Lateral self-assembly of E-cadherin directed by cooperative calcium binding

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Abstract **We report the Ca^{2+} binding characteristics of recombinant Ecad12, a construct spanning the first two repeats of epithelial cadherin, and demonstrate the links between Ca^{2+} binding and dimer formation. Sedimentation equilibrium and dynamic light scattering experiments show that weak dimerization of Ecad12 occurs in the presence of 10 mM Ca^{2+} (K_d^P = 0.17 mM), while no appreciable dimer formation was detected in the absence of Ca^{2+}. Ca^{2+}-induced dimerization was also observed in electron microscopy images of Ecad12. We conclude from Ca^{2+} titration experiments monitored by tryptophan fluorescence and flow dialysis that dimerization does not affect the equilibrium binding constant for Ca^{2+}. However, the value of the Hill coefficient for Ca^{2+} binding increases from 1.5 to 2.4 as the protein concentration increases, showing that dimer formation largely contributes to the cooperativity in Ca^{2+} binding. Based on these observations and previous crystallographic studies, we propose that calcium acts more likely as a geometrical aligner ensuring the proper assembly of cadherin molecules, rather than a simple adhesive.**

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1. Introduction

It has long been recognized that calcium is a key element for maintaining cellular adhesiveness, as shown from live tissues and cultured cells dissociating whenever exposed to a calcium-depleted environment [1,2]. Cadherins are Ca^{2+}-dependent cell adhesion molecules that play a crucial role in homophilic cell-cell association [3]. The modular architecture of cadherin molecules consists of five repeats in the extracellular part, a single transmembrane region and a single intracellular domain linked to the actin filaments of the cytoskeleton via α- and β-catenins. An N-terminal fragment composed of the first two repeats of murine E-cadherin (Ecad12) was found to crystallize as a dimer, while its 3D X-ray structure revealed that the peptide stretch linking the domains of each Ecad12 molecule binds three Ca^{2+} ions, which in turn mediate a network of interactions with the opposite monomer [4]. A previous crystal structure of a single N-terminal domain of neural cadherin was also found to consist of a dimer, but it showed a different mode of interaction with no calcium ions involved in the interface between the monomers [5]. In an effort to understand the role of calcium in cadherin self-assembly, we have investigated in the present study the characteristics of calcium binding by recombinant Ecad12 and demonstrated its link to dimer formation by solution techniques and electron microscopy.

2. Materials and methods

2.1. Sedimentation equilibrium experiments

The experiments were carried out on a Beckman Model E ultracentrifuge. Sample aliquots of 100 μl were loaded into 12-mm double-sector, charcoal-filled Epon cells equipped with sapphire windows. The experiments were performed at 20°C for approximately 48 h before equilibrium photographs were taken. An assumed value of 0.73 was used for the partial specific volume of the sample. The apparent molecular weights were calculated from the slope after fitting the Ln(y) versus r^2 data to a second-degree polynomial equation, using a least-squares technique.

2.2. Light scattering

Molecular weight determinations using light scattering techniques were performed on a Dawn F multi-angle laser light-scattering photometer (Wyatt Technology Corporation, Santa Barbara, CA) according to the methodology previously described [6]. An aliquot of sample was manually injected onto a Pharmacia Superose 12 gel filtration column and the scattering intensity and RI signal of the eluting peaks were monitored. Approximately 10-fold dilution was observed upon injection of the sample of 0.2 mM protein. The RI signal was used to calculate the protein concentration, using an assumed value of 0.185 for the sample dn/dc. For each slice across a peak, a Debye plot was produced ($R_\Theta/K \times c$ versus $\sin^2\Theta/2$), in which the intercept of the extrapolation of scattering intensities to the zero angle and zero concentration yielded the reciprocal molecular mass. The apparent average molecular mass across the whole peak was then calculated from all the individual slices.

2.3. Electron microscopy

Solutions of Ecad12 (100 μg/ml) in 10 mM Tris-HCl (pH 7.5) were adsorbed on copper grids covered with butvar B-76 support film, stained with 2% phosphotungstic acid and air dried. Images were obtained on a transmission electron microscope Zeiss EM902 equipped with an energy filter. Bright field images were recorded at a magnification of 85000 for the apo form and 50000 for the Ca^{2+}-bound form. Selected molecules were digitized with a 64×64 pixel resolution.

2.4. Calcium binding to Ecad12

All buffers and samples were treated with Chelex to remove trace amounts of Ca^{2+}. Flow dialysis experiments were carried out at 25°C as described previously [8]. The protein solution used was 0.17 mM dissolved in 1 ml of buffer (20 mM Tris-HCl (pH 7.5), 100 mM KCl, 2 mM DTT, 100 μM NaN_3). Non-linear regression was used to fit the data to a classical Hill model. The Ecad12 sample used for the fluorescence titration was 1.5 μM dissolved in 2 ml of buffer (0.1 KCl, 50 mM HEPES, pH 7.5, 1 mM DTT) placed in a cuvette at 25°C. The intrinsic fluorescence of the protein (excitation at 290 nm and emission at 305 nm) was measured using an Aminco SLM-8000C fluorimeter. Aliquots of a standard $CaCl_2$ solution (5 mM) were added at

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