The Nanomechanics of Polycystin-1 Extracellular Region*

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Recent evidence suggests that polycystin-1 (PC1) acts as a mechanosensor, receiving signals from the primary cilia, neighboring cells, and extracellular matrix and transduces them into cellular responses that regulate proliferation, adhesion, and differentiation that are essential for the control of renal tubules and kidney morphogenesis. PC1 has an unusually long extracellular region (~3000 amino acids) with a multimodular structure. Proteins with a similar architecture have structural and mechanical roles. Based on the structural similarities between PC1 and other modular proteins that have elastic properties we hypothesized that PC1 functions mechanically by providing a flexible and elastic linkage between cells. Here we directly tested this hypothesis by analyzing the mechanical properties of the entire PC1 extracellular region by using single molecule force spectroscopy. We show that the PC1 extracellular region is highly extensible and that this extensibility is mainly caused by the unfolding of its Ig-like domains. Stretching the native PC1 extracellular region results in a sawtooth pattern with equally spaced force peaks that have a wide range of unfolding forces (50–200 pN). By combining single-molecule force spectroscopy and protein engineering techniques, we demonstrate that the sawtooth pattern in native PC1 extracellular region corresponds to the sequential unfolding of individual Ig-like domains. We found that Ig-like domains refold after mechanical unfolding. Hence, the PC1 extracellular region displays a dynamic extensibility whereby the resting length might be regulated through unfolding/refolding of its Ig-like domains. These force-driven reactions may be important for cell elasticity and the regulation of cell signaling events mediated by PC1.

The human kidney is composed of roughly one million nephrons that must develop and maintain their glomerulus-tubule structure to function properly. Mutations in PC1 lead to autosomal dominant polycystic kidney disease (ADPKD), one of the most common life-threatening genetic diseases (it has an incidence of 1 in 2000 live births), and is a leading cause of kidney failure (1–4). In ADPKD, cysts develop from renal tubules and enlarge dramatically, compress the normal parenchyma and ultimately cause renal failure in more than 50% of the affected individuals (1, 2). In ADPKD the sensing mechanisms for tubule size appear to be lost, and cysts develop and enlarge progressively, in a process that ultimately causes renal failure (3, 5). The function of PC1, as well as the mechanisms whereby mutations in this protein lead to the pathogenesis of the disease, remain unknown. In particular, it is not known how PC1 senses the size of the renal tubules, or how this protein controls cell growth and the development of the tubular phenotype.

PC1 is a large (~520 kDa) membrane protein with an extensive N-terminal extracellular portion, comprising some 3000 amino acids (Fig. 1). This part contains a novel combination of domains: leucine-rich repeats (LRR), a WSC (cell wall integrity and stress response component), a C-type lectin domain, a low density lipoprotein-like domain (LDL domain), 16 copies of a novel immunoglobulin (Ig)-like fold, the polycystic kidney disease (PKD) domain, and a region that extends for ~1000 amino acids that is homologous to a sea urchin protein called receptor for egg jelly (REJ) (6–9). Recent data indicated that PC1 may function as a cell adhesion receptor involved in cell-cell or cell-matrix interactions (10–12). PC1 has also been suggested to function as a mechanoreceptor that senses flow rates in the renal tubules (13, 14).

PC1 has a structure that is very similar to proteins with structural and mechanical roles, such as titin (an elastomeric protein of muscle; Refs. 15 and 16), fibronectin (an elastic extracellular matrix protein; Ref. 17), or NCAM (an elastic cell adhesion protein; Ref. 18). All of these proteins contain Ig-like domains arranged in tandem. Recent single molecule experiments have shown that such proteins are highly extensible and elastic.

Based on the structural similarities between PC1 and other modular proteins that have elastic properties we hypothesized that that PC1 functions mechanically by providing a flexible and elastic linkage between cells. The mechanical properties of PC1 may be vital for maintaining the architectural integrity of the kidney. Mutations may alter PC1 cell adhesion and mechanical properties and lead to the alterations in cell-cell and/or cell-matrix interactions and abnormal tissue development, which are characteristic of ADPKD.

