Experimental Cell Research 358 (2017) 10–13

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Biophysical basis of cadherin mediated cell-cell adhesion

Andrew Vae Priest, Omer Shafraz, Sanjeevi Sivasankar*

Department of Physics and Astronomy, Iowa State University, Ames, IA 50011, USA

ARTICLE INFO

Keywords:
E-cadherin
Strand-swap dimers
X-dimers
Catch bond
Slip bond
Ideal bond

ABSTRACT

Classical cadherin transmembrane cell-cell adhesion proteins play essential roles in tissue morphogenesis and in mediating tissue integrity. Cadherin ectodomains from opposing cells interact to form load-bearing *trans* dimers that mechanically couple cells. Cell-cell adhesion is believed to be strengthened by *cis* clustering of cadherins on the same cell surface. This review summarizes biophysical studies of the structure, interaction kinetics and biomechanics of classical cadherin ectodomains. We first discuss the structure and equilibrium binding kinetics of classical cadherin *trans* and *cis* dimers. We then discuss how mechanical stimuli alters the kinetics of cadherin interaction and tunes adhesion. Finally, we highlight open questions on the role of mechanical forces in influencing cadherin structure, function and organization on the cell surface.

Tissue formation and integrity relies on the ability of cells to sense, transmit and respond to mechanical forces; classical cadherin cell-cell adhesion proteins are integral to these functions [1,2]. Cadherins form load-bearing adhesive complexes that associate with the dynamic actin cytoskeleton, mechanically couple neighboring cells, transmit mechanical forces from the extracellular environment to the cytosol and trigger intracellular signaling events [3]. Deficiencies in cadherin adhesion are associated with the onset and metastasis of several human cancers [4].

The cadherin superfamily of cell-cell adhesion proteins is composed of four major subfamilies: classical cadherins, desmosomal cadherins, protocadherins and atypical cadherins. Of these subfamilies, classical cadherins have been most extensively studied using biophysical methods. This brief review summarizes our current understanding of the biophysics of cadherin-mediated cell-cell adhesion with a focus on the classical cadherin extracellular region. We discuss the structure and homotypic binding kinetics of classical cadherins, both in the presence and absence of mechanical perturbations and examine future directions in this exciting area of research.

1. Structure and equilibrium binding affinities

Classical cadherins are calcium-dependent transmembrane glycoproteins. Cadherin extracellular regions from opposing cells bind in a *trans* orientation to mediate adhesion while their cytoplasmic tails interact with a range of effector proteins, most notably the catenins that link cadherins to the actin cytoskeleton [5,6]. Classical cadherins, which are subdivided into type I and type II, have extracellular regions that are comprised of five tandemly repeated extracellular (EC1-5) domains with three calcium binding sites in each interdomain linker [6]. Type I classical cadherins, which include epithelial (E) and neuronal (N) cadherin, have a conserved HAV tripeptide motif [7] and a conserved tryptophan 2 (Trp 2) residue [8] in the most distal EC (EC1). In contrast, type II classical cadherins, such as vascular endothelial (VE) cadherin, have two conserved Trps (Trp2 and Trp4) and also lack the HAV motif [9,10]. Our review focuses on the homophilic interaction of Type I classical cadherin ectodomains.

Structural studies have revealed that classical cadherin ectodomains interact in two *trans* dimers conformations: strand-swap dimers (S-dimers) and X-dimers (Fig. 1A and B) [8,11–15]. S-dimers were first identified in early crystal structures of N-cadherins [8]. In an S-dimer, opposing cadherins dock a conserved Trp2, located on N-terminal β-strands, into complementary hydrophobic pockets on the EC1 domain of their binding partners (Fig. 1A) [8,11,12,14,15]. Mutations of Trp2 in E- or N-cadherin expressing cells inhibits cell aggregation [16], which shows that strand-swapping is essential for classical cadherin mediated cell-cell adhesion. The driving force for strand-swapping is the strain on the anchored N-terminal β-strands in monomeric cadherins, which is relieved by S-dimer formation. When strain on the β-strands is altered by changing the length of the strand or by mutating anchoring amino acids, S-dimer affinity is significantly changed [15]. It has been proposed that type I classical cadherin binding specificity is tightly controlled by their corresponding binding affinities and a conserved Pro5-Pro6 motif, which prevents hydrogen bond formation between opposing β-strands, precludes unwanted, high affinity *trans* interactions [15]. Indeed, mutating one or both Pro5-Pro6 residues in E- and N-cadherin resulted in the formation of

