Conformational stability and mixed domain binding properties of the EC5 domain of E-cadherin.

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Introduction

Due to their physicochemical properties, large hydrophilic molecules such as protein or peptide drugs cannot readily pass through the transcellular route of the intestinal mucosa and blood-brain barriers. Similarly, the presence of tight junction prevents these large molecules from permeating via the paracellular pathway.

Materials

Plasmid: pERF-cadherin (containing the full-length human E-cadherin, provided by Dr. David Rimm, Yale University).
Expression vector: pET-24d (EMD Biosciences, San Diego, CA).
Companion Cell: E. coli BL21 (Stratagene, La Jolla, CA).
E-cadherin derived peptide: HAV peptide (from groove region of the EC1 domain): SHAVSS

Results

Figure 1. CD and FTIR spectra of EC5 in 25 mM K2HPO4, buffer at pH 7.5. The secondary structure composition of EC5 calculated from CD and FTIR spectra are shown in the table.

<table>
<thead>
<tr>
<th>Spectra</th>
<th>CD</th>
<th>FTIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-helix</td>
<td>7 %</td>
<td>14 %</td>
</tr>
<tr>
<td>β-sheet</td>
<td>32 %</td>
<td>27 %</td>
</tr>
<tr>
<td>β-turn</td>
<td>27 %</td>
<td>20 %</td>
</tr>
<tr>
<td>Random coil</td>
<td>34%</td>
<td>31%</td>
</tr>
</tbody>
</table>

Figure 2. The temperature induced unfolding of EC5 in 25 mM K2HPO4, buffer at pH 7.5, monitored by second derivative UV spectroscopy. Here shows the melting curves of Tyr and Trp residues.

Figure 3. The temperature induced unfolding of EC5 in 25 mM K2HPO4, buffer at pH 7.5, monitored by intrinsic fluorescence spectroscopy and differential scanning calorimetry. Intrinsic fluorescence shows that there are two conformations of EC5 at low temperature as there is only one Trp residue in EC5 and two deconvoluted emission peaks can be found.

Figure 4. 1H-15N HSQC NMR of 0.2 mM 15N labeled EC5 in 25 mM Tris buffer with 5% D2O at pH 7.5. The chemical shifts from the side chains of Asn and Gln are reasonably dispersed in the folded protein and collapse into a single pair of peaks upon denaturation at 65°C. EC5 contains only one Trp residue. Spectra collected at both higher and lower temperatures reveal this residue occupies multiple conformations.

Figure 5. 1H-15N HSQC NMR of 0.2 mM 15N labeled EC5 in 25 mM Tris buffer with 5% D2O at pH 7.5. Left figure: EC5 at the absence of 20 mM HAV peptide. Right figure: EC5 at the presence of 20 mM HAV peptide. Red arrows indicate the chemical shift changes after binding.

Figure 6. 1H-15N HSQC NMR of 0.2 mM 15N labeled EC5 in 100 mM Tris buffer with 5% D2O at pH 7.5. Left figure: at the absence of 10 mM BLG4 peptide. Right figure: at the presence of 10 mM BLG4 peptide. Red arrows indicate the chemical shift changes after binding.

Figure 7. EC1 and EC3 interaction study using size-exclusion chromatography. After mixing EC1 and EC3, both EC1 peak (47.892 min) and EC3 peak (74.289 min) disappear and two new peak (62.384 min and 65.576 min) with higher molecular weight appear.

Conclusions

The temperature induced unfolding experiments using fluorescence spectra suggest that EC5 has two different conformations at room temperature.

EC5 has a high thermal stability, which is possible due to the existence of the two intramolecular disulfide bonds.

Solution state NMR shows good dispersion of cross peaks between 8.5 – 10 ppm at 25°C and provides the tertiary structural information.

Both EC domain derived peptides can interact with EC5 and cause the conformational change. Size-exclusion chromatography suggests the interaction between EC1 and EC5.

References


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