To understand how PC1 senses and mediates mechanical forces a detailed knowledge of its mechanical properties at the molecular level is needed. One technique that has been extremely useful in measuring the mechanical properties of single proteins is single molecule force spectroscopy (19). In this technique one uses the cantilever tip of an atomic force microscope (AFM) to pick up and stretch single molecules from their N and C termini. Such experiments have been extremely useful in analyzing the fundamental biophysical properties of proteins that are exposed to mechanical forces (15–23). Indeed a recent study demonstrated that the first five PKD domains have remarkably high mechanical stability, suggesting that the PC1 extracellular domain may have a load-bearing function where most modules are designed to resist denaturation when exposed to mechanical forces (24). However, these
domains comprise only a small fraction (~15%) of the long PC1 extracellular region, and it is likely that the other PKD domains may have different mechanical stabilities, as shown for other modular Ig-like proteins such as titin (16) and fibronectin (17). Also, the mechanical stability of the long REJ region is not known. This information is essential to better define the putative mechanical role of PC1.

Here, we directly examined the mechanical properties of the entire extracellular region of PC1 using single molecule force spectroscopy. This technique offers the most direct way of studying the stability and elasticity of proteins that are exposed to mechanical forces and hence more closely resemble the conditions found in vivo.

MATERIALS AND METHODS

Cloning and Expression of Fragments of PC1 Extracellular Region Proteins in Mammalian Cells—We cloned and expressed the following constructs (Fig. 1): 1) the full-length human PC1 N-terminal extracellular region (AF20-Fc; amino acids 1–3048), 2) a fragment encompassing the first 412 amino acids containing the signal peptide (SP), LRR domains (amino acids 33–180), the WSC domain (amino acids 181–240), and the first PKD domain (amino acids 268–354) plus the last 180 amino acids (QIF-2; this construct is derived from AF20-Fc by deleting a fragment corresponding to amino acids 413–2963), 3) a fragment containing the SP plus the last 1394 amino acids containing PKD domains 13–16 and the complete REJ region (QIF-4), and 4) a control fragment containing just the SP and the Fc fragment (QIF-8; 296 amino acids long). These constructs were fused into a modified pCI vector (Promega) with an Fc fragment of human IgG1 plus a Stop codon at its C terminus.

PC1 ectodomain is rich in cysteine residues, and hence its folding or structure may be sensitive to oxidizing/reducing conditions as shown for VCAM-1 (25). Hence all the protein constructs, except for the PKD no. 1-I27 polyprotein, were expressed and purified in the absence of DTT or any other reducing agent.

To express the different protein fragments, we transfected HEK293 cells using Lipofectamine2000 (2.5 μl of reagent for 1 μg of DNA; 20 μg of plasmid for each 150-mm plate). After 6 h, the medium was changed to OptiMEM and incubated for 48 h. After 48 h, the medium was harvested, and the proteins were purified using protein G-agarose beads (Amersham Biosciences). The beads were washed three times with 1 ml of PBS with 0.5% Triton X-100, and the proteins were eluted by 0.1 M glycine, pH 2.5. The eluates were neutralized by adding 1 volume of 0.5 M PBS, pH 7.2 (no DTT). The integrity of the proteins was verified by Western blots using a 4–15% SDS gel using anti-human Fc (Jackson ImmunoResearch Laboratories).

Cloning and Expression of PKD Domains 6–10 in Bacteria—A construct coding for PK1 PKD domains 6–10 was obtained by PCR using the pCI-PKD1-FLAG vector (26). The corresponding protein sequence is from EELRGLS at the N terminus to SIFYYYL at the C terminus, with a molecular mass of ~50 kDa. The DNA was cloned into the pQ80E expression vector, which has a His tag at the N terminus, and the vector was transformed into Escherichia coli BL21(DE3) cells (Novagen). Transformed colonies were grown overnight at 37 °C in LB containing 100 μg/ml ampicillin. The overnight culture was reinoculated into fresh LB medium, and the cells were induced with 1 mM isopropyl-1-thio-β- d-galactopyranoside when the A at 600 nm reached 0.6. The cells were collected by centrifugation and sonicated. The insoluble pellet fraction was resuspended in Tris-HCl buffer, pH 8.0 containing 8 M urea. The solubilized pellet was renatured initially by dialyzing against Tris-HCl buffer containing 500 mM urea. The solubilized pellet was then dialyzed against the same buffer without urea and centrifuged. The supernatant of the renatured solubilized pellet was purified by Ni2+ affinity chromatography. The protein was kept at 4 °C. We also attempted to express other protein fragments containing other PKD domains but encountered difficulties at the level of expression and/or purification making them unsuitable for AFM studies.

