Structural and biochemical consequences of disease-causing mutations in the ankyrin repeat domain of the human TRPV4 channel

Hitoshi Inada, Erik Procko, Marcos Sotomayor, and Rachelle Gaudet

Supporting Information

Table S1. TRPV-ARD structures used in this study

Protein	Species	Structure (# mol/a.u.)	ATP binding	References
TRPV1	Rat	2PNN (1), 2NYJ (1)	+	Lishko *et al.,* 2007 (ATP-bound forms)
TRPV2	Rat	2ETA (2), 2ETB (1), 2ETC (2)	–	Jin *et al.,* 2006
	Human	2F37 (2)		McCleverty *et al.,* 2006
TRPV4	Human	Form I 4DX1 (2) without ATP	+	This study
		Form II 4DX2 (2) with 5 mM ATP		
	Chicken	3JXI (4), 3JXI (2)	+	Landouré *et al.,* 2010 (ATP-free forms)
TRPV6	Mouse	2RFA (1)	–	Phelps *et al.,* 2008

Table S2. Structural similarity between TRPV4-ARD and other TRPV-ARDs

TRPV	TRPV1	TRPV2			TRPV6	TRPV4	
Species	Rat	Rat		Human	Mouse	Chicken	
PDB	2PNN	2ETB	2ETC[a]	2F37[a]	2RFA	3JXI[a]	3JXJ[a]
RMSD[b]							
Finger 2[c]	1.596 / 0.279	1.527 / 0.245	1.251 / 1.081	1.577 / 0.227	1.538 / 0.259	0.183 / 1.599	0.213 / 1.617
Finger 3[d]	4.588 / 1.762	5.168 / 2.444	3.652 / 3.941	5.266 / 1.949	5.135 / 1.972	3.587 / 4.732	2.934 / 4.393
Core[e]	1.593 / 1.653	1.378 / 1.457	1.451 / 1.573	1.463 / 1.541	1.865 / 1.736	0.691 / 0.737	0.637 / 0.676

[a] Only the chains with complete loops were used in the analysis. [b] RMSD to human TRPV4-ARD (ATP-free, form I / ATP-bound, form II). [c] Residues 229-240 in hTRPV4. [d] Residues 261-287 in hTRPV4. [e] Residues 152-176, 194-228, 241-260, 288-307, 324-356, 373-394 in hTRPV4.

Table S3. Molecular dynamics simulations of rat TRPV1-ARD

Label	t_{sim} (ns)	Type[†]	Ensemble	SMD atoms	Speed (nm/ns)
TRPV1-ARD-ATP	1.1[‡]	*EQ*	NpT	-	-
	1.6	*SMD*	NVE	L111-Cα/H358-Cα	20
	8.3	*SMD*	NpT	L111-Cα/H358-Cα	2
TRPV1-ARD-Apo	1.1[‡]	*EQ*	NpT	-	-
	1.6	*SMD*	NVE	L111-Cα/H358-Cα	20
	8.3	*SMD*	NpT	L111-Cα/H358-Cα	2

[†] EQ denotes equilibrium simulations and SMD denotes constant velocity steered molecular dynamics.

[‡] These simulations consisted of 1,000 steps of minimization, 100 ps of dynamics with the backbone of the protein restrained (k = 1 kcal/mol/Å^2), and the remaining time as free dynamics in the NpT ensemble (Langevin damping set to 1 ps^{-1}).

Table S4. T_m of wildtype and mutant TRPV4-ARD proteins

Protein	N	Mean T_m	±	Std Dev (°C)	p-Value	
WT	8	37.93	±	0.08	1	
E183K	3	33.78	±	0.06	<.0001	*
K197R	3	35.3	±	0.1	<.0001	*
L199F	3	32.9	±	0.1	<.0001	*
R232C	3	38.1	±	0.1	0.8415	
R269C	3	38.6	±	0.2	<.0001	*
R269H	3	36.8	±	0.3	<.0001	*
E278K	3	37.3	±	0.2	<.0001	*
R315W	3	36.2	±	0.2	<.0001	*
R316C	3	34.7	±	0.3	<.0001	*
I331F	3	35.5	±	0.2	<.0001	*
I331T	3	37.97	±	0.07	1	
D333G	3	36.4	±	0.2	<.0001	*
V342F	3	35.4	±	0.3	<.0001	*

