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Self-chaperoned dimerization of P-cadherin

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Herein we propose that the adhesive dimerization of cadherins is achieved by a mechanism analogous to that of assembly chaperons. This mechanism confers high specificity and fast association rates. These findings will guide therapeutic approaches targeting cadherins in cancer.

1 Introduction

Placental cadherin (P-cadherin) is a member of the classical cadherin family (type I) mediating cell adhesion and tissue formation. Dysregulation of P-cadherin is also a landmark of numerous types of malignant cancers. Therapeutic approaches targeting P-cadherin have shown beneficial effects in disease models. In addition, P-cadherin is a candidate marker for cancer stem cells.

A preliminary characterization of P-cadherin showed it dimerizes in the so-called strand-swap conformation [1]. Later we identified the monomer and the X-dimer conformation at the biochemical level [2]. Herein, we have elucidated the complete mechanism of cell adhesion by human P-cadherin from a structural standpoint [3]. These data are critical for the understanding of cell-adhesion mediated by cadherins, and will help to manipulate cell-adhesion in biological systems.

2 Experiment

Expression of P-cadherin (or E-cadherin) was carried out as described previously [2]. Purified proteins were screened in an Oryx8 system (Douglas Instruments, UK) with commercially available kits at 20 °C. Conditions yielding protein crystals were identified, and then optimized. Protein crystals were harvested, and stored in liquid N_2 until data collection at the Photon Factory. Diffraction data were collected under cryogenic conditions (100 K) at a wavelength of 1.000 Å.

We also employed for kinetics of dimerization, a genetically encoded photoreactive probe, isothermal titration calorimetry, analytical ultracentrifugation, full-length expression in CHO cells, cell aggregation assays, immunofluorescence, and live cell imaging.

3 Results and Discussion

To understand the basis of homo-dimerization by human P-cadherin we determined crystal structures of P-cadherin prior (monomer), at intermediate stages (X-dimer) and after complete dimerization (strand-swap dimer). Surprisingly, there were no structural differences between the initial monomer and the final strand-swap dimer, except for the first two residues at the N-terminus, which are exchanged to form the dimer. In contrast, the intermediate X-dimer generated larger conformational changes than expected. The interaction surface of X-dimer was very extensive, revealing a possible mechanism by which the X-dimer progressively closes on itself by a bending motion. The thermodynamic data obtained by ITC helped to interpret the structural data. By comparing the crystal structures and thermodynamic parameters of P-cadherin and E-cadherin, we suggest that water molecules play a key role in achieving strong dimerization properties in P-cadherin.

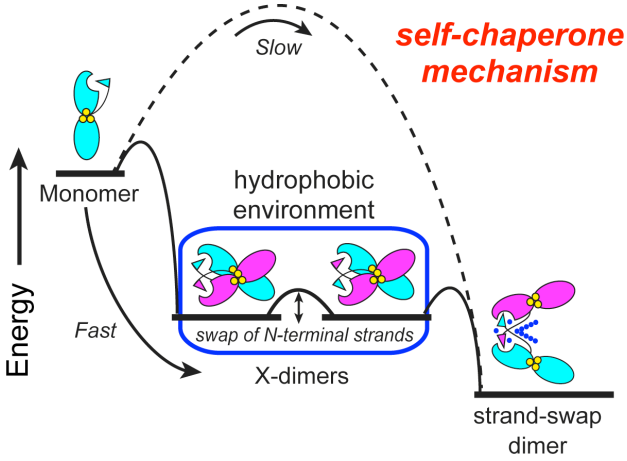

Fig. 1: Self-chaperone mechanism of P-cadherin.

Because P-cadherin accelerates its own assembly by means of a large hydrophobic patch at the protein-protein interface, we termed this mechanism chaperone-like dimerization (Fig. 1). In summary, crystal structures of P-cadherin in multiple conformational states were employed to elaborate the first motion picture of the dimerization of P-cadherin at high-resolution.

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