tails are believed to serve as signaling sensors, possibly allowing TRP channels to be activated or modulated by specific stimuli, such as protein-protein interactions or ligand binding (3), which are then believed to be transduced into conformational changes that regulate opening of the channel pore. However, how these sensor domains communicate with the ion channel pore remains unknown.

We and others have previously shown that, like other TRP channels, the C-terminal cytoplasmic tail of PC2 (PC2-C, residues 704–968) contains several possible functional domains:

\(^3\)The abbreviations used are: ADPKD, autosomal dominant polycystic kidney disease; PC, polycystin; TRP, transient receptor potential; SANS, small angle x-ray scattering; 2DSA, two-dimensional spectrum analysis; CSI, chemical shift index.

**Background:** Polycystin-2, a calcium-permeable TRP channel, is mutated in autosomal dominant polycystic kidney disease. **Results:** Calcium binding by the polycystin-2 EF-hand domain induces discrete conformational and oligomerization state transitions that impact channel gating. **Conclusion:** Polycystin-2 channel activity is regulated by cytoplasmic calcium-induced conformational changes. **Significance:** These studies provide a structural and mechanistic understanding for the impact of calcium binding on channel regulation.
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an EF-hand domain (PC2-EF, residues 720–797) connected by a flexible acidic linker to a coiled coil domain (residues 833–895) (4–9). Based on structural analysis and modeling of these domains, it has been suggested that they have functional roles in Ca\(^{2+}\)-dependent channel activation (via the EF-hand domain) and oligomerization (via the coiled coil domain) (4–9). Although the crystal structures of the isolated PC2 coiled coil are trimeric (7) and that of the related PKD2L is also trimeric (10), several groups have reported PC2-C dimers in solution (11). Moreover, PC2 channels have been shown to function as homotetramers and 2:2 heterotetramers (in conjunction with TRPC1) (12); thus, residues outside of the coiled coil domain play a role in regulation of the oligomerization state of the PC2 cytoplasmic tail (11, 13) and PC2 channels (12).

The NMR structure of the isolated Ca\(^{2+}\)-bound PC2 EF-hand revealed a single Ca\(^{2+}\)-binding site (5), whereas iso- thermal titration calorimetry showed that this domain binds Ca\(^{2+}\) noncooperatively (\(K_d = \sim 200 \mu M\)), and CD spectroscopy showed that PC2-EF undergoes a Ca\(^{2+}\)-induced increase in \(\alpha\)-helicity (4). In the context of the entire C-terminal cytoplasmic tail, however, the affinity for Ca\(^{2+}\) is increased (\(K_d = \sim 10 \mu M\)), and the mode of Ca\(^{2+}\) binding appears to be cooperative, implying that residues outside of the EF-hand domain contribute to Ca\(^{2+}\) binding (4). This therefore raises the potential for synergistic interactions between the EF-hand and coiled coil domains during PC2 channel gating.

Although PC2 channels have been shown to be gated by changes in cytoplasmic Ca\(^{2+}\) levels, the molecular basis of channel gating is unknown. PC2 channel activity is bell-shaped with respect to Ca\(^{2+}\), withactivation by low levels of cytoplasmic Ca\(^{2+}\) and inhibition at higher Ca\(^{2+}\) concentrations. Phosphorylation of the PC2-C acidic linker at Ser-812 modulates the threshold concentration of Ca\(^{2+}\) required for PC2 channel activation, and the Ca\(^{2+}\) dependence of PC2 channel activity requires intact PC2-C (many PC2 ADPKD mutations result in truncations of the C-terminal tail). Furthermore, channel activity can be altered by C-terminal cytoplasmic tail-mediated interactions with other proteins, such as PC1 (14–19). Together, these data suggest that the C-terminal cytoplasmic tail of PC2 plays an important role in PC2 channel gating. The molecular basis for these regulatory mechanisms, however, remains unknown. Here we show by SAXS, NMR, and AUC that Ca\(^{2+}\) binding by the PC2 EF-hand domain induces discrete conformational and oligomerization state transitions in the entire C-terminal cytoplasmic region. Moreover, we demonstrate that mutant PC2 channels unable to bind Ca\(^{2+}\) via their EF-hand domains are completely inactive in single-channel planar lipid bilayers and inhibit Ca\(^{2+}\) release from endoplasmic reticulum (ER) stores upon overexpression in mammalian cells. Based on our results, we propose a molecular model of PC2 channel gating in response to changes in cytoplasmic Ca\(^{2+}\) levels.