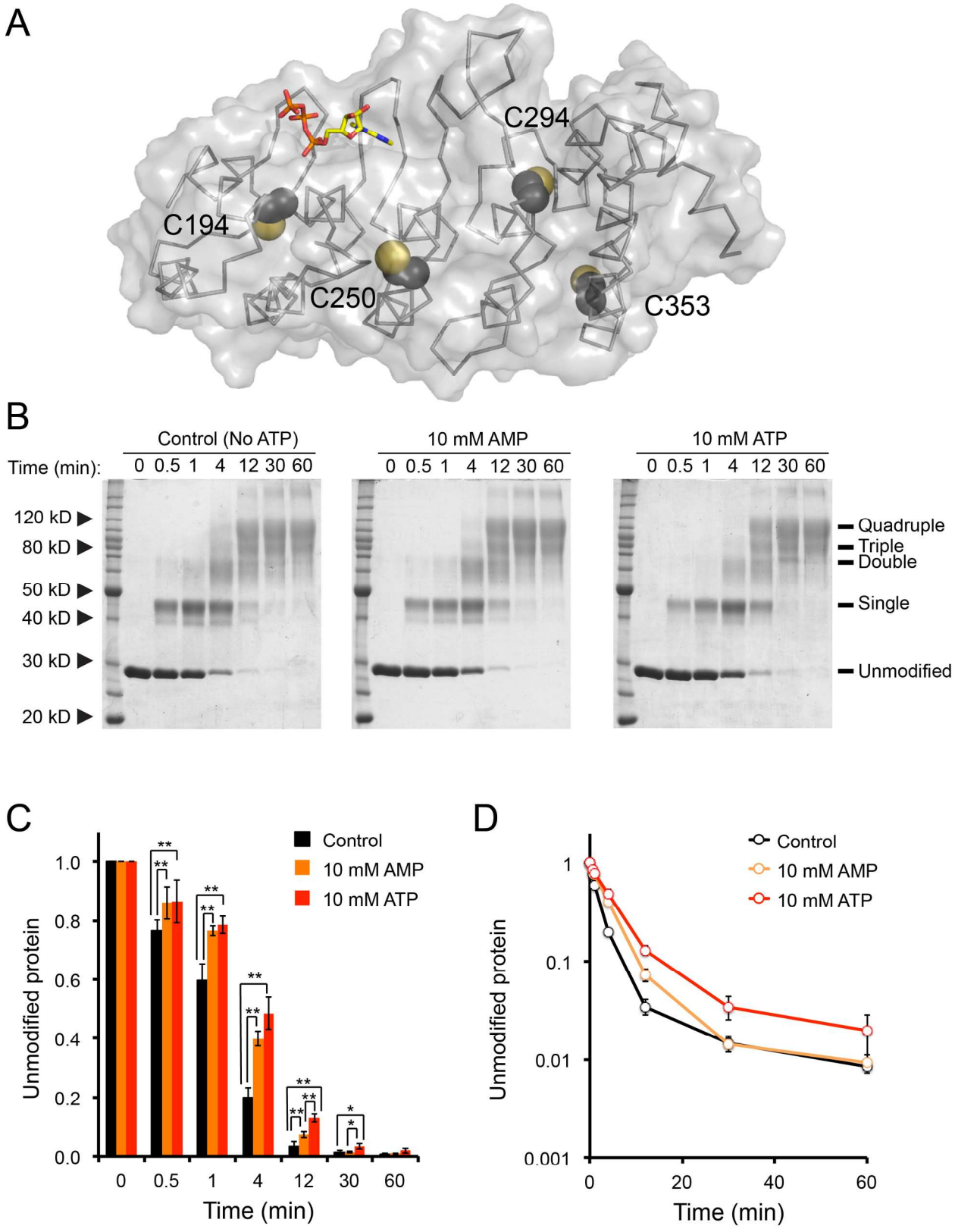
A
C294
C194
C250
C353
B
Control (No ATP)
10 mM AMP
10 mM ATP
Time (min):
0 0.5 1 4 12 30 60
120 kD
80 kD
50 kD
40 kD
30 kD
20 kD
Quadruple
Triple
Double
Single
Unmodified
C
Control
10 mM AMP
10 mM ATP
Unmodified protein
Time (min)
D
Control
10 mM AMP
10 mM ATP
Unmodified protein
Time (min)

Figure S1. ATP binding effect on protein folding/flexibility in hTRPV4-ARD. A, hTRPV4-ARD contains four cysteines (Cys194, Cys250, Cys294, and Cys353; spheres). Bound ATP is shown as yellow sticks. Cys194 corresponds to Cys157 in TRPV1, which is required for activation by chemical modification with allicin, a garlic extract compound.[1] B, Time course for modification of hTRPV4-ARD cysteines by PEG-maleimide (mPEG). Reactions containing hTRPV4-ARD (8.5 μM) in 150 mM NaCl, 20 mM Tris (pH 7.0), 0.5 mM mPEG-5 kDa (Creative PEGWorks) and 10 mM nucleotide as indicated, were incubated at room temperature, stopped by addition of DTT to 110 mM, and analyzed by Coomassie-stained 12% SDS-PAGE. hTRPV4-ARD modification at cysteine residues resulted in electrophoretic mobility shifts on a Coomassie-stained SDS-gel. Unmodified protein is reduced and modified proteins (single, double, triple, and quadruple) are increased in a time-dependent manner. Shown is a representative Coomassie-stained gel from one of three experiments. The statistical significance of the change in unmodified protein with respect to time point 0 was determined by a multiple comparison test using Tukey-Kramer method, with $p < 0.05$ and $p < 0.01$ indicated by * and **, respectively. C, Amount of unmodified protein in (B) is quantified as the mean ± standard deviation. D, Same data as in (C) with vertical axis in log scale.