Cloning and Expression of a PKD Domain 1-Titin I27 Heteropolyprotein—We cloned and expressed in bacteria a heteropolyprotein based on the first PKD domain (PKD no. 1, residues Val268–Glu354) and the titin immunoglobulin domain 27 (I27). The I27 domain has been extensively studied by force spectroscopy and hence serves as an internal fingerprint (16, 17). We assembled an I27-PKD no. 1 heteropolyprotein using a multiple step cloning technique that makes use of four restriction sequences (BamHI, BglII, BstY, and KpnI) to three multiples of the I27-PKD no. 1 dimer (21). The polyprotein was cloned in an E. coli recombination-defective strain, Sure-2 (Stratagene), and expressed in the BLR (DE3) strain (Novagen). The cells were lysed with Triton X-100 and then purified by Ni2+ or Co2+ affinity chromatography. The protein was kept in PBS containing 5 mM DTT and 0.2 mM EDTA at 4 °C.

Single Molecule AFM—The mechanical properties of single PC1 proteins were studied using a home-built single molecule atomic force microscope (AFM) that consists of a detector head (Digital Instruments) mounted on top of a single axis piezoelectric positioner with a strain gauge sensor (P841.10, Physik Instrumente). The P841 has a total travel of 15 μm, and it is attached to two piezo-electric positioners (P280.10A, Physik Instrumente) that are used to control the x and y positions. This system has a z-axis resolution of a few nanometers and can measure forces in the range of 10–10,000 pN. The monitoring of the force reported by the cantilever, and the control of the movement of the piezoelectric positioners, are achieved by means of two data acquisition boards (PCI 6052E, PCI 6703, National Instruments) and controlled by custom written software (LabView; National Instruments and Igor, WaveMetrics). With this system, it is possible to measure the force as a function of the extension of the protein (force-extension mode) or measure the elongation of the protein at a constant force (force-feedback mode; Ref. 27). The spring constant of each individual cantilever (MLCT-AUHW: silicon nitride gold-coated cantilevers; Veeco Metrology Group, Santa Barbara, CA) was calculated using the equipartition theorem (28). Cantilever spring constant varied between 20–80 pN/μm. Unless noted, the pulling speed of the different force extension curves was in the range of 0.5–0.7 nm/ms.

Single Protein Mechanics—In a typical experiment, a small aliquot of the purified proteins (~1–50 μl, 10 μg/ml) was allowed to adsorb to clean glass coverslips (for ~10 min) and then rinsed with PBS, pH 7.4 (no DTT). We found that PC1 proteins molecules adsorbed well to glass, mica, or gold-coated glass coverslips. We obtained identical data with these different substrates. However, almost all the experiments reported here were done on clean glass coverslips. The glass coverslips were cleaned by sonication in acetone for 20 min following by boiling then for 10 min in 3 N KOH and then 30% H2O2. Between each step, the coverslips were rinsed and sonicated with MilliQ water (>18.2 megohm × cm). Before use, the coverslips were dried in a stream of clean N2 gas. Segments of a protein were picked up randomly by adsorption to the cantilever tip. The probability of picking up a protein was typically kept low (less than one in 50 attempts) by controlling the amount of protein used to prepare the coverslips.

Analysis of Force Extension Curves—The elasticity of the stretched proteins were analyzed using the worm-like chain (WLC) model of polymer elasticity (29) shown in Equation 1,
study its mechanical properties, we used a home-built single molecule atomic force microscope (17, 19, 22, 27). In a typical experiment, a small aliquot of the purified N-terminal region was allowed to adsorb to a clean glass coverslip (for ~10 min) and then rinsed with PBS pH 7.4. Segments of the PC1 ectodomain are then picked up randomly by adsorption to the cantilever tip, by pressing it down onto the sample for 1–2 s at forces of several nanoNewtons, and stretched for several hundred nanometers.

