Instrument-Dependent Cadherin Monolayer Interactions

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Abstract We introduce two theoretical models for predicting forces between cadherin-coated sphere and plane. The first model is based on a continuum theory previously developed for the interaction of large objects embedded in liquids. The second model takes into account size effects, which are relevant in the cadherin measurements, as the size of the tip is frequently comparable to the size of geometrical features of the cadherin. We use the models to compare force–separation curves in atomic force microscopy and surface force apparatus.

Keywords Cadherin · Surface force apparatus · Atomic force microscopy

1 Introduction

Cadherin is an individual protein chain that folds into a sequence of domains. On the surface of the cell, there are five compact domains. The adhesive site depends on a tryptophan amino acid. Calcium ions bind at the intersection between domains, offering stability and proper adhesive function. In the interior of the cell, a domain interacts with catenins and other proteins that bind to the cytoskeleton. Cadherins extend from the surface of the cell and attach to cadherins on a neighboring cell [1]. Thus, cadherins are transmembrane, cell surface proteins. The primary role of cadherin as a mediator in cell adhesion has been demonstrated in a variety of relevant situations: cell sorting during embryogenesis, maintaining the architecture of tissue in adult organisms, cell–cell recognition, and in several pathologies [2].

The microscopic mechanisms that lead to initial recognition and final adhesion are less well understood. To clarify this problem, experiments measuring cadherin–cadherin

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interactions have been made [3] between cadherin-coated surfaces using the surface force apparatus [4, 5] and using atomic force microscopy [6].

In this paper, we inspect the force–distance curves obtained by the two techniques, describe their qualitative trends, and propose theoretical model systems to convert force–separation curves across techniques.

2 Background of Previous Experiments and Motivation

In many situations of practical interest, the interacting surfaces are or can be well approximated by planes and spheres. That is the case in the interaction of colloidal particles in paint and the painted surface [7] (sphere–plane), Casimir forces between the plates of a capacitor [8] (plane–plane), and fat globules in milk [9] (sphere–sphere). In this study, we are interested in two geometries of cadherin–cadherin interaction detection. In the first case, cadherin is attached to the cross-elements of the surface force apparatus. Leckband et al. have shown that these measurements correspond to plane–plane interactions [3]. In the second case, cadherin is attached to a flat surface and to the tip of an atomic force microscope [10], giving rise to a plane–sphere interaction [6].

In the plane–plane configuration, Leckband’s group observed that, in addition to an overall decay (with a decay length of about 20 nm), the force–separation curve presents oscillations with a number of minima that depends on the cadherin fragments being probed. When using the entire extracellular fragment of cadherin (EC1–5), they found three clear minima [3]. However, the EC12 fragment only shows a single minimum [5]. In the experiments done by Silberzan’s group with the atomic force microscope, they measured forces for two different graftings. One of them, the nitrilo tri acetic acid grafting, corresponds to highly oriented cadherin on the tip and plane and is the same conformation used in Leckband’s group with the EC12 fragment. Consistently, Silberzan’s force–separation curves also show one minimum related to cadherin binding [6].

Hence, comparing measurements on the same cadherin fragments, E12, both SFA and AFM provide qualitatively similar force–separation curves. These results are consistent with the intuitive physical picture in which the EC12 pair sticks at a location between the globular domains.

Ultimately, one is concerned with the interactions between cadherin fractions *in vivo* under a variety of buffer conditions. The shape of the minima should give substructural information to help understand the onset of sticking and thus facilitate the development of a mechanism for kinetics. Thus, one should be interested in gaining insight into the differences between the force–separation measurements obtained via AFM and SFA. We address this issue theoretically by explicitly computing SFA and AFM interaction forces for cadherin configurations. In the first model, we use a continuum approach that has been successfully used for computing interaction forces between macroscopic objects embedded in a medium made up of comparatively small particles (colloids). However, recognizing that cadherin fragments and their separations are of sizes comparable to the AFM radius of curvature (about 10 nm), we also propose a second, minimalist discrete model.

3 Continuum Model

We have developed an analytical expression [11] that allows calculating interaction forces between spheres and planes from the corresponding forces between planes [12]. Consider

two parallel planes separated by a distance x . Call $E(x)$ the energy per unit area stored in the system. Then, [11] shows that, under the same conditions, the force between a sphere and a plane is

$$F(D) = 2\pi a[E(D) + E(D + 2a)] - 2\pi \int_D^{D+2a} E(x)dx \quad (1)$$

where D is the shortest distance between the sphere and the plane, and a is the radius of the sphere.

We shall use this expression to study the connection between the measurements of cadherin interactions by the surface force apparatus (plane–plane) and the atomic force microscope (sphere–plane).

We propose a model expression for the cadherin force per unit area in the plane–plane geometry, namely

$$\Gamma(x)_{\text{plane-plane}} = \frac{\mu}{x^3} - \frac{\gamma}{x^2} + \lambda e^{-\alpha x} \cos(\beta x). \quad (2)$$

This form is chosen because, by an appropriate choice of parameters, it can fit the experimental data. In this study, we use data for the entire extracellular fragment of cadherin, EC1–5 (data provided by S. Sivasankar and D. Leckband). It has decaying and oscillatory features, allowing for multiple minima. Γ is obtained from the experimental force by dividing by the area covered by the cadherin.