**Experimental Procedures**

**Purification of Recombinant PC2 Fragments**—Fragments of human polycystin-2, PC2-EF (720–797) and PC2-C (704–968), were PCR-amplified from human PC2 cDNA, cloned into pET-28 (a+) (Novagen), and transformed into BL21(DE3) CodonPlus RIL (Stratagene) for bacterial expression as previously described (4). Bacterial cultures were grown in either \(^{15}\)N or \(^{13}\)C,\(^{15}\)N isotope-enriched M9 minimal medium (for NMR experiments) or LB medium, containing 50 \(\mu\)g/ml kanamycin and containing 30 \(\mu\)g/ml chloramphenicol to an \(A_{600} \sim 0.6\) at 37 °C, induced with isopropyl-\(\beta\)-d-thiogalactopyranoside at a final concentration of 1 \(\mu\)M and shifted to 18 °C for \(\sim 18\) h. The cells were harvested and resuspended in Buffer A (20 mM Tris, 500 mM NaCl, pH 8.0), lysed by freeze thaw/sonication with lysozyme (\(\sim 1\) mg/ml) (Sigma), and clarified by centrifugation. Supernatant was loaded onto a 1-ml HisTrap column (GE Bio- sciences). The column was washed with 30 column volumes of Buffer A and then with 10 column volumes of Buffer A with 50 mM imidazole. Purified PC2 fragments were eluted in Buffer A with 500 mM imidazole and applied to a Bio-Rad desalting column for buffer exchange. Protein concentration was quantified using the Bradford colorimetric protein assay.

**Analytical Ultracentrifugation**—Sedimentation equilibrium experiments were performed with a Beckman Optima XL-I at the Center for Analytical Ultracentrifugation of Macromolecular Assemblies (University of Texas Health Science Center at San Antonio, Department of Biochemistry). PC2-C was first stripped of Ca\(^{2+}\) using 100 mM EDTA and then run over a Superdex-200 and desalted into Ca\(^{2+}\)-free buffer over a Bio- Rad 6000 molecular weight cutoff column. The sample was split into three, and CaCl\(_2\) was added back in two samples to concentrations of 100 \(\mu\)M and 5 \(\mu\)M. The samples were analyzed in 10 mM MOPS, 150 mM NaCl, pH 7.2, in the presence 1 mM EDTA, 100 \(\mu\)M CaCl\(_2\), or 5 \(\mu\)M CaCl\(_2\). Sedimentation velocity experiments were performed at 40,000 rpm and 20 °C. Absorbance samples were spun in two-channel epon/charcoal centerpieces in the AN-60-T1 rotor. A loading concentration of 0.9 \(A_{280}\) for PC2-C was scanned at 231 nm with 0.001-cm step size setting and no averaging. The concentration of PC2-C used was 94 \(\mu\)M for PC2-C (\(\varepsilon = 9530 \)A\(_{280}\) mol\(^{-1}\) cm\(^{-1}\)) the extinction coefficient at 280 nm was estimated from the protein sequence according to the method by Gill and von Hippel (20). AUC experiments were analyzed with UltraScan version 9.5 (21). Hydrodynamic corrections for buffer conditions were made using UltraScan (21). The partial specific volumes of PC2-EF and PC2-C were estimated in UltraScan and were found to be 0.722 and 0.717 ccm/g, respectively. 2DSA and Monte Carlo analyses were calculated on a Linux Beowulf cluster at the Bioinformatics Core Facility (Department of Biochemistry, The University of Texas, Health Science Center at San Antonio) and on Lonestar/Teragrid (Texas Advanced Computing Center, University of Texas at Austin). Velocity data were analyzed with 2DSA (22) combined with Monte Carlo analysis (23), direct nonlinear least squares fitting of finite element solutions of the Lamm equation (24) combined with the enhanced van Holde-Weischet method as implemented in UltraScan (25). In the 2DSA plot peaks occur at molecular mass values of 65–70 kDa/\(ff_o\)\(\sim 1.3\), 100–110 kDa/\(ff_o\)\(\sim 1.9\), and 120–130 kDa/\(ff_o\)\(\sim 2.1\). The molecular weight of PC2-C including N-terminal His tag (residues MGSSHHHHHHSSGLVPRGSHMASM) is 32.5 kDa.

