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Biophysics of Cadherin Adhesion

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Abstract

Classical cadherins are the principle adhesive proteins at cohesive intercellular junctions, and are essential proteins for morphogenesis and tissue homeostasis. Because subtype-dependent differences in cadherin adhesion are at the heart of cadherin functions, several structural and biophysical approaches have been used to elucidate relationships between cadherin structures, biophysical properties of cadherin bonds, and cadherin-dependent cell functions. Some experimental approaches appeared to provide conflicting views of the cadherin binding mechanism. However, recent structural and biophysical data, as well as computer simulations generated new insights into classical cadherin binding that increasingly reconcile diverse experimental findings. This review summarizes these recent findings, and highlights both the consistencies and remaining challenges needed to generate a comprehensive model of cadherin interactions that is consistent with all available experimental data.

Introduction

The assembly and regulation of cohesive intercellular junctions is central to morphogenesis and tissue homeostasis. The calcium-dependent, transmembrane classical cadherins are the major architectural proteins at intercellular junctions. They mediate cell-cell cohesion by binding cadherins on adjacent cells via their extracellular regions, which comprise five, tandemly arranged extracellular (EC) domains, numbered 1–5 from the N-terminus (EC1–5) (Fig. 1A). Different classical cadherin subtypes have different sequences but similar overall structures. The subtypes are expressed at different morphogenetic stages and in different tissues, and are named according to the tissues from which they were first isolated. Their sequence differences are thought to underlie differences in affinities and adhesion linked to their distinct functions.

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A challenge is to therefore characterize cadherin-binding and to determine how affinity differences impinge on cadherin-dependent cell functions. The primary adhesive interface is called the “strand-swapped-dimer” because it involves the mutual insertion of a conserved tryptophan at position 2 (Trp2) into the hydrophobic pocket on EC1 of the apposing protein (Fig. 1B) [1]. All biophysical and cell adhesion data support the view that this is a *trans* adhesive bond. Mutating Trp2 to alanine (W2A) nearly abolishes cadherin-mediated adhesion, although W2A mutants localize to cell-cell junctions and the ectodomains weakly aggregate beads [1,2].

Measurements cadherin binding affinities

Biophysical methods

Because adhesive differences between cadherin subtypes are central to cadherin-specific functions, efforts focused on characterizing cadherin bonds, using solution-binding measurements, cell-cell binding kinetics, and adhesion-based methods. Analytical Ultra Centrifugation (AUC) characterizes the binding equilibria of soluble macromolecules [3]. *Sedimentation equilibrium* AUC quantified the state of cadherin oligomerization and the three-dimensional (3D) affinities K_A 's [3]. *Sedimentation velocity* AUC assessed whether the equilibration kinetics were fast or slow on the ~45min measurement timescale [3]. *Surface Plasmon Resonance* (SPR) measurements compared relative 3D K_A values for EC1–2 binding to EC1–2 on sensor chips. However, cadherin dimerization complicates the SPR analyses due to competing dimerization equilibria in solution, on the chip, and between soluble and immobilized proteins. Thus, SPR measurements determined relative, but not absolute K_A values for cadherins, using homophilic K_A 's from AUC data as references [4]. A difference between AUC and SPR is that AUC measured binding between identical cadherins, whereas SPR also measured heterophilic K_A 's.

The micropipette adhesion (MPA) assay determined two-dimensional (2D) affinities between membrane-bound cadherins, in the native context of the cell membrane. This approach measured binding kinetics between two apposing cells held by micropipettes. MPA measurements quantified the time-dependent intercellular binding probability P , which is the number of cell-cell binding events divided by the total number of times the cells are brought into contact. Here, $P(t)$ reflects the number of intercellular bonds [5]. Fitting the kinetic data to a model for the binding mechanism gives the affinity and dissociation rate. Because this approach characterizes binding between membrane-bound proteins in intercellular gaps, it determines the two-dimensional (2D) affinity. MPA studies characterized several membrane proteins, including cadherins [6–8].

Homophilic and heterophilic binding

Sedimentation equilibrium AUC measurements of the K_A 's of wild type (WT) and mutant classical cadherin homodimerization used two-domain constructs (EC1–2), except for C-cadherin (EC1–5) [1]. Wild type mouse E-cadherin EC1–2 expressed in mammalian and in bacterial cells have similar 3D K_A 's of $1.03 \times 10^4 \text{ M}^{-1}$ and $1.25 \times 10^4 \text{ M}^{-1}$, respectively. The homophilic affinity of mouse N-cadherin EC1–2 is ~four fold higher at 25°C (Table 1) [4].

