Regulation of Exocytosis by Complexin

Ok-Ho Shin

Complexin (Cpx), which is expressed mainly in the nervous system, binds efficiently to the SNARE complex that is composed of either Syntaxin 1 or Syntaxin 3. Both Syntaxin 1 and Syntaxin 3 are involved in mediating synchronous neurotransmitter release in the nervous system. Cpx stabilizes the SNARE complex upon binding, and potentiates the efficacy of synchronous exocytotic process. Cpx is consisted of N-terminal, accessory α-helix, central α-helix, and C-terminal domains. Each domain of Cpx has a distinct function that is differentially involved in the regulation of priming, clamping, and activating in the exocytotic process. These functions of Cpx domains coordinately potentiate the efficacy of synchronous exocytotic process.

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Key words: Complexin; Exocytosis; SNARE Complex; Syntaxin; Transmitter release


Abbreviations

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Cpx: Complexin (also known as Synaphin); GST: glutathione S-transferase; IGF-1: insulin-like growth factor-1; KD: knock-down; KO: knock-out; LTP: long-term potentiation; SNAP-23 or -25: synaptosomal-associated protein 23 or 25; SNARE: soluble N-ethylmaleimide-sensitive factor attachment protein receptor; SNARE complex: a ternary complex composed of Syntaxin, Synaptobrevin, and either SNAP-23 or SNAP-25; Syb2: Synaptobrevin 2 (also known as vesicle-associated membrane protein 2, VAMP2); Syt1: Synaptotagmin 1; Syt10: Synaptotagmin 10; WT: wild-type.

INTRODUCTION

Ca²⁺-triggered exocytosis is an essential cellular process that mediates transmitter release to the extracellular space for intercellular communications particularly in neurons[1-3]. The same or similar processes are used for intracellular trafficking to deliver membrane patches or proteins to the plasma membrane[4,5]. Stimulations of neurons cause an increase in cytosolic Ca²⁺ concentrations and trigger fusions of synaptic vesicles into the plasma membrane to release transmitters to the extracellular space[1-3]. Synaptotagmin 1 (Syt1), a vesicular Ca²⁺ binding C2 domain protein, regulates Ca²⁺-triggered neurotransmitter release by interacting with membranes and the SNARE complex in a Ca²⁺-dependent manner[6,7]. Synaptobrevin 2 (Syb2; also known as vesicle-associated membrane protein 2, VAMP2), a vesicular SNARE protein, forms a ternary SNARE complex with Syntaxin and SNAP-25 that are located in the target plasma membrane[8]. The interaction between Syt1 and the membrane is essential for the Ca²⁺-binding to the C2 domains of
Syt1 in physiological Ca\(^{2+}\) concentrations\[^{7,9}\]. Meanwhile, the Ca\(^{2+}\)-dependent affinities of interactions between Syt1 and SNARE complexes are correlated with the efficacies of the corresponding exocytosis\[^{9}\]. Complexin (Cpx; also known as Synaphin) binds to the assembled SNARE complex, stabilizes the SNARE complex upon binding, and potentiates the efficacy of Syt1-regulated neurotransmitter release\[^{11-14}\].

**CPX IS EXPRESSED MAINLY IN THE NERVOUS SYSTEM**

Cpx is initially identified as a cytosolic protein that interacts with the SNARE complex\[^{12,13}\]. Cpx binds to the assembled SNARE complex composed of Syntaxin 1, Syb2, and SNAP-25 with a high affinity\[^{11-13}\]. The structure of Cpx-bound SNARE complex revealed that Cpx interacts directly with all components of the SNARE complex\[^{13}\]. Consistent with this observation, Cpx does not bind to partially assembled SNARE complexes\[^{13,15}\]. Cpx family has four isoforms (Cpx1-4) that are expressed mainly in the nervous system such as the brain, spinal cord, and retina (Figure 1A)\[^{13,16}\]. However, trace amounts of Cpx1-3 isoforms are also expressed in the liver, pancreas, kidney, spleen, skeletal muscle, and lung\[^{13,14}\]. Cpx1 and Cpx2 are soluble cytosolic proteins\[^{12,13}\]. Meanwhile, the C-terminals of Cpx3 and Cpx4 are farnesylated, and this modification is required for the targeting of these Cpx isoforms to the presynaptic plasma membrane\[^{14}\].