**Small Angle X-ray Scattering**—PC2-C and PC2-EF were analyzed by SAXS in the presence and absence of 20 \(\mu\)M Ca\(^{2+}\) at Beamline F3 of the Cornell High Energy Synchrotron Source.
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Ca$^{2+}$-free samples of PC2-C and PC2-EF were prepared as described above for AUC. Exposure times of 2–80 s were used, and exposures were recorded in triplicate. Two-dimensional scattering data were corrected for buffer scatter, CCD dark current, and detector nonuniformity. Ag-Behenate was used to calibrate sample to detector distances. Ag-Behenate and leupeptin and pepstatin (Calbiochem). NMR spectra were recorded in the presence and absence of 20 mM Ca$^{2+}$.

NMR Spectroscopy—NMR samples contained 1 mM PC2-EF with 2 mM Tris, 150 mM NaCl, pH 7.4, along with 5% D$_2$O, 0.05% NaN$_3$, and 10 µM each of the protease inhibitors PMSF (Sigma) and leupeptin and pepstatin (Calbiochem). NMR spectra were recorded in the presence and absence of 20 mM Ca$^{2+}$. All of the NMR experiments were collected at 30 °C on a Varian INOVA 600 MHz spectrometer and a room temperature, 5-mm, triple resonance (1H, 13C, and 15N-tuned) probe equipped with triple-axis (XYZ) pulsed magnetic field gradients. All NMR spectra were acquired using pulse sequences from the Varian BioPack user library and processed using NMRPipe (30).

Sequential backbone and aliphatic side chain assignments for apo-PC2-EF were determined by manual analysis of two-dimensional $^1$H, $^{15}$N heteronuclear single quantum coherence and three-dimensional HNCACB and $^{15}$N-total correlation spectroscopy-heteronuclear single quantum coherence NMR experiments collected using $^{13}$C, $^{15}$N-labeled apo-PC2-EF. NMR chemical shift assignments for apo-PC2-EF have been deposited in the BioMagResBank with the accession number 18268. Apo-PC2-EF secondary structural propensity was calculated from patterns of backbone atom chemical shifts using the CSI (31) and TALOS (32) software packages. NOE correlations between nearby protons in three-dimensional $^{15}$N-NOESY heteronuclear single quantum coherence spectra were also used to aid assignment of secondary structure elements for apo-PC2-EF.

Single-channel Recordings in Planar Lipid Bilayers—PC2-x-z (1771A/E774A) was created from a full-length PC2 template using the QuikChange site-directed mutagenesis system (Qia-gen). Vesicles enriched in ER membranes from mammalian cells (LLC-PK1 cells) transiently transfected with PC2 alleles (wild type PC2 or PC2-x-z) were used for single-channel recording experiments. PC2 channel-containing microsomes were prepared as previously described (33). The presence of PC2 in microsomal preparations was confirmed by Western blotting. For single-channel recording experiments, wild type PC2- or PC2-x-z-containing microsomal vesicles were fused to lipid bilayers containing phosphatidylethanolamine and phosphatidylcholine (3:1 w/w) dissolved in decane (40 mg lipid ml$^{-1}$). A potassium chloride gradient, with 600 mM potassium chloride on the side of vesicle incorporation (cis side) and 0 mM potassium chloride on the opposite side (trans side), was used to facilitate and monitor fusion. The experiments were performed using 250 mM HEPES-Tris at pH 7.35 on the cis side and 250 mM HEPES with 55 mM Ba(OH)$_2$ at pH 7.35 on the trans side of the bilayer. The experiments were recorded under voltage-clamp conditions. The trans-side is the ground (reference electrode); the cis-side is the input electrode. Using cellular conventions, Ca$^{2+}$ flux from the ER lumen into the cytoplasm was defined as an inward current, where inward currents are shown as downward deflections. The data were filtered, digitized, and analyzed using pClamp software (Axon Instruments). The concentration of free Ca$^{2+}$ on the cytosolic side of the protein (cis-side of the lipid bilayer) was varied to activate PC2 channels as described (11).