MPA measurements quantified homophilic affinities of different classical cadherins [7,9,10]. The strand swapping mechanism (Fig. 1B) predicts that $P(t)$ would rise exponentially to a limiting plateau (see Fig. 2F, solid line) [7]. However, the binding kinetics of the full-length classical cadherins occur in two stages (Fig. 2F): a fast, initial EC1-dependent step rises to a low binding probability P_1 , followed by a 2–5s lag, and a slower rise to a second, higher binding probability P_2 [7,9,10]. The lag and second rise require the full ectodomain (Figs. 2F, G) [7].

A kinetic model for the strand-swapping mechanism does not describe the entire kinetic profile, but it describes the fast, first step [11]. Thus, model fits of the strand-swapping model to the first step (Fig. 2F, solid line) gave the two-dimensional affinities and dissociation rates for EC1–EC1 bonds (Table 2)[9–11]. The relative affinities of homophilic N- and E-cadherin bonds differ from AUC measurements, but the 2D affinities correlated with the segregation of cells that express these proteins and with adhesion measurements [10,12]. The difference may arise from small sequence differences between the proteins studied, because single amino acid differences can alter the K_A 's.

Cadherins were widely assumed to only form homophilic bonds, largely based on in vitro cell-sorting results [13]. However, adhesion measurements and equilibrium binding studies showed that classical cadherins also form heterophilic bonds [4,9,12,14,15]. Generally, heterophilic affinities and bond energies are intermediate between the homophilic bonds of the two cadherin subtypes [4,9,10,12,14] (Table 2).

Binding site mutations and the structural origins of affinity differences

Studies identified structural elements that modulate strand-swapping affinities. Mutating the conserved Trp2 to Ala (W2A) decreases the 3D K_A of E-cadherin EC1–2 by ~11 fold [16]. In MPA measurements, the 2D K_A between the full-length W2A mutant and WT C-cadherin was ~100 fold lower than WT C-cadherin [7].

Strain in the closed, self-docked monomer arises because the Trp2 anchors the short, swapping-strand at one end and a Ca^{2+} -Glu11 ion pair anchors the other end. This strain provides the driving force for strand expulsion and exchange with an apposing protein [17]. Therefore, mutations such as W2F that relieve strain in the A-strand of cadherin *monomers* decrease the *dimerization* K_A (Table 1) [17]. Reducing strain by lengthening the swapping strand by one or two Ala ('Ala-Ala extension') also decreased the affinity (Table 1) [17].

A salt bridge between Glu89 and the N-terminus of the swapped strand also stabilizes the strand dimer, so that replacing Glu89 (E89A) or deleting two N-terminal residues ('Asp-Trp deletion') decreased the affinity (Table 1) [16]. A conserved proline-proline motif in the A-strand strand ensures that the strand-swapped dimer cannot form a continuous hydrogen-bonded β -sheet between apposing proteins. Therefore, mutating Pro5, Pro6, or both (P5AP6A) increased the 3D affinities of E- and N-cadherin (Table 1) [17,18].

Micropipette measurements demonstrated that polar side chains in the Trp2 binding pocket also modulate 2D affinities [10]. C-cadherin point mutations, based on sequence differences between C- and N-cadherin, altered the 2D K_A 's and the segregation patterns of cells expressing these mutants, relative to wild type C-cadherin. The C-cadherin mutant S78A reduced the 2D affinity for WT C-cadherin six-fold. Cells expressing this mutant sorted out from WT C-cadherin expressing cells but intermixed with cells expressing N-cadherin. The M92I mutant reduced the 2D affinity for WT C-cadherin two-fold, and caused cells to intermix with both C-cadherin and N-cadherin expressing cells. A third mutant K8NS10P, postulated to alter the orientation of bound Trp2, had no detectable effect on the affinity for WT C-cadherin, but slightly altered GTPase signaling triggered by homophilic ligation [10].

All classical cadherins share the strand-swapping motif, but small sequence differences affecting A-strand flexibility and W2 docking alter the K_A 's. Such differences may account, in part, for subtype-specific differences in cell-cell cohesion, signaling, and cell sorting.

