Characterization of the binding affinity between the lynx protein and neuronal nicotinic acetylcholine receptors

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Abstract

Anxiety disorder is one of the most prevalent psychiatric illnesses in the United States. The lynx family proteins, a subset of the Ly6/uPAR superfamily expressed in multiple regions of the brain, have been shown to modulate the cholinergic system and be involved in the pathogenesis of anxiety disorders. To characterize the mechanism, we studied the interaction between lynx family proteins and neuronal nicotinic acetylcholine receptors (nAChRs). The binding affinity between lynx1 and α4β2-nAChRs and lynx2 and α7-nAChRs were characterized using atomic force microscopy (AFM). The result shows that the rupture forces between lynx1 and α4β2-nAChRs were relatively low (10-40 pN), indicating the binding might not be stable. Fitting the dynamic force spectrum (DFS) to the Bells-Evans model [1] yielded an activation barrier width of 0.92 nm and k_{off} of 0.59 s^{-1}. In contrast, the binding strength between lynx2 and α7-nAChRs was stronger, ranging from 60 to 90 pN. The lynx2-nAChR interaction has an activation barrier width of 0.61 nm and k_{off} of 6.8 x 10^{-4} s^{-1} These results indicate that the lynx proteins (lynx1 and lynx2) can bind with nAChRs specifically. However, the lynx2 interaction is stronger and presumably more stable. Ongoing experiments will further identify the structural and biophysical mechanisms underlying lynx-nAChR interactions.

Experimental Methods

Lynx protein expression

- Received lynx1&2 genes (**Fig. 1**) from collaborators.
- Transfected lynx genes (cDNA) into HEK 293T cell line.
- Purified proteins by size-exclusion chromatography (Ni-NTA chromatography).
- Confirmed protein quality by western-blot.

Figure 1. Human lynx2 wild-type (WT) amino acid sequence.

α7- and α4β2-nAChR expression

Human α7- and α4β2-nAChRs were expressed on cell membranes by transfecting the cDNA of each receptor into the SH-EP1 cell line. These vectors also carried a hygromycin B resistance gene for characterization. 0.13 mg/mL hygromycin B was thus added for positive selection of cells that contained the cDNA genes. This selective medium decreased the growth rate of cells by 2-3 times compared to that of the WT SH-EP1 cells.

AFM cantilever functionalization

Functionalizing the AFM cantilever is a critical process in this experiment. To coat the AFM tip with lynx protein, we add two objective substrates: APTES (3-Aminopropyl) triethoxysilane) and acetal-PEG27-NHS. In summary, the functionalized probe contains three layers: APTES, acetal-PEG27-NHS and the lynx protein (**Fig. 2**)

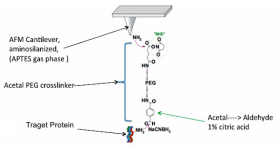

Figure 2. Schematic of the probe-protein binding assay setup.

Experimental Methods

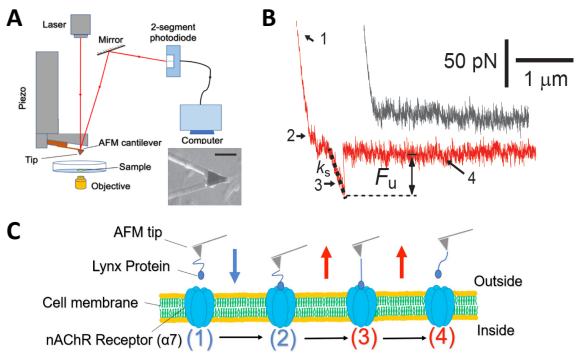

Figure 3. The unbinding force detection of lynx-nAChR interactions. **(A)** The schematic of the custom-built AFM with 2-segment photodiode detection. Inset: micrograph showing an AFM microcantilever functionalized with lynx2 above SH-EP1 cells expressing α7-nAChRs. **(B)** Representative pulling traces. The grey (upper) trace recorded no interaction, and the red (lower) trace shows the rupture force of a single lynx 2- α7-nAChR complex. F_u is the unbinding force. k_s is the system spring constant derived from the slope of the force-displacement trace. The cantilever retraction rate of the measurements was 1.8 μm/s. The four stages of stretching and rupturing a single ligand-receptor complex are labeled on the red trace. **(C)** The four stages of a force measurement: 1. The functionalized cantilever moving downward to allow contact with the cell membrane. 2. A small constant force (300 pN) applied onto the membrane, providing the time and space for a ligand-receptor interaction to take place. 3. The AFM tip retracting from the cell membrane. If the protein is bound to a receptor, there will be an adhesive force to resist separation between the tip and cell membrane. 4. The ligand-receptor bond rupturing, and the cantilever unbending.

Results & Conclusions

Results

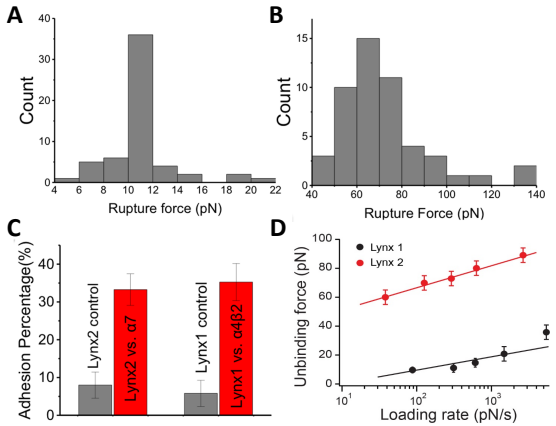

Figure 4. The binding affinity based on the unbinding force of lynx-nAChR interactions. **(A)** The histogram of the most probable unbinding force at 90 pN/s between lynx1 and α4β2-nAChRs. **(B)** The histogram of the most probable unbinding force at 120 pN/s between lynx2 protein and α7-nAChRs. **(C)** Adhesion frequency between lynx2 (left) or lynx1 (right) and their corresponding nAChRs. The control experiment was conducted between lynx and cells not expressing nAChRs. An adhesion frequency of 30% ensured the detection of single molecular interactions. **(D)** The unbinding forces of the lynx2- (red) and lynx1- (blue) nAChR interactions plotted against the corresponding loading rates. The unbinding forces of lynx1-α4β2-nAChRs were around 10-40 pN, whereas those of lynx2-α7-nAChRs were 60-90 pN. Fitting the data to the Bell-Evans model [1] yielded an activation barrier of 0.92 nm and k_{off} of 0.59 s^{-1} for lynx1, and an activation barrier of 0.61 nm and k_{off} of 6.8×10^{-4} s^{-1} for lynx2.

[1] E. Evans, K. Ritchie, Dynamic strength of molecular adhesion bonds. Biophys. J. 72, 1541-1555 (1997).

Conclusions

- The lynx proteins (both lynx1 and lynx2) can bind to nAChRs specifically, which supports the hypothesis that lynx proteins may influence brain activity via interaction with nAChRs. Combining with other behavioral experimental results, lynx proteins play a crucial role in the anxiety disorders.
- Lynx2-α7-nAChR binding is stronger and more stable than that of lynx1-α4β2-nAChRs binding, indicating that the lynx2-α7-nAChR interaction may be more functionally important in regulating nAChRs.