Live Cell Imaging—SH-SY5Y live cell imaging was performed as previously described (34). Briefly, SH-SY5Y cells transfected with 2 µg of pC DNA 3.1 empty vector, full-length wild type PC2, or PC2-x-z were incubated for 30 min at 37 °C in 5% CO$_2$ in HEPES buffer containing 0.1% Pluronic F-127 and 5 µM fluo-4 (Molecular Probes, Invitrogen). dsRed was cotransfected to distinguish cells that had been transfected. HEPES buffer contained 20 mM HEPES, 130 mM NaCl, 4.7 mM KCl, 1 mM MgSO$_4$, 1.2 mM KH$_2$PO$_4$, 5 mM glucose, 1.3 mM CaCl$_2$, pH 7.4. A Zeiss LSM 510 META scanning laser confocal microscope equipped with a C-Apochromat X40/1.2 NA water immersion objective (Zeiss, Thornwood, NY) and 488-nm excitation, 505–530-nm emission bandpass filter was used. For carbachol stimulation experiments, the cells were activated with 200 µM carbachol in Ca$^{2+}$-containing HEPES buffer to induce a transient release of Ca$^{2+}$ from intracellular stores. 5 µM thapsigargin, an inhibitor of the intracellular Ca$^{2+}$ pump (sarcoplasmic/endoS omplac ical reticulum Ca$^{2+}$-ATPase), was added to indicate that the intracellular stores were filled, and the cells were viable. The Ca$^{2+}$-induced fluorescence intensity ratio F/I$$_0$ was plotted as a function of time in seconds, with $F$$_0$ calculated as the average of the first 10 points of the base line. For each coverslip of cells monitored, 15–20 cells were analyzed; four coverslips, recorded on separate days, were included in the analy-
sis. The data are presented as average of all cells analyzed. The response duration was defined as the time the Ca$^{2+}$/H$^+$ signal remains consecutively over 50% of the peak value.

RESULTS AND DISCUSSION

We propose that PC2 channels are gated by discrete conformational and oligomerization state changes in their C-terminal cytoplasmic tails in response to changes in cytoplasmic Ca$^{2+}$ levels. To investigate the conformational dynamics and oligomerization state of the C-terminal tail of PC2 in solution, we performed SAXS, NMR, and AUC experiments. In addition, functional studies were conducted using PC2 in planar lipid bilayers or expressed in cell lines derived from mammalian cells.

PC2 EF-hand Domain Undergoes a Global Conformational Change upon Binding Ca$^{2+}$—To serve as a Ca$^{2+}$-sensitive channel regulator, the PC2 EF-hand domain would be expected to respond structurally to changes in cytoplasmic Ca$^{2+}$. To determine whether PC2-EF undergoes a global conformational change upon Ca$^{2+}$ binding, we conducted SAXS on the purified EF-hand domain alone. SAXS enables solution state analysis of macromolecular envelope changes (i.e. global conformational changes) in response to ligand binding (35). SAXS experiments were conducted on PC2-EF at multiple concentrations, in both a Ca$^{2+}$-free, apo state and in the presence of excess (20 mM) Ca$^{2+}$. A notable reduction in the radius of gyration, $R_g$ (from 30 to 22 Å) was observed between Ca$^{2+}$-bound and Ca$^{2+}$-free PC2-EF (Table 1). In addition, the maximum interatomic distances, $D_{max}$, for PC2-EF decreased from 84 to 61 Å upon the addition of Ca$^{2+}$. The $\chi^2$ value between SAXS data for Ca$^{2+}$-bound PC2-EF and the NMR structure of Ca$^{2+}$-bound PC2-EF (Protein Data Bank code 2K6Q) (5) was determined to be 1.5 by CRYOSOL (29). Ab initio envelope reconstructions of the SAXS data in the program DAMMIF (36) show that the Ca$^{2+}$-bound reconstruction for PC2-EF corresponds very well to the NMR structure of PC2-EF (Protein Data Bank code 2K6Q) (5) (Fig. 1C). In contrast, ab initio envelope reconstructions for the Ca$^{2+}$-free PC2-EF suggest a partially unfolded protein (Fig. 1C). Previously, we showed by NMR that PC2-EF is an atypical EF-hand domain containing only one Ca$^{2+}$-binding site (5) and that PC2-EF undergoes an increase in helicity from 27% to 45% upon the addition of Ca$^{2+}$ by circular dichroism (4). Therefore, we hypothesize that these macromolecular envelope changes upon the addition of Ca$^{2+}$ to PC2-EF result from a transition from a Ca$^{2+}$-unbound to Ca$^{2+}$-bound state, analogous to conformational changes observed in other EF-hand proteins on binding Ca$^{2+}$ (37). Thus, PC2-EF appears able to respond structurally to changes in cytoplasmic Ca$^{2+}$ levels.