Fig. 2A shows several examples of force-extension curves obtained after pulling random stretches of the PC1 ectodomain. These traces show regularly spaced force peaks, sawtooth patterns, with a wide range of peak forces and a mean of 107 ± 58 pN (n = 193 force peaks; range: ~50–200 pN; Fig. 2B). The first 20–50 nm of these force extension curves displays several peaks that are irregular in amplitude and spacing; these events occur randomly in the force-extension curves probably because of nonspecific interactions, and were not further analyzed in the study.

To analyze the spacing between peaks in the sawtooth patterns we used the WLC model for polymer elasticity, which predicts the entropic restoring force (F) generated upon the extension (x) of a polymer (29; see “Materials and Methods”). The thin lines in Fig. 2A correspond to fits of the WLC equation to the curve that precedes each force peak. We found that the separation between force peaks is 27.8 ± 3.3 nm (n = 102 force peaks; Fig. 2C). This value corresponds very well with the expected increase in contour length of a 90 amino acid PKD domain: 90 amino acids × 0.35 nm (length of an amino acids) − 4 nm (size of folded PKD domain, Ref. 31) = 27.5 nm. In addition, these sawtooth patterns are very similar to those found for other modular protein containing tandem Ig-like domains, such as native titin (15, 16), native tenascin (22), native fibronectin (17), and recombinant PKD constructs (24). Hence, these sawtooth patterns most likely correspond to the sequential unfolding of PKD domains. So far the largest number of force peaks that we have seen is twelve, less than the total number of PKD domains, 16. This could be simply because of the random nature of the attachment of the protein to the cantilever tip making the likelihood of picking up a full-length protein very low. Alternatively, it is possible that some of the PKD domains have a low mechanical stability, and thus the unfolding forces will be below the resolution limit of our instrument (~10 pN).

In summary, our data show that the native extracellular region of PC1 is highly extensible and that this extensibility results from the unfolding of mechanically stable structural folds such as PKD domains. However, based on these data we cannot exclude the possibility that other regions, such as the REJ region, may also contribute to the extensibility and unfolding patterns.

The Unfolding of PC1 Extracellular Domains Is Reversible.—To test whether PC1 extracellular domains refold after mechanical unfolding, we repeatedly stretched and relaxed the PC1 extracellular region. Fig. 3 shows an experiment in which a single molecule remained attached to the AFM tip allowing for repeated extension and relaxation cycles (up to 15 cycles in this experiment over a period of ~3 min). After each extension, the molecule was allowed to relax completely (the relaxation traces is shown in gray, Fig. 3A, trace i). To measure the refolding kinetics, we used a double pulse protocol in which the pulse interval was varied (17, 22), where the protein is first stretched to count the number of unfolded domains and then is quickly refolded to zero length within ~1 s. Fig. 3 shows consecutive force-extension recordings obtained with time delays of 1, 5, 10, and 20 s (traces ii–v, respectively). As the delay between stretching pulses is increased more domains recover from unfolding. The ratio of the unfolding peaks between the second and first pulls is a measure of the fraction of domains that refolded. By varying the time between stresses we could then measure the refolding rate (Fig.

**Figure 1.** PC1 native fragments used for single molecule spectroscopy experiments. A, schematic diagram of different fragments expressed in mammalian cells. AF20-Fc contains the complete extracellular domain of human with the adjacent GPS domain of PC1 (3,048 amino acids), fused with Fc of human IgG1 at the C terminus. QIF-2 contains the LRR domains plus the first PKD domain; QIF-4 contains PKD domains 13–16 plus the complete REJ region, QIF-8 is a control fragment containing just the signal peptide, SP, and the Fc fragment. The domain structure of full-length PC1 is shown on top, with domains indicated. The vertical lines denote transmembrane segments. The numbers indicate the amino acid positions of deletion boundaries. B, Western blot of PC293 cells purified fusion proteins. The proteins expressed in HEK293 cells are purified from the culture media using G-protein-conjugated agarose beads and probed either with anti-Fc (left panel) or anti-LRR antibody (right panel).

\[ F(x) = \left( k_b T / p \right) \times \left[ 0.25 \times (1 - x/L_c)^{-2} - 0.25 + x/L_c \right] \quad (\text{Eq. 1}) \]

where \( F \) is force, \( p \) is the persistence length, \( x \) is end-to-end length, \( L_c \) is the contour length of the stretched protein. The adjustable parameters are the persistence length, \( p \), and the contour length, \( L_c \).
For a simple two-state model, the folding probability is given by Equation 2,

\[ P(f) = 1 - e^{-kf_0 t} \]  

