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

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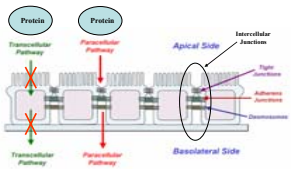
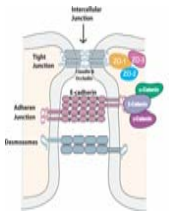
Introduction



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.



- The adherens junctions have an important function in the formation of intercellular junctions.
- The adherens junctions are formed by E-cadherins, which are Ca^{2+}-dependent cell surface glycoprotein.
- The extracellular domain of E-cadherin consists of five repetitive domains, EC1 to EC5.
- The existence of EC5 protein in the cell culture can inhibit the E-cadherin-mediated cell-cell adhesion, which suggests the binding of EC5 with other EC domains. However, the basic structural properties of this domain are not fully investigated.

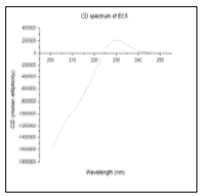
Objectives

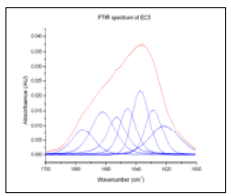
- To physically characterize the structure and the thermal stability of the EC5 domain.
- To understand the mixed domain interactions of the extracellular domain of E-cadherin.
- To modulate E-cadherin interactions in the adherens junctions of biological barriers for enhancing the permeation of large hydrophilic molecules such as protein drugs.

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).*
- Competent Cell: Epicurian coli BL21 *(Stratagene, La Jolla, CA).*
- E-cadhein derived peptide: HAV peptide (from groove region of the EC1 domain): SHAVSS
  BLG4 peptide(from the bulge region of the EC4 domain): TYRIWRDTAN

Results





Spectra	CD	FTIR
α –helix	7 %	14 %
β –sheet	32 %	27 %
β –turn	27 %	28 %
Random Coil	34%	31 %

Figure 1. CD and FTIR spectra of EC5 in 25 mM K_2HPO_4 buffer at pH 7.5. The secondary structure composition of EC5 calculated from CD and FTIR spectra are shown in the table.

Results (Cont.)

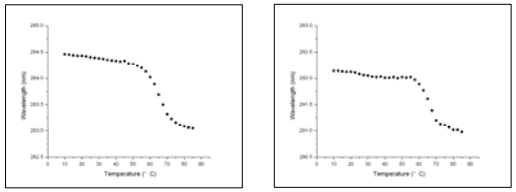


Figure 2. The temperature induced unfolding of EC5 in 25 mM K_2HPO_4 buffer at pH 7.5, monitored by second derivative UV spectroscopy. Here shows the melting curves of Tyr and Trp residues.

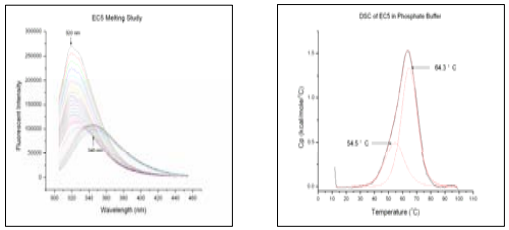


Figure 3. The temperature induced unfolding of EC5 in 25 mM K_2HPO_4 buffer at pH 7.5, monitored by intrinsic fluorescence spectroscopy and differential scanning calorimetry. Intrinsic fluorescence shows that there are two conformations of EC5 at low temperature as there is only one Trp residue in EC5 and two deconvoluted emission peaks can be found.

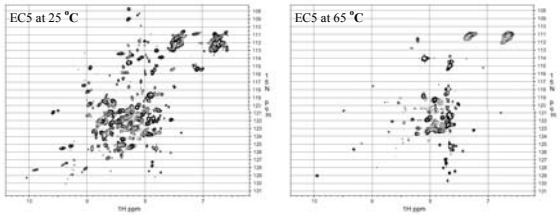


Figure 4. ^1H-^{15}N HSQC NMR of 0.2 mM ^{15}N labeled EC5 in 25 mM Tris buffer with 5% D_2O at pH 7.5. The chemical shifts from the side chains of Asn and Gln are reasonably disbursed in the folded protein and collapse into a single pair of peaks upon denaturation at 65 °C. EC5 contains only one Trp residue. Spectra collected at both higher and lower temperatures reveal this residue occupies multiple conformations.

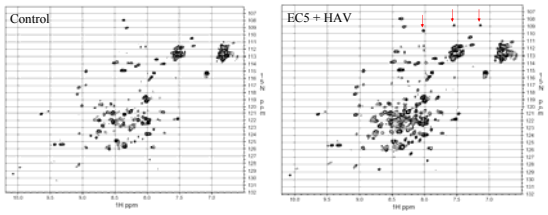


Figure 5. ^1H-^{15}N HSQC NMR of 0.2 mM ^{15}N labeled EC5 in 100 mM Tris buffer with 5% D_2O at pH 7.5. Left figure: EC5 at the absence of 20 mM HAV peptide. Right figure: EC5 at the presence of 20 mM HAV peptide. Red arrows indicate the chemical shift changes after binding.

Results (Cont.)

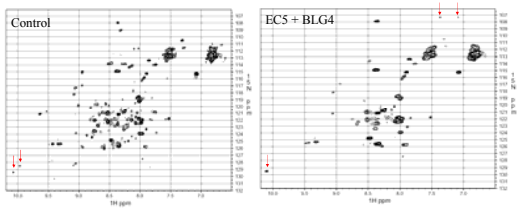


Figure 6. ^1H-^{15}N HSQC NMR of 0.2 mM ^{15}N labeled EC5 in 100 mM Tris buffer with 5% D_2O at pH 7.5. Left figure: at the absence of 10 mM BLG4 peptide. Right figure: at the presence of 10 mM BLG4 peptide. Red arrows indicate the chemical shift changes after binding.

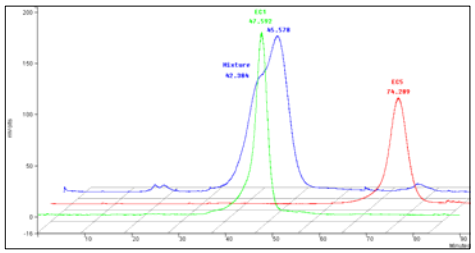


Figure 7. EC1 and EC5 interaction study using size-exclusion chromatography. After mixing EC1 and EC5, both EC1 peak (47.592 min) and EC5 peak (74.289 min) disappear and two new peak (42.384 min and 45.578 min) with higher molecular weight appear.

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 possibly 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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Acknowledgements

Financial Supports:

NIH (EB-00226)

NSF (DBI-0088931 and EPS-9874732)