

Citation Spotlight

N-terminal insertion and C-terminal ankyrin-like repeats of α -latrotoxin are critical for Ca^{2+} -dependent exocytosis

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" α -Latrotoxin, a potent stimulator of exocytosis from neurons and neuroendocrine cells, has been studied intensively, but the mechanisms of its actions are poorly understood. Here, we developed a new method to generate active recombinant α -latrotoxin and conducted a structure/function analysis of the toxin in stimulating Ca^{2+} -dependent exocytosis. α -Latrotoxin consists of a conserved N-terminal domain and C-terminal ankyrin-like repeats. After cleavage of an N-terminally fused purification tag of glutathione S-transferase (GST), the recombinant toxin strongly stimulated exocytosis, whereas the GST-fused toxin was much less potent. The GST-fused toxin bound to the receptors [neurexin 1 α ; CL1 (CIRL/latrophilin 1)] as efficiently as did the GST-cleaved toxin but was much less effective in inserting into the plasma membrane and inducing cation conductance. The toxin with deletion of the last two ankyrin-like repeats still bound the receptors but could neither stimulate exocytosis nor induce cation conductance efficiently. The abilities of the mutated toxins to stimulate exocytosis correlated well with their abilities to induce cation conductance, but not their binding to the receptors. Our results indicate that

(1) C-terminal ankyrin-like repeats and a free (unfused) N terminus are both required for the toxin to form pores, which is essential for Ca^{2+} -dependent exocytosis, and (2) receptor binding alone is not sufficient to stimulate Ca^{2+} -dependent exocytosis."

To express active recombinant α -latrotoxin, the authors transformed Origami™ B(DE3) competent cells. They state that "Previously, α -latrotoxin expressed in bacteria has lacked biological activity, apparently because of the strong reductase activities in *E. coli*. Such reductase activities would prevent the formation of disulfide bonds within α -latrotoxin, which are essential for binding to its surface receptors." "Thus, we hypothesized that the use of a recently developed strain of *E. coli*, Origami

(commercially available from Novagen, Madison, WI), in which both thioredoxin reductase and glutathione reductase are inactivated, would improve the production of active α -latrotoxin."

After comparing recombinant α -latrotoxin to native latrotoxin for the ability to stimulate norepinephrine release from pheochromocytoma 12 cells in a dose-dependent manner, Li and this group conclude that they "succeeded in the generation of active α -latrotoxin using the (Origami) strain of *E. coli*." ■

Product	Size	Cat. No.	Price \$
Origami™ B(DE3) Competent Cells	0.4 ml	70837-3	73
	1 ml	70837-4	134

Components

0.4 ml	1 ml	Component
• 2 × 0.2 ml	5 × 0.2 ml	Origami B(DE3) Competent Cells
• 2 × 2 ml	4 × 2 ml	SOC Medium
• 2 ng	2 ng	Test Plasmid

Origami™ host strains are K-12 derivatives that have mutations in both the thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) genes, which greatly enhance disulfide bond formation in the *E. coli* cytoplasm. Origami B host strains are derived from a *lacZY* mutant of BL21 to enable precise control of expression levels by adjusting the concentration of IPTG. The mutations in *trxB* and *gor* are selectable on kanamycin and tetracycline, respectively; therefore, these strains cannot be used with plasmids that can only be selected with kanamycin or tetracycline. These strains also include the *lon* and *ompT* deficiencies of BL21, which increase protein stability.