Intermediates in the strand swapping reaction pathway

Despite the emphasis on strand-swapping, EC1–2 is the minimum unit necessary for cadherin adhesion. Studies of T-cadherin suggested why [16,19]. T-cadherin is unusual because it lacks the Trp2 required for strand swapping. In crystals, T-cadherin EC1–2 fragments associate through extensive nonpolar contacts at the EC1–2 junction [16,19]. This structure is termed the “X-dimer”, because it adopts a tetrahedral configuration (Fig. 1C). Similar interfaces in structures of E-cadherin EC1–2 [1] and its W2A mutant [19], which cannot form the strand dimer, suggest that this X-dimer is a general binding interface of classical cadherins.

The X-dimer may be an intermediate in the strand dimerization pathway [16]. The K14E mutant impedes X-dimer formation. Although WT E-cadherin EC1–2 dimerizes in SPR measurements, the K14E X-dimer mutant does not bind to the K14E mutant or to WT EC1–2 [16]. However, in slower sedimentation equilibrium AUC measurements, mutants that cannot form X-dimers bind with affinities that are indistinguishable from WT protein (Table 1) [16]. In sedimentation velocity experiments, the WT protein exhibits a rapidly exchanging monomer-dimer equilibrium, but the monomer-dimer inter-conversion of X-dimer mutants is slow on the ~45min measurement timescale [16]. This indicates that the X-dimer affects the kinetics, but not the thermodynamics, of strand dimerization [16].

Dynamic fluorescence measurements indicated that the K14E mutant also slows cadherin exit from cell-cell junctions. This would be expected, if the K14E mutation increases the transition state energy for both forward and reverse dimerization reactions. These findings suggests that cadherin also switches between the strand-swapped dimer and X-dimer when exiting junctions [20].

Strand-swap and X-dimers in the context of biophysical studies

Fluorescence Resonance Energy Transfer (FRET) [21] and sub nanometer single-molecule localization [22] were used to measure cadherin conformations in complexes in solution, and provided further evidence for both strand and X-dimers. In FRET experiments, specific EC domains were labeled with donor and acceptor fluorophores. FRET changes quantify subnanometer distances less than 10nm [22], but dyes separated by >10 nm do not undergo FRET.

Single molecule FRET measurements with N-terminally labeled cadherins showed that most monomers homodimerize via their EC1 domains [23], but studies also identified a W2-independent “initial encounter complex”. Because the W2A mutant blocks strand dimerization, W2A mutants would interact, only if cadherins dimerized via the putative X-dimer [24]. The single molecule FRET characterization of a kinetically trapped W2A complex strongly suggests that cadherins first form an X-dimer “encounter complex”, prior to strand dimerization [24].

The strand and X-dimer models agree qualitatively with prior force measurements, which demonstrated that cadherins undergo different binding interactions, which involve different structural regions (reviewed in [13,25]). Surface force apparatus (SFA) and atomic force microscopy (AFM) measurements identified three adhesive bonds between cadherin extracellular domains (Fig. 2A, E). SFA measurements quantified the lengths of cadherin complexes, and showed that cadherin ectodomains adhered at intermembrane distances of 25.1 ± 0.3 nm, 31.7 ± 0.8 nm, and 39 ± 1 nm (Fig. 2A). Adhesion at 39nm is due to the strand swapped dimer, based on the complex dimension and on the elimination of this bond by the W2A mutation (Fig. 2A, B) [25,26]. Adhesion at 32nm is consistent with the X-dimer (Fig. 2A, C). W2A mutants also adhere at 32nm, although the adhesion is lower than WT

cadherin [26]. The D103A mutation at EC1–2 junctions also likely inhibits X-dimer formation because the mutant retains the interdomain structure but does not form the strand swapped dimer [27]. Consistent with this interpretation, the D103A E-cadherin mutant ablates adhesion at 39nm [25], although this could also be due to allosteric modulation of Trp2 docking [28].

Single bond rupture measurements, e.g. with an AFM corroborate strand and X-dimer formation (Fig. 2B–D). AFM measurements lack the distance information of the SFA, but distinguish bonds based on their rupture forces and how the forces depend on the rate at which bonds are pulled apart [29]. EC1–5 fragments form three cadherin bonds with different strengths and dissociation rates [14,23,26,30–33]. Importantly, EC1–2 fragments only formed two, distinct bonds (Fig. 2D). The W2A mutation eliminated one of the latter bonds, which is attributed to the strand swapped dimer. The residual bond between W2A fragments is attributed to the X-dimer [23,26].