![Figure 1: SAXS analysis of Ca$^{2+}$-induced global conformational changes in the PC2 EF-hand domain. A, SAXS patterns for PC2-EF in the apo (left panel) and Ca$^{2+}$-bound (right panel) states. Fitted scattering data calculated from the DAMAVER PC2-EF without Ca$^{2+}$ model and the PC2-EF NMR structure (Protein Data Bank code 2K6Q) (5) are shown in green (apo) and red (Ca$^{2+}$-bound). B, P(r) curves for apo (green) and Ca$^{2+}$-bound (red) PC2-EF show decreases in both the radius of gyration and maximum interparticle distance upon binding Ca$^{2+}$. C, calculated dummy atom model of apo PC2-EF shows a broadly unstructured protein (left panel, green spheres). Calculated dummy atom model of Ca$^{2+}$-bound PC2-EF (right, red mesh) shows high similarity to the NMR structure of PC2-EF (Protein Data Bank code 2K6Q) (5) (yellow spheres). The same scale is used. PyMOL was used to make structural figures.](image-url)
**Ca^{2+}** Binding Induces Folding State Transition in PC2 EF-hand—To determine whether **Ca^{2+}**-induced conformational changes correspond to a discrete structural transition, as expected for ion channel gating regulation, residue-specific structural changes in PC2-EF on titration with **Ca^{2+}** were investigated by NMR (Fig. 2). Resonance-specific chemical shifts were assigned for apo-PC2-EF using conventional NMR methods and compared with chemical shift assignments for **Ca^{2+}**-bound PC2-EF (5) (Fig. 2A). Then, using the 13C chemical shift index (CSI) and Cα chemical shift assignments, CSI changes between apo and **Ca^{2+}**-bound PC2-EF were mapped onto the NMR structure of **Ca^{2+}**-bound PC2-EF (Protein Data Bank code 2K6Q) (5). Strikingly, the α1-α2 helix-loop-helix motif remains intact in the **Ca^{2+}**-free state, and helix α2 and the first half of helix α3 remain unchanged by ligand binding. The helix-loop-helix motif and the α2-α3 turn may catalyze folding of the EF-hand motif in the presence of **Ca^{2+}**. CSI plots for Cα atoms in apo PC2-EF and **Ca^{2+}**-bound PC2-EF are shown (D). The helix-loop-helix motif and the α1-α2 turn may catalyze folding of the EF-hand motif in the presence of **Ca^{2+}**. Helix-loop-helix.

**Ca^{2+}** Binding Induces Folding State Transition in PC2 EF-hand—To determine whether **Ca^{2+}**-induced conformational changes correspond to a discrete structural transition, as expected for ion channel gating regulation, residue-specific structural changes in PC2-EF on titration with **Ca^{2+}** were investigated by NMR (Fig. 2). Resonance-specific chemical shifts were assigned for apo-PC2-EF using conventional NMR methods and compared with chemical shift assignments for **Ca^{2+}**-bound PC2-EF (5) (Fig. 2A). Then, using the 13C chemical shift index (CSI) and Cα chemical shift assignments, CSI changes between apo and **Ca^{2+}**-bound PC2-EF were mapped onto the NMR structure of **Ca^{2+}**-bound PC2-EF (Fig. 2B). Significant conformational changes occur in helices α1, α3, and α4 of PC2-EF on binding **Ca^{2+}**. These changes correspond predominantly to residues in the C terminus of α3 (Ile-758 – Tyr-762), the **Ca^{2+}**-binding loop between α3 and α4 (Asp-763–Thr-771), and residues at the N terminus of α4 (Glu-772–Lys-784). Strikingly, the α1-α2 helix-loop-helix motif remains intact in the **Ca^{2+}**-free state (albeit with less helical propensity in α1) and may serve as a template to catalyze folding of the EF-hand upon **Ca^{2+}** binding. Based on these results, the PC2 EF-hand domain appears to undergo a folding-unfolding transition on binding **Ca^{2+}**, consistent with its proposed role as a **Ca^{2+}** sensor in PC2 channel gating.

The C-terminal Cytoplasmic Tail of PC2 Transitions from Extended Oligomer to Compact Dimer upon Binding **Ca^{2+}**—To serve as a **Ca^{2+}**-responsive channel gating module, the C-terminal cytoplasmic tail of PC2 would be expected to undergo significant, discrete structural changes as a function of cytoplasmic **Ca^{2+}** levels. To investigate changes in the molecular shape and oligomerization state of PC2-C upon **Ca^{2+}** binding, we conducted high speed velocity sedimentation experiments on PC2-C by AUC, using a combination of 2DSA and Monte Carlo analysis, as implemented in the program UltraScan (23). AUC provides information on the hydrodynamic properties of macromolecules, including their oligomerization state and
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FIGURE 3. AUC analysis of Ca\(^{2+}\)-induced conformational and oligomerization state changes in PC2-C. A, AUC velocity sedimentation analysis of PC2-C in the presence of equimolar amounts of Ca\(^{2+}\) reveals a mixture of oligomerization and conformation states ranging from a compact dimer to an extended tetramer. \(ff_o > 1\) indicates an elongated shape. B, saturating concentrations of Ca\(^{2+}\) (50:1) convert PC2-C into a predominantly compact dimeric globular state.

