Letter to the Editor: Sequence-specific resonance assignments and partial unfolding of extracellular domains II and III of E-cadherin

Jean-René Alattia, Frances K. Tong, Kit I. Tong & Mitsuhiko Ikura

Division of Molecular and Structural Biology, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, 610 University Avenue, Toronto, ON, Canada M5G 2M9

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Biological context

Epithelial cadherin (E-cadherin) is a transmembrane receptor that plays a vital role in Ca\(^{2+}\)-dependent homotypic cell–cell adhesion processes (Takeichi, 1991). The extracellular domain of E-cadherin contains five tandem repeats of about 100 amino acid residues, and is responsible for recognizing cadherin molecules of the same type in a calcium-dependent manner (for a review, see Alattia et al., 1999). The determination of the three-dimensional structure and dynamics of the cadherin extracellular domain in the presence and in the absence of Ca\(^{2+}\) should help us understand how cadherin interacts with Ca\(^{2+}\) ions and mediates cell adhesion functions.

Methods and results

A recombinant protein containing extracellular domains II and III of mouse E-cadherin (residues 109–333; MW 24 kDa) was cloned and expressed using a modified version of the pET 15b expression system (Novagen) and purified to homogeneity using the manufacturer’s standard procedure. Unlabeled, 15 N-labeled, or 13 C/15 N-labeled protein solution was prepared in 20 mM [\(^2\)H\sub{11}-]Tris-HCl (pH 7.2), 100 mM KCl, 1 mM [\(^2\)H\sub{12}]EDTA, 0.5 mM AEBSF, 95% H\sub{2}O/5% D\sub{2}O. All NMR experiments were performed on Varian INOVA 600 and 500 spectrometers.

Backbone C\(^{\alpha}\), N, H\(^N\), C\(^{\beta}\) and side-chain C\(^{\beta}\) resonances were assigned using HNCA(CB) and CBCA(CO)NNH supplemented with HNCO and CB-CACO(CA)HA. The aliphatic side-chain proton and carbon assignments were obtained by employing CC(CO)NNH TOCSY and HCCH-TOCSY, together with 15 N-edited NOESY-HSQC and TOCSY-HSQC (for details of the NMR experiments, see Bax et al. (1994) and Kay et al. (1997) and references cited therein). 1H and 13 C chemical shifts were referenced relative to TSP, 15 N chemical shifts were referenced relative to 15 N urea (78.98 ppm). Weighted-average secondary chemical shifts were calculated using the procedure described by Metzler et al. (1996).

Extent of assignments and data deposition

In the course of our NMR analysis, it became apparent that a number of resonances were missing in the 1H-15 N HSQC (Figure 1a and b) and other spectra (data not shown). Upon completion of the backbone assignment, we found that many residues in domain III (259–262, 288–291, 293–303) display significantly broadened or no signals in the NMR spectra under the conditions employed. On the other hand, almost all resonances in domain II as well as remaining residues in domain III were relatively sharp, permitting straightforward sequence-specific assignments. Interestingly, the domain II resonances were highly dispersed in the HSQC spectrum, whereas the domain III resonances clustered around the ‘random coil’ region (7.7–8.5 ppm) in the 1H dimension (Figure 1a and b). Consistent with these data, a secondary shift analysis using C\(^{\alpha}\) and C\(^{\beta}\) resonances (Figure 1c) strongly suggests that domain II is well folded but domain III is largely unfolded. The significance of this unfolded nature of domain III remains to be determined.

Ca\(^{2+}\) ions have been shown to bind the linker region between two adjacent domains (Overduin et al.,...
1995; Nagar et al., 1996). The present NMR study was performed on the domain II–III construct in the absence of Ca$^{2+}$, which is a prerequisite for characterizing the effect of Ca$^{2+}$ binding to the sites located between domains II and III. Our preliminary data indicated that addition of 10 mM CaCl$_2$ dramatically broadened the HSQC spectrum of this construct, hampering detailed NMR analysis of the Ca$^{2+}$-bound state. The $^1$H, $^{13}$C and $^{15}$N chemical shifts for the Ca$^{2+}$-free E-cadherin domain II–III construct have been deposited in the BioMagResBank under accession number BMRB-4457.

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References