(Eq. 2)

This function describes well the folding data of Fig. 3B with \( k_f^0 = 0.095 \) s\(^{-1}\). This value for the mechanical refolding rate is much lower than those reported for titin Ig domains (16) or FnIII domains (22, 23), which refold at rates of about 1 s\(^{-1}\). An unusual feature of the refolding pattern is that the domains tend to misfold. For example, the second and third traces in Fig. 3A show low force peaks (marked by asterisks) with large increases in contour length (thin lines in Fig. 3A, trace iii). As shown by the histogram in Fig. 3C, these low force unfolding events (asterisks) contribute to an increase in contour length of 60 nm or 90 nm, values that \( \sim 2 \) and 3 times longer than that of a correctly folded Ig-like domain. We interpret these events as the unfolding of mechanically weak structures that may consist of several (2–3) misfolded Ig-like domains. Hence, these data show that when PC1 extracellular domains are unfolded by a mechanical stretching event, they can slowly refold after the protein is fully relaxed.

**Mechanical Properties of Recombinant PC1 Extracellular Region Fragments**—Our data show that the extracellular region of PC1 is highly extensible and that this extensibility results from the unfolding of stable structural folds (PKD, LRR, or REJ domains). To determine the origin of the sawtooth patterns obtained for the full-length ectodomain, we analyzed the mechanical properties of several recombinant proteins (inset in Fig. 4). We cloned and expressed these different fragments in mammalian cells and \( E. coli \) (see “Materials and Methods”). QIF-2 contains the LRR and WSC domains plus the first PKD domain (PKD no. 1), 6–10 contains PKD domains 6–10 and QIF-4 contains PKD domains 13–16 plus the complete REJ region.
Our force-spectroscopy data show that each fragment has a distinct mechanical fingerprint (Fig. 4). For example, the QIF2 protein extends for only ∼100 nm (114 ± 27 nm, n = 43) with occasional extra force peaks before detachment that correspond to the unfolding of PKD no. 1 (unfolding forces: 157 ± 44 pN, n = 9; Lc = ∼28 nm; Fig. 4A, trace ii). These data suggest that, under our conditions, LRR and WSC domains (amino acids 33–240) readily extend under a stretching force displaying a featureless force-extension curve.

Stretching the QIF-4 construct resulted in sawtooth patterns with 3–10 force peaks (Fig. 4E) with a wide range of unfolding forces of 50–250 pN (mean: 152 ± 59 pN, n = 161). The large number of observed force peaks is surprising because this construct has only four PKD domains. Hence, this indicates that these extra force peaks must originate from the REJ region. The REJ domain accounts for more than 30% of the total number of amino acids in the ectodomain. However, nothing is known about the structure or properties of this region. Previous work suggested that this region represents a novel sequence that contains no repeating motifs, and it does not show any homology to any known fold (6, 32). However, our data show that the REJ is also made of Ig-like domains that have ∼90 amino acids. Indeed, recent analysis of this region using homology molecular modeling techniques revealed that the REJ region may contain structural elements similar to FnIII domains, and this is the subject of evaluation in a separate report.3

Theoretically, the REJ could have as many as 10 FnIII domains (∼1000/92 amino acid domain). This is very significant because it suggests that most of the PC1 ectodomain is made of Ig-like domains, which are designed to resist stretching forces. Another interesting feature of these recordings is that the force peaks are typically preceded by a long spacer of 100–200 nm (Fig. 4E, traces i and ii). We interpret this initial spacer as the stretching of an unstructured polypeptide chain in the REJ region (of ∼200–500 amino acids), and the force peaks as the sequential unfolding of PKD and the unfolding of novel Ig-like domains present in the REJ region.

We also made a construct containing PKD domains 6–10. This protein was expressed in E. coli. Stretching this protein resulted in sawtooth patterns with up to 4 force peaks (Fig. 4C) and unfolding forces of ∼100 pN (102 ± 50 pN, n = 39; Fig. 4D). In sum, our data show that the PC1 extracellular region is mainly made of Ig-like domains that have a wide range of mechanical stabilities (range of unfolding forces: 50–250 pN).

