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Homophilic interaction and deformation of E-cadherin and cadherin 7 probed by single molecule force spectroscopy

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ABSTRACT

Cadherin-mediated adhesion plays a crucial role in multicellular organisms. Dysfunction within this adhesion system has major consequences in many pathologies, including cancer invasion and metastasis. However, mechanisms controlling cadherin recognition and adhesive strengthening are only partially understood. Here, we investigated the homophilic interactions and mechanical stability of the extracellular (EC) domains of E-cadherin and cadherin 7 using atomic force microscopy and magnetic tweezers. Besides exhibiting stronger interactions, E-cadherin also showed more efficient force-induced self-strengthening of interactions than cadherin 7. In addition, the distributions of the unbinding forces for both cadherins partially overlap with those of the unfolding forces, indicating that partial unfolding/deformation of the cadherin EC domains may take place during their homophilic interactions. These conformational changes may be involved in cadherins physiology function and contribute to the significant differences in adhesive strength mediated by type I and type II cadherins.

1. Introduction

Selective and robust cell–cell adhesion plays a key role in maintaining tissue structural integrity and specific architecture in multicellular organisms [1,2]. In most tissues, cell–cell adhesion is dominated by a class of transmembrane proteins named cadherins [1,2]. Dysregulation of cadherin function correlates with tumour cell invasion and distant dissemination [1–4]. The cadherin superfamily comprises distinct families and subfamilies [5,6]; one of these is the classical cadherins. E-cadherin, the prototypic member of classical type I cadherins, is an essential component of epithelial adherens junctions and contributes to a fully polarized state in the cell through the formation of a circumferential actin belt. In contrast, classical type II cadherins, such as cadherin 7, show significantly weaker adhesion and are mainly expressed in mesenchymal tissues [6,7].

Type I and type II cadherins demonstrate similar domain organization: a cytoplasmic region, a transmembrane region, and an extracellular region [8,9]. The primary sequence of the extracellular region differs significantly between type I and type II cadherins [10]. The forces required to separate cell doublets expressing type II cadherins are much weaker than for those of type I-expressing cells, a property linked to their extracellular region [6]. Nonetheless, the extracellular segments of type I and type II cadherins share a similar 3D structure that comprises five tandem repeats, called extracellular cadherin (EC) domains, herein referred to as EC1 to EC5. Each EC domain consists of about 110 amino acids forming seven β-strands that are organized into two β-sheets [5,11,12].

Crystallographic data suggest the formation of X-dimers and strand-swapping dimers by the homophilic interaction of classical

Abbreviations: EC, extracellular cadherin; EC1 to EC5 domains, the first to the fifth extracellular cadherin domains; AFM, Atomic Force Microscopy; SMD, Steered Molecular Dynamics; TIRF, Total Internal Reflection Fluorescence.

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type I cadherins *in vitro* [12–14]. In a two-step adhesive binding experiment, cadherins were shown to initially form X-dimers and then convert to strand-swapping dimers [12]. A similar pathway were also proposed by Rakshit et al., as in the atomic force microscopy (AFM) studies [15], they found that strand-swapping dimers formed slip bonds, and X-dimer of E-cadherin formed catch bonds [15]. The latest steered molecular dynamics simulations results suggest that tensile force can deform cadherin EC domains to form long-lived hydrogen bonds to tighten the X-dimer contact [16]. Crystallographic studies also show that type II cadherins form similar strand-swap dimers [13,14]. In their strand-swapping dimers, the buried accessible surface area was found larger than that of type I cadherins [13,14] and, the dissociation constants (k_d) measured by ultracentrifugation [17] imply that the binding energy of type II cadherins is higher than that of type I cadherins. On the contrary, type I cadherins expressed cells show stronger unbinding forces [6,7]. Nevertheless, direct comparison between type I and type II cadherins at the molecular level is lacking, while this is important for understanding the distinct adhesion mechanism between them.

In the AFM study of E-cadherin X-dimers and strand-swapping dimers, Rakshit et al. proposed a model of reorientation of the EC domains by tensile forces to lock the dimer more tightly by an alternate binding site as the mechanism of the catch-bond behaviour. Meanwhile, quite a few studies also indicate that force plays important role in assisting cadherin-mediated adhesion processes. E-cadherin-mediated adhesion occurs under an actomyosin-generated tension force *in vivo* [18], force can enhance E-cadherin-mediated adhesion [19–21], and force can also increase the junction size in cadherin adhesions [22,23]. In addition, studies indicated that the cells can respond to the activation of E-cadherin EC domains (conformational change for binding) to regulate adhesion [24]. Therefore, EC domains and the homophilic interactions of their pairs response to mechanical forces is essential for cell–cell interaction.

Here, we used AFM to compare the homophilic interactions between E-cadherin and cadherin 7 at the single-molecule level varied under the external force dynamics. While both cadherins showed slightly time-dependent strengthening in their homophilic interactions, the strengthening effect by additional mechanical stretching is much more noticeable for E-cadherin than for cadherin 7. The elasticity of the EC domains of both cadherins were also carried out using AFM and magnetic tweezers, and the results indicated that the force to partially unfold/deform the EC domains showed a larger overlap with the unbinding force of the dimers for E-cadherin than cadherin 7.