**CPX BINDS EFFICIENTLY TO THE ASSEMBLED SNARE COMPLEXES THAT ARE COMPOSED OF EITHER SYNTAXIN 1 OR SYNTAXIN 3**

Eukaryotic cells express at least four different Syntaxin isoforms (Syntaxin 1, 2, 3, and 4) that are involved in mediating various types of exocytotic processes\[^{19}\]. Systematic analyses of interactions between Cpx and SNARE complexes by employing both glutathione S-transferase (GST)-pulldowns and a yeast four-hybrid interaction assay showed that Cpx binds efficiently to the SNARE complexes that are composed of either Syntaxin 1 or Syntaxin 3\[^{13,16}\]. Cpx does not bind efficiently to the SNARE complexes that are composed of either Syntaxin 2 or Syntaxin 4\[^{13,16}\]. Consistent with these findings, the Syntaxin 1 residues that interact directly with Cpx are conserved in Syntaxin 3, but not in Syntaxin 2 and Syntaxin 4 (Figure 1B).

**SNARE COMPLEXES THAT ARE INVOLVED IN MEDIATING SYNCHRONOUS RELEASE ARE PROBABLY ASSOCIATED WITH CPX**

Stimulated neurons release neurotransmitters in two distinct modes, synchronous and asynchronous (Figure 2A). Synchronous release causes rapid actions, while asynchronous release triggers long-lasting actions of the target cells\[^{12,21}\]. Both modes of release are required to maintain the functions of biological systems. In addition, neurons are also capable of releasing neurotransmitters without evoked stimulations but still mainly in a Ca\(^{2+}\)-dependent manner, which is called spontaneous release (Figure 2A)\[^{12,21}\]. Four Cpx isoforms (Cpx1-4) are expressed mainly in the nervous system such as the brain, spinal cord, and retina, where neurotransmitters are released in a synchronous manner (Figure 2A)\[^{13,16}\]. In particular, membrane-bound Cpx3 and Cpx4 are expressed in retinal ribbon synapses where strong synchronous neurotransmitter release is required\[^{17}\]. Cpx binds efficiently to the SNARE complexes that are composed of either Syntaxin 1 or Syntaxin 3\[^{13,16}\]. These Syntaxin isoforms are expressed at presynaptic nerve terminals where neurotransmitters are released in a synchronous manner\[^{16,17}\]. Neurons also express other Syntaxin isoforms such as Syntaxin 2 and Syntaxin 4, which are not involved in mediating synchronous neurotransmitter release\[^{22,23}\]. Therefore, the differential binding of Cpx to SNARE complexes is probably required for the regulation of subsequent interaction between the SNARE complex and responsible Ca\(^{2+}\)-sensors. Cpx-deficient neurons, in the presence of Syt1, display a significant reduction of the efficacy of Syt1-regulated synchronous release\[^{14}\]. However, these neurons still maintain the mode of synchronous release\[^{14}\]. Meanwhile, Syt1-deficient neurons, in the presence of Cpx, change the mode of release to asynchronous from synchronous release\[^{26}\]. These observations suggest that Syt1 is a key regulator for synchronous neurotransmitter release and that Cpx potentiates the Syt1-regulated synchronous release (Figure 2B).

**CPX IS INVOLVED IN REGULATING VARIOUS TYPES OF EXOCYTOTIC PROCESSES**

Cpx-deficient neurons consistently display a significant reduction of the efficacy of synchronous neurotransmitter release in many different release systems (Table 1). Cpx is also involved in regulating large dense-core vesicle exocytosis\[^{25,26}\] and rapid delivery of AMPA receptors to the plasma membrane\[^{21}\]. Cpx-deficient chromaffin cells show a significant decrease in both evoked norepinephrine release and the pool size of releasable vesicles\[^{25,26}\]. In olfactory neurons,
Cpx-deficiency is associated with an impairment of Synaptotagmin 10 (Syt10)-regulated insulin-like growth factor-1 (IGF-1) release[27]. These findings suggest that Cpx is involved in regulating various types of exocytotic processes in the nervous system. Trace amounts of Cpx1-3 isoforms are also expressed in many non-neuronal tissues such as the liver, pancreas, kidney, spleen, skeletal muscle, and lung[13,16]. Therefore, Cpx is also likely involved in regulating various synchronous exocytotic processes occurring in these non-neuronal cells.