These findings collectively reveal an increasingly consistent view of cadherin EC1–2 dimerization (Fig. 2). There are apparent differences between the magnitudes of cadherin bond properties determined from solution binding versus adhesion measurements. However, other examples of differences between solution binding and force-dependent measurements include catch bonds, which have no apparent strength in the absence of force but strengthen when pulled [34–36]. It will be interesting to determine whether cadherins also form catch bonds.

Additional cadherin interactions

Cadherins may also form cis dimers, but conclusive evidence has been elusive (reviewed in [13]). There is experimental evidence that additional cadherin EC domains contribute to adhesion, in incompletely understood ways. For example, genetic analyses of E-cadherin mutations associated with inherited gastric cancers identified clusters of mutations along the entire extracellular domain [37]. A few of the most deleterious mutations are within EC2 and EC3 [38,39]. In some cancers, abnormally high N-glycosylation on EC4 and EC5 impairs E-cadherin-dependent cell adhesion and signaling [40,41].

In addition to the two EC1–2 bonds, force measurements of full-length cadherin ectodomains identified an additional, stronger bond (Figs. 2A, E) (reviewed in [2,13]). AFM measurements of C-cadherin domain deletion mutants identified the domains required for the three cadherin bonds [26]. Measured forces between EC1–4 fragments (Fig. 2E), for example, exhibit three, distinct peaks, e.g. bonds, different from EC1–2 binding (Fig. 2D) [26]. The role of this additional, EC3-dependent interaction remains controversial, but cell-binding kinetics provides clues.

The second rise in the cell binding probability (Figs. 2F, G) also requires EC3 [25]. Studies with N-glycosylation mutants suggest this step may reflect slower-forming lateral interactions. Ablating N-glycosylation sites in the N-cadherin ectodomain increased lateral N-cadherin dimers on cells, and enhanced ERK signaling [42]. Removing three N-glycans on EC2 and EC3 eliminated the lag, and accelerated the second step [11]. The altered kinetics and increased lateral dimerization following selective N-glycan removal suggest that N-cadherin forms an additional interaction(s) on cell surfaces that is modulated by N-glycosylation. The structural basis for this behavior remains to be determined.

In a proposed cis-binding interface, inferred from ectodomain structures, EC1 contacts EC2 near the EC2–3 junction of an adjacent protein [1,43]. Mutations at this interface disrupted cadherin ordering at inter-vesicle junctions [43]. However, this interaction was undetected by NMR, electron microscopy, or solution binding measurements [1,4]. Single-molecule

FRET measurements did not detect cis bonds between cadherins expressed as Fc-dimers, despite the close ectodomain proximity in this construct [23]. Bacterially expressed VE-cadherin ectodomains form hexamers, in an EC4-dependent manner [44]. However, only non-glycosylated VE-cadherin oligomerizes, so the complex may not be physiological [45].

Computer modeling may explain some differences between solution binding (3D) and surface binding and adhesion (2D). Simulations revealed cooperativity between cis and trans bonds that may be necessary for cadherin junction assembly [46]. The simulations suggest that the entropy reduction due to the confinement of trans bonds in intermembrane (2D) gaps could enhance otherwise weak cis bonds [47], such as the putative EC1/EC2 bond or adhesive bonds detected in force measurements. In this model, trans binding augments cis interactions, which in turn bias the equilibrium in favor of trans bonds. Intriguingly, the MPA data (Fig. 2F, G) may support this model qualitatively. The two-stage binding kinetics is consistent with a model in which trans binding (first step) switches on lateral interactions that in turn increase trans binding (second step). Although there may be different interpretations of the interaction(s) underlying the kinetics, further studies with putative cis dimer mutants should test this concept.

Conclusions and Future Directions

Recent structures, biophysical studies, and computer simulations generated new insights into classical cadherin binding mechanisms, and revealed several parallels between these and previous findings that reconcile, in part, apparent differences between structural and biophysical data (Fig. 2). Differences remain, and a comprehensive picture has yet to emerge that reconciles all available data. Future studies with these and other approaches will likely resolve apparent differences discussed here, and provide a more comprehensive functional and structural understanding of classical cadherins.