TABLE 2

Calculated and observed molecular weights for PC2-C by AUC

| Molecular mass (kDa) | Theoretical | 0:1 | 1:1 | 50:1 |
|----------------------|-------------|-----|-----|------|
| Monomer              | 32.5        | --- | --- | ---  |
| Dimer                | 65          | --- | 65–70| 65–70|
| Trimer               | 97.5        | 100–110| --- | ---  |
| Tetramer             | 130         | 120–130| --- | ---  |

A dash indicates "not observed."

overall molecular shape (21). Color gradients were used to show the relative concentrations of PC2-C species present in solution under specific conditions, in a pseudo-three-dimensional plot (Fig. 3). We conducted three experiments, at 0 mM, 100 \(\mu\)M, and 5 mM Ca\(^{2+}\), approximately corresponding to 0:1, 1:1, and 50:1 ratios of Ca\(^{2+}\) to PC2-C (which was at a concentration of 94 \(\mu\)M). PC2-C aggregated in the 0 mM Ca\(^{2+}\) condition, preventing analysis by AUC; however, this aggregation was reversible, and the protein could be resolubilized with addition of Ca\(^{2+}\). In the 1:1 Ca\(^{2+}\) condition, PC2-C was observed in multiple states at approximately equal concentrations. In contrast, in the presence of excess Ca\(^{2+}\) (50:1 condition), a single state predominated. We used 2DSA analysis to investigate the frictional ratios and molecular weights of PC2-C species present in the 1:1 and 50:1 Ca\(^{2+}\) conditions. Frictional ratios depend on the molecular shape of a sample, where an \(ff_o \approx 2\) indicates an elongated sample, and \(ff_o \approx 1\) indicates a compact, spherical shape. For the 1:1 Ca\(^{2+}\) condition (where molar concentrations of Ca\(^{2+}\) and PC2-C were approximately equivalent), 2DSA analysis indicates that PC2-C (MW 32.5 kDa) was in three distinct states (Table 2): 1) extended tetramer, with an \(ff_o \approx 2.1\) and a molecular mass of \(\sim 120–130\) kDa; 2) extended trimer, with an \(ff_o \approx 1.9\) and a molecular mass of \(\sim 100–110\) kDa; and 3) compact dimer, with an \(ff_o \approx 1.3\) and a molecular mass of \(\sim 65–70\) kDa. Consistent with these results, the oligomerization state of PC2 has previously been reported as dimeric (11), trimeric (7), and homo/hetero-tetrameric (12). Remarkably, in the presence of excess Ca\(^{2+}\) (50:1 condition), a single compact dimer was observed, with an \(ff_o \sim 1.3\) and a molecular mass of \(\sim 65–70\) kDa. Thus, the C-terminal cytoplasmic tail undergoes discrete conformational and oligomerization state changes in response to changes in Ca\(^{2+}\) levels, consistent with its proposed role as a Ca\(^{2+}\)-sensitive gating module.

Ca\(^{2+}\)-induced Conformational Changes in PC2 EF-hand Are Propagated throughout C-terminal Cytoplasmic Tail of PC2—To regulate channel gating, the PC2 EF-hand domain would be expected to sense changes in cytoplasmic Ca\(^{2+}\) and transduce those changes to the PC2 channel. To determine whether the conformational changes observed for the PC2 EF-hand alone are propagated throughout the entire C-terminal cytoplasmic tail of PC2, we conducted SAXS analysis on PC2-C in both the Ca\(^{2+}\)-free state and in the presence of excess (20 mM) Ca\(^{2+}\) (Fig. 4). Similar to our SAXS results for the isolated PC2 EF-hand, a significant change in \(R_g\) (from \(\sim 72\) to \(\sim 62\) Å) was observed between Ca\(^{2+}\)-bound and Ca\(^{2+}\)-free PC2-C. Remarkably, \(D_{\text{max}}\) for PC2-C reduced \(\sim 35\) Å, from 225 Å to 190 Å, upon the addition of Ca\(^{2+}\). Envelope reconstructions were conducted for PC2-C using DAMMIF, and the resulting ab initio model with P4 symmetry (Fig. 4C) suggests that PC2-C exits as a dimer of dimers in solution. The resulting \(D_{\text{max}}\) values are in agreement with the lengths of the previously determined trimeric crystal structure of the PC2 coiled coil domain (Protein Data Bank code 3HRN) (7) (\(\sim 90\) Å) and the NMR structure of PC2-EF (Protein Data Bank code 2K6Q) (5) (\(\sim 40\) Å), especially when one considers that these structures comprise only 53% of the 265 PC2-C residues (141 residues; 78 from the EF-hand and 63 from the coiled coil). These significant reductions in \(R_g\) and \(D_{\text{max}}\) in the presence of excess Ca\(^{2+}\) correlate with a compression of the overall structure of PC2-C that could be mediated by Ca\(^{2+}\)-induced folding and interdomain interactions, as we previously suggested (5).