Abbreviations: EC, extracellular; S-dimer, strand-swap dimer; AUC, analytical ultracentrifugation; FRET, fluorescence resonance energy transfer; NMR, nuclear magnetic resonance

* Corresponding author.
E-mail address: sivasank@iastate.edu (S. Sivasankar).

http://dx.doi.org/10.1016/j.yexcr.2017.03.015
Received 25 February 2017; Accepted 9 March 2017
Available online 12 March 2017
0014-4827/

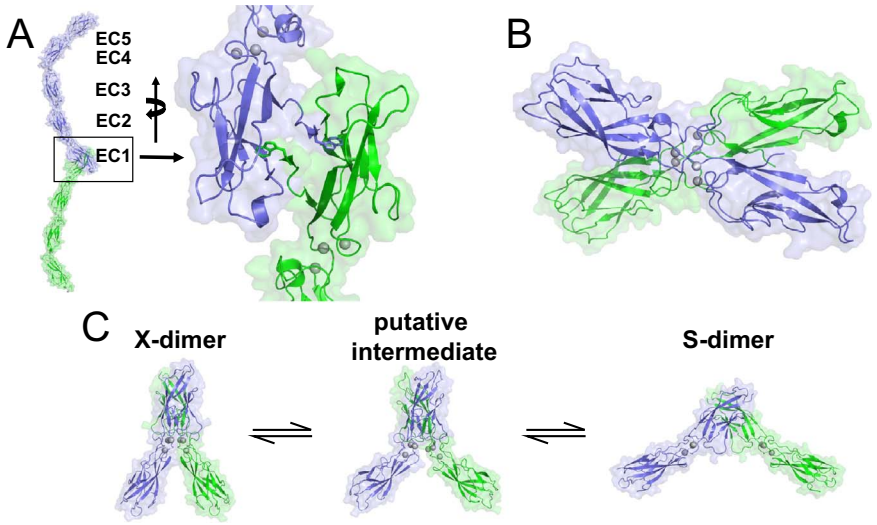

Fig. 1. Structure of classical cadherin *trans* dimers. **(A)** E-cadherin monomers from opposing cells (green and blue) bind in a S-dimer conformation. S-dimers are formed by the exchange of conserved Trp-2 residues between opposing EC1 domains (PDB: 3Q2V). Bound Ca^{2+} are denoted by gray spheres. **(B)** X-dimer interface consists of surface interactions between EC1 and EC2 domains near the Ca^{2+} binding site (PDB: 3LNH). **(C)** Model for E-cadherin conformational interconversion. Cadherins interconvert between an X-dimer (left) and S-dimer (right) through an intermediate state (middle; simulated structure and interconversion pathway from reference [23]).

higher affinity homodimers [15].

In contrast to S-dimers, classical cadherin X-dimers are formed by extensive surface interactions along the EC1-2 interdomain linker region such that the *trans* dimeric assembly of the elongated molecules resembles the shape of an 'X' (Fig. 1B) [14]. Early evidence for X-dimers was provided by two crystal structures of E-cadherin EC1-2 fragments, containing small N-terminal extensions that revealed non-strand-swapped dimeric association [17,18]. Small-angle X-ray scattering and site directed mutagenesis data with human P-cadherin, another type I cadherin, indicated existence of the X-dimer similar to that of E-cadherin [19], although, the P-cadherin X-dimer was undetectable in analytical ultra-centrifugation (AUC) measurements [20]. X-dimers were also observed by macromolecular crystallography for the non-classical T-cadherin [13]. Single-molecule fluorescence resonance energy transfer (FRET) experiments showed that when strand-swapping was ablated by mutating Trp2 to an Ala, E-cadherins formed a dimer, presumably the X-dimer, which served as an "encounter complex" prior to S-dimer formation [21]. Nuclear magnetic resonance (NMR) studies with E-cadherin fragments confirmed that cadherins initiate dimerization through an X-dimer and subsequently switch to form an S-dimer [22]. While it had been believed that X-dimers exist only transiently as intermediates in the formation and rupture of strand-swap dimers [14,21], recent biophysical studies [23] demonstrate that purified E-cadherin ectodomains constantly shuttle between X-dimer and S-dimer conformations.