The Mechanical Properties of PKD Domain No. 1—To examine the mechanical properties of PKD domains in more detail, we studied the mechanical property of one PKD domain, PKD no. 1. We selected this domain because its structure is known (31). For this we made a polyprotein chimera, which contains three repeats of PKD no. 1 and a titin Ig domain (Ig domain 27 from human cardiac titin). The I27 domain has been extensively studied by force spectroscopy and hence serves as an internal fingerprint (16, 17). Also, I27-based protein chimeras have been found to express well in bacteria; this strategy has proven to be useful in

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3 F. Qian, A. Oberhauser, and F. Fraternali, unpublished observations.
the analysis of several protein domains with single molecule force spectroscopy, such as the FnIII domains from fibronectin (27).

Fig. 5 shows the mechanical characterization of the (PKD1-I27)₃ polyprotein. We used two different techniques to characterize its mechanical properties: length clamp (Fig. 5A) and force ramp (Fig. 5C). Fig. 5A shows that stretching of single (PKD1-I27)₃ polyproteins give force-extension curves with a sawtooth pattern with equally spaced force peaks. The sawtooth pattern is well described by the WLC equation (continuous lines). An unfolding force frequency histogram shows a mean of ~170 pN (173 ± 38 pN, n = 150). Fig. 5C shows the stepwise unfolding of (PKD1-I27)₃ polyprotein using the force-ramp method (top trace). The lower trace shows the time course of the applied force. The downward transients are caused by the feedback lag. We found that many recordings showed two levels of unfolding forces (Fig. 5A): low force peaks (~160 pN) and high force peaks (~200 pN; marked with dotted lines). Because the high force peaks have the characteristic fingerprint of the I27, we attribute the low force peaks to PKD no. 1 domain unfolding. However, other recordings did not show such clear two levels of unfolding forces. This indicates that PKD no. 1 domain has a similar stability to titin I27 domains, as was already shown by Forman et al. (24).

We also used the force-clamp mode of the single molecule AFM for stretching (PKD1-I27)₃ polyproteins (Fig. 5C). The advantage of this mode is that it is possible to measure the force dependence of the unfolding probability of single protein domains and it gives a direct measurement of kinetic parameters, such as the rate constant at zero force, $\alpha_o$, and the distance to the transition state, $x_{u}$ (27, 33). In these experiments we applied a force that increases linearly with time (Fig. 5C, bottom trace) and observe the unfolding of single domains as a stepwise elongation of the proteins (Fig. 5C, top trace). Fig. 5C shows the stepwise elongation of a single polyprotein observed after increasing the force at a rate of 200 pN/s. Unfolding steps are seen in the range of 100–180 pN. We found that the step sizes had a mean value of about 20 nm (21.5 ± 3 nm, n = 38; at these forces the unfolded domains are only partially extended). The bottom trace shows the time course followed by the stretching force, which changes linearly with time. By measuring the force at which each unfolding event takes place we can calculate the distribution function for the probability of unfolding as a function of the applied force. Fig. 5D shows the probability, $P_u$, as a function of the applied force. The data show that the probability of unfolding domains depends on the applied force. The probability of unfolding changes from $P_u = 0.1$ to 0.9 over ~100 pN.
To analyze the data of Fig. 5D quantitatively, we used a simple two-state kinetic model for mechanical unfolding. In this model, a protein is exposed to a force that increases linearly with time, simulating the conditions of our force-ramp experiment. According to this model the probability of observing an unfolding event at a given force, \( P_u(F) \), is given by Equation 3,

\[
P_u(F) = 1 - e^{-\frac{\alpha_0}{a} \int_0^F \frac{F}{RT} df} 
\]

(Eq. 3)

where \( a \) is the rate of change of the applied force (\( a = 200 \) pN/s in our experiments), \( \alpha_0 \) is the rate of unfolding at zero force, \( \Delta x_u \) is the distance to the transition state, and the other symbols have their usual meaning. For this protein we found that the \( \alpha_0 \) versus force data were well described by a single rate constant, \( \alpha_0 = 2.3 \times 10^{-4} \) s\(^{-1}\) (continuous line). This rate of unfolding at zero force is very similar to that of I27 (3.3 \( \times 10^{-4} \) s\(^{-1}\); Refs. 21 and 27). Hence, by using two independent techniques we find that PKD no. 1 has relatively high mechanical stability, which is similar to that of titin I27 domains.