Overall, our AUC and SAXS data demonstrate that the global conformation of PC2-C transitions from a mixture of extended oligomers to a single compact dimer species in the presence of increasing Ca\(^{2+}\) concentrations. These results are consistent with a role for the PC2 C-terminal cytoplasmic tail in regulating PC2 channel gating in response to changes in cytoplasmic Ca\(^{2+}\) levels.
Ca$^{2+}$ binding by PC2 EF-hand Is Required for Single PC2 Channel Activity in Planar Lipid Bilayers—We previously measured the single-channel properties of wild type PC2 channels in response to increasing concentrations of Ca$^{2+}$/H11001 (14, 15). Although PC2 channels have been shown to be gated by changes in cytoplasmic Ca$^{2+}$/H11001 levels, the molecular basis of channel gating is unknown. Based on the results presented above, we propose that discrete conformational and oligomerization state changes in the C-terminal cytoplasmic tail of PC2 provide a plausible molecular mechanism for channel gating. If Ca$^{2+}$/H11001-induced conformational changes in the PC2 EF-hand domain are required for channel gating, elimination of the Ca$^{2+}$/H11001 binding in PC2-EF should have an effect on the single channel properties of PC2 channels. To test this, we created a PC2 construct (PC2-x-z) with a mutated EF-hand domain (T771A/E774A) that is unable to bind Ca$^{2+}$/H11001 (4). These point mutations were chosen using the canonical sequence of EF-hand binding domains and eliminate Ca$^{2+}$/H11001 binding by mutation of two key residues in the Ca$^{2+}$/H11001-binding loop. Microsomes were prepared from cells overexpressing either PC2-x-z or wild type PC2, and the presence of PC2-x-z and wild type PC2 was confirmed by Western blotting (not shown). Single channel experiments were done using multiple PC2-x-z-containing samples and were conducted in parallel on wild type PC2 channels. Fusion of microsomes to bilayers was monitored using a potassium gradient and monitoring the appearance of potassium currents. Only bilayers exhibiting at least 30 pA of current because of potassium channel activity were included in the analysis. Once stable current activity was observed, the potassium-containing solution was replaced with potassium-free solution, leaving Ba$^{2+}$/H11001 as the only permeant ion present. No channel activity was observed in microsomes prepared from cells overexpressing PC2-x-z when the cytoplasmic free Ca$^{2+}$/H11001 concentration was 0.1 nM, a concentration at which wild type PC2 channels were active (Fig. 5A). The Ca$^{2+}$/H11001 dependence of channel activity showed that PC2-x-z channels were not active at any of the Ca$^{2+}$/H11001 concentrations at which wild type PC2 channels display activity (Fig. 5B, n = 5 for each condition). Additional single channel bilayer experiments were performed on PC2-x-z channels using a range of voltages (0 to $-30$ mV) that can activate wild type PC2 channels (33), and no activity was observed. Together, these results suggest that Ca$^{2+}$/H11001 binding to the EF-hand domain of PC2 can alter the conformation of this domain and regulate channel activity.

Expression of PC2-x-z Channels Inhibits Ca$^{2+}$/H11001 Release from Intracellular Stores—We have previously shown that PC2 functions as a Ca$^{2+}$/H11001 release channel in the ER membrane (33). PC2 channels are believed to be homo-tetramers, with the C-terminal cytoplasmic tail forming oligomeric intertail interactions.