Classical cadherin *trans* dimer dissociation constants in solution are on the order of micromolar, despite their functional role in mediating robust cell-cell adhesion. The dissociation constant (K_D) for the full-length extracellular region of C-cadherin (EC1-5) is 64 μM as determined by AUC [24]. Similarly, AUC gives K_D values of about 97 μM [14,20,25], 26 μM [20], 14 μM [20], 127 μM [20], and 31 μM [20] for the EC1-2 domains of E-, N-, R-, C-, and P-cadherin, respectively. In contrast to S-dimers, X-dimers have markedly weaker affinities. Specifically, E-cadherin mutations that abolished strand-swapping had significantly higher dissociation constants with the largest being 916 μM [14], suggesting cadherins favor an S-dimer conformation in solution.

Besides binding in *trans* conformations, cadherins on the same cell are also believed to interact in a *cis* geometry [11,26]. *Cis* dimers were first proposed, based on the crystal structure of N-cadherin [8]. However, a subsequent crystal structure of C-cadherin proposed a new interface for the *cis* dimer [11] that contradicted this model. Indeed, crystal structures of E-, N-, and C-cadherin showed the same conserved *cis* interface, which primarily consists of an asymmetric junction between the EC1 domain of one protomer and the EC2 domain of its partner cadherin [26]. Furthermore, mutations at the *cis* interface of E-cadherin abolished *cis* dimerization in crystals [26], and AUC was unable to detect *cis* dimers suggesting that this interaction was weak [26]. Consequently, *cis* interactions were not observed in NMR measurements of E-cadherin EC1–2 [27] or in single molecule FRET experiments where E-cadherins were placed in close *cis* proximity [28]. Based on Monte Carlo simulations, it has been proposed that cadherin *cis* dimer formation requires prior *trans* dimerization [29,30]; when *trans* dimers are formed, the conformational flexibility of the extracellular region is reduced which lowers the entropic penalty associated with *cis* interactions [29]. This interplay of *trans* and *cis* contacts results in the formation of 2D cadherin lattices at intercellular junctions [26]. Indeed, both cells expressing E-cadherin and liposomes coated with E-cadherin ectodomains formed ordered, junction-like arrangements. In contrast, E-cadherin *cis* mutants failed to form ordered structures in liposomes and stable junctions in cells [26].

Cis interactions are thought to be important in stabilizing intercellular junctions by forming cadherin clusters. Cadherin clusters are believed to form by rapid diffusion of cadherins and by a slower mechanism of endocytosis and exocytosis [31,32]. Single molecule studies show that clustering enhances adhesion by increasing cadherin avidity [28]. However, direct evidence for the role of *cis*-dimers in cluster formation is lacking. A recent study of E-cadherin on bilayers has shown that intracellular linkage to actin cytoskeleton, and not *cis* interactions, stabilize cadherin clusters [33]. Moreover, super resolution microscopy measurements showing that the mesh-like cortical F-actin network corral non-adherent cadherins into nanometer scale clusters [34], raise questions on whether prior *trans* dimerization is indeed required for cadherin clustering. Micropipette measurements [35] and computational studies [36] also show that glycosylation of the cadherin ectodomain plays an important role in *cis* dimer formation. These studies bring into question the molecular role of *cis* dimers in cadherin function.

2. Mechanical force alters the kinetics of cadherin adhesion

Since cadherins are exposed to mechanical stimuli during the course of their normal physiological function, it is crucial to understand