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Figure 1. Cadherin structures. (A) Crystal structure of the extracellular region of *Xenopus* C-Cadherin showing the W2 residue (green VDW structure), and calcium ions (yellow VDW structures). (B) Strand-swapped dimer between E-cadherin EC1–2 fragments. Here the W2 residues (gray VDW structures) bridge the apposing EC1 domains. (C) X-dimer of the W2A mutant of E-cadherin EC1–2 fragments. The adjacent domains form a tetrahedral structure with extensive contacts at the inter-domain junction.

Figure 2. Parallels between biophysical and structural data

The red arrows highlight the corresponding features in adhesion-based measurements and structural data. (A) Force versus distance measurements with a surface force apparatus showing cadherin adhesion at three membrane distances, indicated by the minima in the curves and outward directed arrows. (B) The strand dimer interface and orientation of trans-bonded cadherins between apposing membranes. (C) X-dimer complex between apposing cadherin interactions. The structures of the trans and X-dimers (center) are compared with cadherin binding signatures from three different biophysical measurements discussed in the text, or X-dimer (center) and different features in surface force measurements (top), AFM data (bottom left), and cell binding kinetics (bottom right).

Table 1

Homo-dimerization affinities from AUC measurements at 25°C

Protein	Description	KA (x 10 ⁴) M ⁻¹	Ref.
Xenopus C-cadherin EC1–5			
WT	Wild Type	1.56	[48]
Mouse E-cadherin EC1–2			
WT	Wild Type (human)	1.25	[49]
WT	Wild Type	1.04	[4]
WT	Wild Type	1.01	[16]
W2A	Strand-swap mutant	0.11	
Ala-Ala N-terminal extension	Strand-swap mutant	0.12	
E89A	Strand-swap mutant	0.34	
Asp-Trp deletion at N-terminus	Strand-swap mutant	0.15	
K14E	X-dimer mutant	1.17	
K14S	X-dimer mutant	1.04	
Y142R	X-dimer mutant	1.30	
W2A K14E	Double mutant	monomer	
W2F	Reduced strain on A*/A strand	0.41	[17]
Ala inserted between 2 and 3	Reduced strain on A*/A strand	0.07	
AlaAla inserted between 2 and 3	Reduced strain on A*/A strand	0.51	
E11D	Enhanced strand-swapping	1.40	
P5A P6A	Swapping strand stabilization	27	
P5S P6S	Swapping strand stabilization	34	
P5G P6G	Swapping strand stabilization	37	
P5A	Swapping strand stabilization	34	
P5G	Swapping strand stabilization	21	
Mouse N-cadherin EC1–2			
WT	Wild Type	3.88	[4]
P5A P6A	Swapping strand stabilization	27	[17]

Table 2
Two dimensional cadherin affinities and dissociation rates from cell binding kinetics

Cadherin on Test Cell	Density(μm^{-2}) [§]	Cadherin- Fc on Red Cell	Density(μm^{-2}) [¶]	2D Affinity($\times 10^{-4} \mu\text{m}^2$)	Dissociation rate (s^{-1})	Ref.
C-cadherin	18	C-cadherin	10	11 $\pm$ 2	0.6 $\pm$ 0.2	[7]
C-cadherin	7	C-EC1245	10	30 $\pm$ 9	0.3 $\pm$ 0.1	
C-cadherin	7	C-EC12	155	1.4 $\pm$ 0.5	0.9 $\pm$ 0.2	
C-cadherin W2A	24	C-cadherin	452	0.12 $\pm$ 0.05	0.10 $\pm$ 0.03	[10]
N-cadherin	15	N-cadherin	69	1.9 $\pm$ 0.3	1.1 $\pm$ 0.4	
E-cadherin	16	E-cadherin	44	3.3 $\pm$ 0.5	1.0 $\pm$ 0.3	
C-cadherin	14	N-cadherin	38	3.5 $\pm$ 0.2	1.3 $\pm$ 0.3	[10]
C-cadherin	18	E-cadherin	33	3.3 $\pm$ 0.9	1.3 $\pm$ 0.4	[9]
N-cadherin	16	E-cadherin	33	2.6 $\pm$ 0.4	1.2 $\pm$ 0.5	

[§] Density (#/square micron) of full-length cadherin on the Chinese hamster ovary cell and

[¶] Density (#/square micron) of Fc-tagged cadherin immobilized and oriented on the apposing red blood cell.