FIGURE 4. SAXS analysis of Ca$^{2+}$/H11001-induced global changes in PC2-C. A, SAXS patterns for PC2-EF in the apo (left panel) and Ca$^{2+}$/H11001-bound (right panel) states. Fitted scattering data calculated from the DAMAVER P4 models are shown in green (apo) and red (Ca$^{2+}$/H11001-bound). B, P(r) curves for apo (green) and Ca$^{2+}$/H11001-bound (red) PC2-C show a reduction in D$_{max}$ and conformational changes. C, calculated dummy atom models (DAMAVER, P4) of apo (green) and Ca$^{2+}$/H11001-bound (red) PC2-C compared with the structures of the EF-hand (Protein Data Bank code 2K6Q) (5) and coiled coil (Protein Data Bank code 3HRN) (7). Of 265 residues in the PC2-C construct, 78 correspond to the EF-hand domain, 63 correspond to the coiled coil domain, and 124 correspond to regions whose high resolution structure has not yet been determined. These 124 residues are predicted to be disordered (4). The lengths of the NMR and crystal structures are indicated. AUC and SAXS analyses of PC2-C suggest that conformational changes occur upon the addition of Ca$^{2+}$/H11001 that result in a transition from an extended state to a compact state. D, schematic hypothesis for PC2 channel regulation. Based on our AUC, NMR, and SAXS experiments, we hypothesize that Ca$^{2+}$/H11001 binding to the EF-hand domain of PC2 can alter the conformation of this domain and regulate channel activity.
In the previous components of the present study, we found that the C-terminal cytoplasmic tail of PC2 undergoes conformational and oligomerization state changes upon Ca\(^{2+}\) binding and that Ca\(^{2+}\) binding by the C-terminal tail is necessary for PC2 channel activity. To further investigate the role of Ca\(^{2+}\) binding by the PC2 C-terminal tail in PC2 channel function, we conducted live cell imaging of cytoplasmic Ca\(^{2+}\) levels in SH-SY5Y cells expressing either PC2-x-z or wild type PC2. From Western blot analysis (not shown), the magnitude of PC2-x-z and wild type PC2 overexpression was comparable and was similar to the 5–10-fold increase shown previously (33). Overexpression of wild type PC2 increased the averaged cytoplasmic Ca\(^{2+}\) levels upon stimulation of ER Ca\(^{2+}\) release by 38\% when compared with the response from empty vector controls (Fig. 6). Because the agonist was removed after 225 s, it is not possible to compare the response duration. However, previous reports have shown that expression of wild type PC2 increases the response duration as much as 10-fold (33). In contrast, when cells expressing PC2-x-z were monitored, there was no increase in the response amplitude when compared with the response in cells expressing the empty vector (Fig. 6). Because the agonist was removed after 225 s, it is not possible to compare the response duration. However, previous reports have shown that expression of wild type PC2 increases the response duration as much as 10-fold (33). In contrast, when cells expressing PC2-x-z were monitored, there was no increase in the response amplitude when compared with the response in cells expressing the empty vector (Fig. 6). Also, there was a large decrease in the response duration measured at 50% of the amplitude maximum (220 s for the vector control and 120 s for PC2-x-z) (Fig. 6). The lack of response amplification and the reduced duration is consistent with the failure of PC2-x-z to carry current, as shown in Fig. 5 and with experiments showing the lack of amplification when the entire C-terminal domain of PC2 is removed (33). The present results also suggest that the mutated PC2 (PC2-x-z) behaves as a dominant negative regulator of Ca\(^{2+}\) release. The mechanism for the decreased Ca\(^{2+}\) release from intracellular stores is not understood but could occur because the Ca\(^{2+}\) dependence of the interaction between PC2 and its protein partners is inactivated.

**Conclusions**—We have shown that the addition of Ca\(^{2+}\) to the C-terminal cytoplasmic region of PC2 results in conformational transitions and oligomerization state changes, initiated by Ca\(^{2+}\) binding to the EF-hand domain and that Ca\(^{2+}\) binding by the EF-hand is necessary for PC2 channel activity. Based on our results, we propose a model where changes in cytoplasmic Ca\(^{2+}\) levels induce
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discrete changes to the conformation and oligomerization state of the cytoplasmic region of PC2, which correlate with PC2 channel gating (Fig. 4D). Finally, our model helps to explain the pathogenicity of ADPKD-associated truncation mutations to the PC2 C-terminal cytoplasmic region. Together, our results support our hypothesis that PC2 channels are gated by discrete conformational and oligomerization state changes in the C-terminal cytoplasmic tail in response to changes in cytoplasmic Ca\(^{2+}\) levels.

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