2. Material and methods

2.1. Protein cloning

The Ecad and Cad7 genes were cloned into pFB-Sec-NH vector (Addgene) using ligation-independent cloning [25]. The forward and reverse primers for Cad7 were 5' –TACTTCCAATCCATGAGCTGGGTTTGGAATCAGTTC-3′ and 5′-TATCCACCTTTACTGTCACTCTGCATTGCAGGTCTGG-3′, and for Ecad, 5′-TACTTCCAATCCATGGACTGGGTCATCCCTCCC-3′ and 5′-TATCCACCTTTACTGTCACGCCTTCATGCAGTTGTTGA-3'. The construct contains baculovirus gp64 signal peptide followed by an N-terminal hexahistidine tag and TEV protease cleavage site. Bacmid production, insect cell transfection and virus production were performed as previously described [26]. The early passage of virus particles (P0) was amplified to P2 and was used to infect 2 L of log phase (3 × 106 cells/ml) insect cells for recombinant protein expression. The multiplicity of infection (MOI) was kept between 2 and 3 and the culture was incubated at 140 RPM, 27 °C for 56 h.

2.2. Protein purification

The culture was spun at 4000 × g for 30 min and the supernatant was collected. Protease inhibitor cocktail (Calbiochem) was added to the media (100 μl per 1 L media). For optimal binding, the pH of the supernatant was adjusted to 7.5 using a solution of 500 mM Tris pH 8.0 and 1.5 M NaCl. Ni-NTA Agarose (10 ml; Life Technologies) was added to the supernatant and incubated with rotation at 80 RPM for 1 h (4 °C). The supernatant was then subjected to second protein absorption with 5 ml Ni-NTA beads. The beads were collected and loaded into gravity columns and washed with 20 column volumes (CV) of wash buffer (50 mM Tris, pH 8.0, 500 mM NaCl and 2 mM imidazole, pH 8.0). The target protein was eluted with elution buffer (50 mM Tris, pH 8.0, 500 mM NaCl and 250 mM imidazole pH 8.0). Two CV fractions were collected per time until no protein was detected (absorbance, 280 nm) in the elution buffer. The eluted protein was subjected to buffer exchange (PD10 column, GE Healthcare) and digested with TEV protease (1:40 ratio of mg TEV protease:mg protein) at 4 °C overnight [27]. The sample was then loaded onto a gravity column packed with Ni-NTA agarose beads for the removal of the free His-Tag and TEV protease. The flow-through containing the target protein was collected. The fractions containing the target protein were collected and concentrated to 5 ml using 10K MWCO concentrator (Vivaspin 20 ml, Sartorius Stedim Biotech) before being subjected to size-exclusion chromatography (SEC). SEC was conducted in the AKTA Xpress system (GE Healthcare) using a HiLoad 16/60 200 Superdex prep-grade column equilibrated in GF buffer (20 mM HEPES, 300 mM NaCl, 10% (v/v) glycerol). Elution peaks were collected in 2 ml fractions and the purity of the protein was analysed on SDS-PAGE. The protein sample was again concentrated using a 10K MWCO concentrator (Vivaspin 20 ml, Sartorius Stedim Biotech).

2.3. Buffers and substrates

Unless otherwise stated, a 25 mM HEPES, 125 mM NaCl and 3 mM $CaCl_2$ buffer with a pH adjusted to 7.2 was used. Quartz (UQG optics, Cambridge, Cambridgeshire, UK) slides were cleaned by washing successively in a sonicator with deionized water, ethanol and deionized water again for 20 min each step, and then treated by air plasma (Expanded Plasma Cleaner, Harrick Plasma, St. Ithaca, NY, USA) for 5 min before use.

Three different coating methods were used in experiments. For AFM unbinding experiments, NTA/Ni^{2+}-coated AFM cantilever and quartz slides were prepared. The cantilever and slides were coated with biotin by incubating in 0.1 mg/ml biotin-labelled BSA (Sigma–Aldrich St. Louis, MO, USA) overnight. The 0.4 mM biotin-PEG-SVA (Laysan Bio, Arab, AL, USA) solution was labelled with NTA by reacting with 1 μg/mL Nα,Nα-bis(carboxymethyl)-L-lysine hydrate (Sigma Aldrich) overnight. The NTA-labelled PEG was diluted and mixed with 0.1 mg/mg streptavidin (Sigma Aldrich) at a 1:4 M ratio for 30 min. Finally, the biotin-coated cantilever and slides in the first step were coated with this mixture solution for 30 min, followed by 100 mM $NiSO_4$ for 30 min.

For AFM unfolding experiments, NTA/Ni^{2+}-coated quartz slides were prepared in five steps by incubating sequentially in 1 M NaOH solution for 15 min, propylmethyldimethoxysilane (Alfa Aesar, Ward Hill, MA, USA) solution (1% propylmethyldimethoxysilane, 4% water, 95% ethanol) for 15 min; 0.05% glutaraldehyde (Sigma Aldrich) solution for 1 h; 1 μg/mL Nα,Nα-bis(carboxymethyl)-L-lysine hydrate solution for 30 min, and finally in 100 mM $NiSO_4$ solution for 30 min [28]. The slides were washed thoroughly with deionized water between the steps.