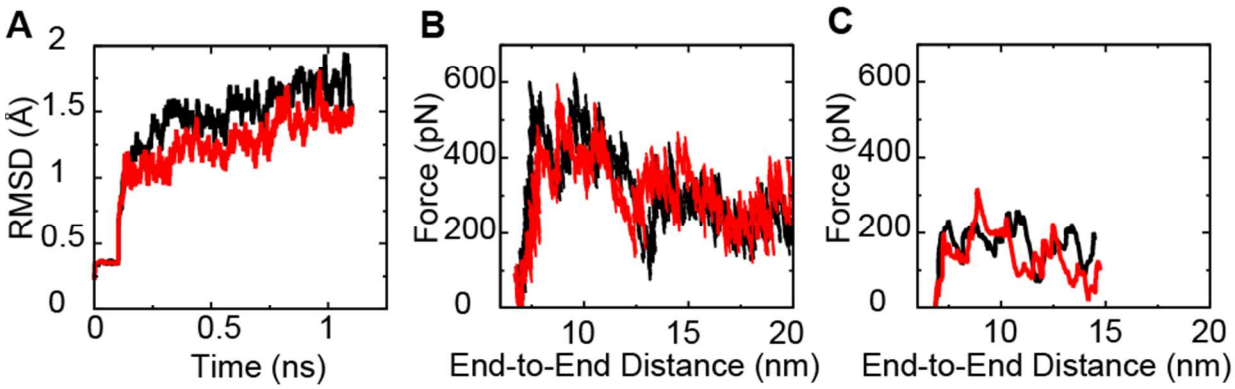

Figure S2. Stability of TRPV1-ARD in equilibrium and SMD simulations. A, Root mean square deviation (RMSD) is shown versus time during equilibrium simulations in the presence (red) and absence (black) of ATP. B and C, Force required to stretch and unfold TRPV1-ARD is shown as a function of the protein's end-to-end distance for simulations performed at 20 (B) and 2 nm/ns (C) with (red) and without (black) bound ATP.

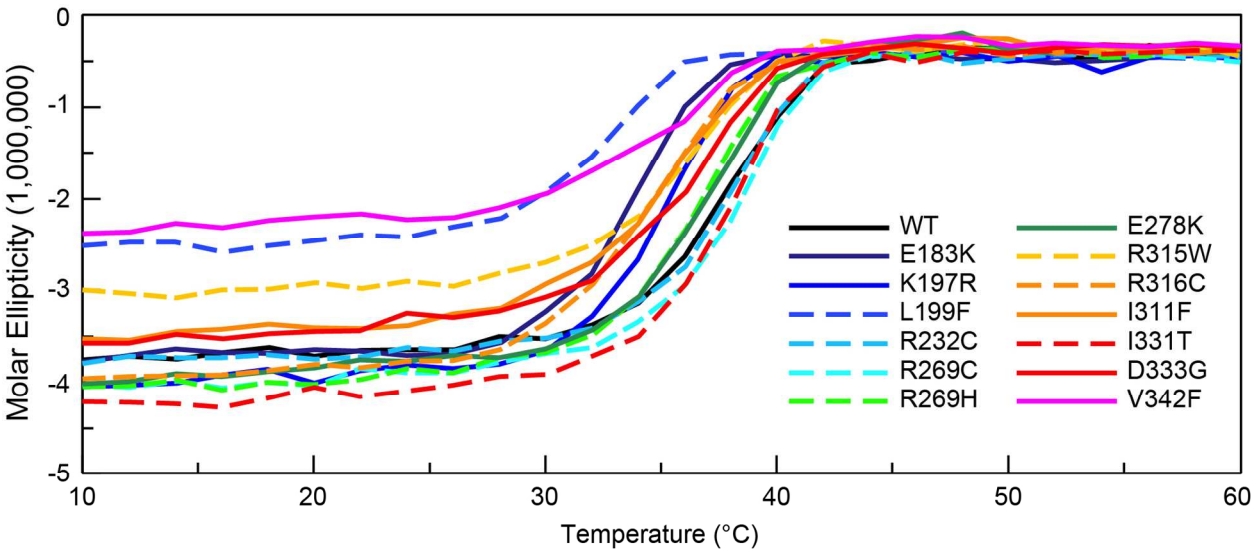

Figure S3. The thermal stability of wildtype and mutant TRPV4-ARD proteins. The molar ellipticity at λ = 222 nm was measured as the protein solutions were heated by 1 ºC/min.

Supplementary References

1. Salazar, H., Llorente, I., Jara-Oseguera, A., Garcia-Villegas, R., Munari, M., Gordon, S. E., Islas, L. D., and Rosenbaum, T. (2008) A single N-terminal cysteine in TRPV1 determines activation by pungent compounds from onion and garlic, *Nat Neurosci 11*, 255-261.