**CPX DOMAINS HAVE DISTINCT FUNCTIONS THAT COORDINATELY POTENTIATE THE EF-FICACY OF SYNCHRONOUS RELEASE**

Cpx-deficient cortical neurons and neuromuscular junctions show an increase in spontaneous release and a decrease in the pool size of releasable vesicles, in addition to a significant reduction of the efficacy of synchronous release (Table 1). These observations suggest that Cpx is involved in regulating at least three different steps of the exocytotic process: priming of vesicles, clamping of spontaneous vesicle fusions, and activation of Ca\(^{2+}\)-triggered vesicle fusions (Figure 3A). However, Cpx-deficient hippocampal neurons do not show any increase in spontaneous release or decrease in the pool size of releasable vesicles[14,28]. These differences among different neurotransmitter release systems are probably caused by the differential expression of other Ca\(^{2+}\)-sensors or regulatory proteins. To resolve this discrepancy, isolated/purified vesicle fusion assays, such as in vitro flipped SNARE cell fusion assay and single vesicle-vesicle microscopy imaging, are developed and employed[29-37]. The investigation of Cpx functions by employing these methods supports the multiple roles of Cpx in the regulation of priming, clamping, and activating during the Ca\(^{2+}\)-triggered vesicle fusion process. Cpx is consisted of N-terminal, accessory a-helix, central a-helix, and C-terminal domains (Figure 3B). And each domain of Cpx has a distinct function that is differentially involved in the regulation of priming, clamping, and activating during Ca\(^{2+}\)-triggered vesicle fusions. These distinct functions of Cpx domains coordinate to potentiate the efficacy of synchronous release (Figure 3B)[13,20,29-37].

**Figure 2** Regulation of different modes neurotransmitter release by Cpx. A. Evoked neurons release neurotransmitters in either synchronous or asynchronous mode. Syt1 and Syntaxin 3 are the major Syntaxin isoforms expressed at presynaptic nerve terminals and they are involved in mediating synchronous neurotransmitter release. Cpx binds efficiently to the SNARE complexes that are composed of either Syntaxin 1 or Syntaxin 3. Therefore, Cpx is probably involved in regulating the interactions between SNARE complexes and Ca\(^{2+}\)-sensors for exocytosis. Neurons also spontaneously release neurotransmitters without evoked stimulations, but still mainly in a Ca\(^{2+}\)-dependent manner. B. Syt1-deficient (Cpx\(^{3,4}\)) neurons change the mode of release from synchronous to asynchronous. Meanwhile, Cpx-deficient (Cpx\(^{1,2}\)) neurons do not change the mode of release, even though the efficacy of synchronous release is significantly reduced. Cpx, Complexin; KO, knock-out; Syt1, Synaptotagmin 1; WT, wild-type.

**Table 1** Effects of Cpx deficiency on various exocytotic processes.

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‡ Complexin, ‡ knock-out, § knock-down, α a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, † long-term potentiation, †† Synaptotagmin 10, †‡ insulin-like growth factor-3, ▲ increase, ▼ decrease.
Shin OH. Complexin & exocytosis

CONCLUSION

Complexin binds efficiently to the assembled SNARE complex that is composed of either Syntaxin 1 or Syntaxin 3, stabilizes the SNARE complex upon binding, and potentiates synchronous neurotransmitter release[11,14,15,32]. In stimulated neurons, Syt1 interacts with membranes and the Cpx-bound SNARE complex in a Ca²⁺-dependent manner to mediate synchronous neurotransmitter release[31]. The efficacies of Syt1-regulated synchronous release are correlated with the strengths of interactions between Syt1 and SNARE complexes[33]. Therefore, it is reasonable to assume that Cpx most likely facilitates the interaction between synchronous Ca²⁺-sensors and SNARE complexes, at a minimum, by selecting, tagging, and stabilizing the assembled SNARE complex to potentiate the efficacy of synchronous release.

CONFLICT OF INTERESTS

The Author has no conflicts of interest to declare.

REFERENCES

23. Kennedy MJ, Davison IG, Robinson CG, and Ehlers MD. Syntaxin-4 defines a domain for activity-dependent exocytosis in den-


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