**DISCUSSION**

Recent evidence suggests that PC1 acts as a mechanosensor, receiving signals from the primary (luminal) cilia, neighboring cells and extracellular matrix and transduces them into cellular responses that regulate proliferation, adhesion, and differentiation that are essential for the control of renal tubules and kidney morphogenesis (13, 14). Understanding the role of mechanical forces in regulating PC1 function requires an understanding of the conformational changes that this protein undergoes in response to a stretching force. Using single molecule force spectroscopy, we demonstrated that the PC1 N-terminal extracellular region is highly extensible and that this extensibility is mainly caused by the unfolding and refolding of its Ig-like domains.

A recent study demonstrated that the first 5 PKD domains (PKD no. 1 and PKD no. 2–4) unfold at forces of 180–200 pN (24). These authors found that the PKD domain 1 is exceptionally strong, with force trace unfolding peaks indistinguishable from those of I27 (~200 pN), and domains 2–4 display a similar mechanical strength (~180 pN). Based on these data the authors predicted the other PKD domains may have a similar mechanical stability. However, we found that PKD domains display a wide range of mechanical stabilities requiring ~50 pN of force to unfold the weakest domains and ~250 pN for the most stable domains. Our data show that PKD domains 6–10 and 13–16 tend to unfold at lower forces than PKD domains 1–5. This mechanical hierarchy might be important for the cell adhesion and elastic function of the PC1 extracellular region.

PC1 is ideally suited for a role as a cell adhesion protein with mechanosensing properties. These include the ability to: 1) sense and transduce mechanical forces into biochemical signals (such as calcium influx and transcription factors) (13, 14), 2) interact with components of the ECM (heterophilic) (11) and neighboring PC1 (homophilic interactions) (10, 12), and 3) interact with the cytoskeleton via intermediate filaments (34).

PC1 has been reported to function as a cell adhesion receptor engaged in homophilic and ECM interactions. The LRR and C-type lectin domains of PC1 bind strongly to several ECM proteins in vitro (11), and PKD domains are reported to be involved in homophilic intercellular interactions of MDCK cells and cystic cell lines (10, 12). Disruption of cell-cell adhesion during tubular morphogenesis may be an early initiating event for cyst formation in ADPKD.

It is likely that PC1 must be under mechanical tension, and that PC1 domains are likely to deform or unfold during these mechanical interactions. Unfolding and refolding of PC1 domains may be a mechanism by which the interaction between cells is maintained as the distance between cells changes, as proposed for other proteins containing Ig-like domains (17, 22, 35, 36). At the same time, the application of a mechanical force may trigger the activation of intracellular signals as demonstrated for integrin (37), cadherin (38), and now also for PC1 (13, 14). It is possible that PC1, by linking neighboring cells via trans homophilic interactions, might sense the mechanical coupling between cells during the normal operation of the renal tubules where changes in flow affect the tubule diameter (39). These force-triggered events might be important for the activation of the associated Polycystin-2 channel or other intracellular signaling events related to cell growth and tissue development (e.g. JAK/STAT pathway; Ref. 40).

To date, about 250 mutations have been identified in the PKD1 gene (Refs. 2, 42–44; a complete list of published PKD1 mutations can be found at the Cardiff Human Gene Mutation Data base). Many are either point mutations or deletions/insertions mutations that introduce frame shifts and stop codons leading to premature termination. The most likely effect of these types of such mutations is a complete loss of normal PC1 function. However, there are also ~100 missense mutations that result in non-conservative amino acid substitutions involving residues that form part of the LRR, PKD repeats, and the REJ domain. Some of these missense mutations are predicted to alter the structure and stability of PKD or LRR domains (42, 44). However, it is not known, how missense mutations may alter the properties of PC1 domains. Mutations may cause changes in conformation, disrupt the domain structure (and cause denaturation), or affect their surface properties, as has been suggested for other Ig-like cell adhesion receptors (41). We hypothesize that pathogenic missense mutations in the PC1 ectodomain may disrupt its mechanical signal transduction properties and hence lead to abnormal tissue development and cyst formation.

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