Integration of (2) provides the energy per unit area in the plane–plane geometry,

$$E(x)_{\text{plane-plane}} = \frac{\mu}{2x^2} - \frac{\gamma}{x} + \frac{\lambda e^{-\alpha x}}{\alpha^2 + \beta^2} [\alpha \cos(\beta x) - \beta \sin(\beta x)]. \quad (3)$$

This is now substituted in (1) to provide the force-separation curve in the sphere–plane geometry

$$\begin{aligned} F_{\text{AFM}} = & 2\pi a \left(\frac{1}{2D^2} + \frac{1}{2(a+D)^2} - \frac{\gamma}{D} - \frac{\gamma}{a+D} \right) \\ & - \frac{a + D(2a+D)\gamma(\log[D] - \log[2a+D])}{2aD(2a+D)} \\ & + 2\pi a \left(\frac{e^{-Da} \alpha \lambda \cos[D\beta]}{\alpha^2 + \beta^2} + \frac{e^{-(a+D)a} \alpha \lambda \cos[a+D]\beta}{\alpha^2 + \beta^2} \right. \\ & \left. - \frac{e^{-Da} \beta \lambda \sin[D\beta]}{\alpha^2 + \beta^2} - \frac{e^{-(a+D)a} \beta \lambda \sin[(a+D)\beta]}{\alpha^2 + \beta^2} \right) \\ & - \frac{1}{(\alpha^2 + \beta^2)^2} \left(2 e^{-2(a+D)a} \pi \lambda \left(\begin{aligned} & e^{(2a+D)a} (\alpha^2 - \beta^2) \cos[D\beta] \\ & - e^{Da} (\alpha^2 - \beta^2) \cos[(2a+D)\beta] \\ & + 2\alpha\beta (-e^{(2a+D)a} \sin[D\beta] + e^{Da} \sin[(2a+D)\beta]) \end{aligned} \right) \right). \end{aligned} \quad (4)$$

Although cumbersome, the right-hand side of (4) can be readily evaluated. Indeed, Fig. 1 shows a comparison between the theoretical surface force apparatus and the atomic force microscopy force–separation curves [for parameters in (2) that give rise to the correct experimental SFA curve]. The input plane-plane model force (red) appears with three minima as required by experimental data. The blue curve is generated from Eq. (1), and corresponds to the sphere-plane interaction, corresponding to an atomic force microscope

Fig. 1 Force–separation curves for the surface force apparatus configuration (red) and for the atomic force microscope configuration (blue)

configuration for a 10-nm tip. In the AFM curve, the first minimum is easily detectable, the second minimum becomes very shallow, and the third is almost imperceptible. Nonetheless, it is at least clear that the AFM curve is smoother than the corresponding SFM one.

Fig. 2 Configuration of the system for the discrete model. The cadherins are represented by rods sticking out from the plane and the sphere of variable radius a

4 Discrete Model

Equation (1) was derived to obtain interaction forces between macroscopic objects embedded in solutions. In that case, the small electrolytes can be treated as a continuum model. However, in the cases of interest in this paper, the cadherin ranges and separation are similar to typical AFM radius of curvature, around 10 nm. Thus, one would be interested in knowing if the results from the previous section, namely, the smoothing of the force–separation shape in the AFM case, can be extended to more realistic models, which take into consideration these size effects.

Fig. 3 Force curves for E12 on plane and tip of radius as indicated. As the tip radius decreases, there is loss of information

To answer this question, we introduce a minimalist model that emphasizes the size effect while keeping details at a minimum. Specifically, we propose a cadherin–cadherin potential of the form

$$V_{CC}(x) = - \sum_{n=1}^N (2N - 2n) \delta \left(x + \frac{d}{2} - nd \right) \quad (5)$$

where δ is the Dirac-delta function, N is the number of fragments, d is the separation between the fragments and x is the relative position of the cadherins, with $x=0$ corresponding to the molecules in perfect register. The term $2N-2n$ is equal to the number of fragment–fragment “bonds.” In this way, the potential will be deeper when many fragments interact (at small x) as compared with a small number of interacting fragments (at larger x).

In Fig. 2, we show a schematic of the system. The cadherin molecules are located on the flat and spherical surfaces. The spherical surface has a radius a , which is allowed to vary from 10 nm to infinity while preserving the size of the patch. To compute the interaction force between the plane and the sphere, we explicitly sum the individual forces between cadherin pairs as given in (5).

Figure 3 shows results for the interaction force between to E12 cadherin fragments. For very large tip radius, corresponding to a SFM configuration, the force curve shows a single minimum corresponding to the stick location. As the radius of the tip decreases, the minimum is reduced in extension until, at 10 nm, it disappears completely. Thus, in this discrete model, we also reach the conclusion that AFM measurements tend to smooth out the force curves. This is due to the fact that, unlike in the SFM configuration, in AFM, the molecules attached to the tip sense the molecules on the plane at different distances, and there is an averaging effect that manifests itself as a smoother force curve.

5 Conclusions

We presented continuum and discrete models to compare cadherin force curves in SFM and AFM. In both models, we found that the AFM smoothes out the SFM force curves due to a broader distance distribution of the proteins. In either case (SFM or AFM), the models can be used to extract cadherin–cadherin forces, ultimately the desired information. This can be done by fitting experimental curves to the force expressions provided in this paper.

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