IUBMB *Life*, 59(2): 84–89, February 2007

informa
healthcare

Research Communication

The Positively Charged Residues in the Fragment 71–77 of Complexin is Required for its Binding to SNARE Complex

Jingguo Liu, Yong Wei, Ting Guo, Xin Xie, Jiansen Jiang and Sen-fang Sui
Department of Biological Sciences and Biotechnology, State-Key Laboratory of Biomembrane and Membrane Biotechnology, Tsinghua University, Beijing, China

Summary

Complexin is a cytoplasmic protein that plays an important role in the neurotransmitters release triggered by action potential. Previous studies suggested that complexin performs its functions through interaction with the SNARE complex. The crystal structure of complexin/SNARE complex revealed that complexin binds to SNARE core complex in an anti-parallel conformation with its residues 48–70. However, the functions of the flanking sequences are unclear. In this paper, we demonstrate that the fragment 71–77 of complexin is indispensable for its binding to the SNARE complex. Moreover, this interaction can be impaired by abolishing the positive charges in the fragment 71–77, which suggests that the positive charges in the fragment 71–77 are important for the interaction between complexin II and the SNARE complex.

IUBMB *Life*, 59: 84–89, 2007

Keywords Complexin; SNARE complex; interaction; positively charged residues; Fluorescence Resonance Energy Transfer.

Abbreviations: SNAREs, soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors; SNAP-25, synaptosomal-associated protein of 25 kDa; VAMP, vesicle associated membrane protein, or synaptobrevin; Syx, syntaxin; CPX, complexin; FRET, Fluorescence Resonance Energy Transfer.

INTRODUCTION

Complexins are a small family of highly hydrophilic proteins enriched in the brain, with four isoforms identified (*1–4*). Genetic and physiological studies show that complexins have an important role during the Ca^{2+}-dependent neurotransmitter release. Long-term potentiation (LTP) was impaired in complexin II knockout mice while neurons lacking complexin I/II show dramatically reduced neurotransmitter release efficiency due to decreased Ca^{2+} sensitivity (*5, 6*). Injection of recombinant complexin into neurons of Aplysia buccal ganglia and overexpression of complexin in PC12 cells reduce the secretion while injection of antibodies into neurons of Aplysia buccal ganglia results in increased release (*3, 7*). Peptides that prevent complexin binding to the SNARE complex also inhibit evoked neurotransmitter release (*8*). Overexpression of complexin in isolated bovine adrenal chromaffin cells can shorten the opening time course of fusion pore (*9*). Recent studies showed that complexin arrests the hemifusion of liposomes reconstituted with SNAREs or cells mediated by 'flipped' SNAREs, and this inhibition can be relieved by synaptotagmin (*10, 11*). However, how complexins perform this function is still unknown.

So far, the SNARE complex, as the minimal machinery for exocytosis, is the only partner to which complexin binds (*1, 12–14*). It contains three proteins, syntaxin, SNAP-25 and VAMP, which bind to each other to form a highly twisted and parallel four-helical bundle through the SNARE motif adjacent to the transmembrane domain (*15, 16*). Biochemical and biophysical studies show that complexins bind rapidly to SNARE core complex in an anti-parallel alpha-helical conformation to the groove between syntaxin and VAMP adjacent to the transmembrane domain (starting from −3 layer onwards) and might stabilize the *trans*-SNARE complex formed between the synaptic vesicle and the presynaptic membrane (*17–19*). In addition, complexin can promote the interaction of the transmembrane domain of syntaxin and VAMP to form slow migration of SNARE complex (*20*). All these evidences support that complexin performs its function through interaction with SNARE complex.

Although X-ray studies revealed that the fragment 48–70 of complexin I is the domain that binds to the SNARE complex, the functions of the flanking regions are still

Received 13 December 2006; accepted 14 January 2007
Address correspondence to: Sen-fang Sui, Department of Biological Sciences and Biotechnology, State-Key Laboratory of Biomembrane and Membrane Biotechnology, Tsinghua University, Beijing 100084, China. Fax: +86 10 62793367.
E-mail: suisf@mail.tsinghua.edu.cn

ISSN 1521-6543 print/ISSN 1521-6551 online © 2007 IUBMB
DOI: 10.1080/15216540701216692

unknown. In this paper, we demonstrate that the fragment 71–77 is necessary for complexin to bind to the SNARE complex. Abolishing the positively charged residues in the fragment 71–77 inhibits the interaction between complexin and the SNARE complex, which suggests that fragment 71–77 of complexin II may interact with the SNARE complex through electrostatic interactions.

MATERIALS AND METHODS

Materials

cDNAs of rat VAMP, SNAP-25 and syntaxin were kindly provided by E. R. Chapman. cDNA of complexin II was a gift from D. Fasshauer. Affinity purified rabbit antiserum of complexin was from Synaptic Systems. BCA protein assay kit was from Pierce.

Plasmids Construction and Recombinant Proteins Preparation

The pET-28a vector encoding for thrombin cleavable amino-terminal His_6-tagged fusion proteins was used for the expression of the following constructs: VAMP (1–94), Syntaxin H3 (180–253), SNAP-25N (1–100) with four Cys replaced by Ala, SNAP-25C (125–206), complexin II (CPX II), CPX II (1–71), CPX II (1–77), CPX II (1–77) A with Lys 73,74,75 replaced by Ala, CPX II (1–77) E with Lys 73,74,75 replaced by Glu, CPX II (47–134) and CPX II (55–134). Syntaxin H3 (180–253) were cloned to pGEX-4T vector encoding for thrombin-cleavable GST fusion proteins. CPXII, CPXII 71, CPXII 77, CPXII 77A and CPXII 77E were fused with YFP at their N-terminals and cloned to pET-28a vector, respectively. The corresponding fusion proteins were named by YFP-CPXII, YFP-CPXII71, YFP-CPXII77, YFP-CPXII77A and YFP-CPXII77E. In addition, SNAP-25N (1–100), with four Cys replaced by Ala, was fused with CFP at its C-terminal and cloned to pET-28a vector to generate the SNAP-25N-CFP fusion protein.

All recombinant proteins were expressed in *E. coli* BL21 (DE3) cells according to standard protocols. All N-terminal His_6-tagged fusion proteins were purified by Ni^{2+}-Sepharose affinity chromatography and eluted with buffer A (20 mM Tris-Cl (pH 7.8), 150 mM NaCl, 2 mM β-ME) supplemented with 250 mM imidazole and 0.5% Triton X-100. After dialysis against buffer A at 4°C overnight, the fusion proteins were further purified by ion exchange chromatography using Mono-Q or Mono-S columns on an FPLC system (Pharmacia). The GST fusion proteins were purified by glutathione-Sepharose chromatography and eluted by 20 mM reduced glutathione in buffer A supplemented with 0.5% Triton X-100 and dialyzed against buffer A at 4°C overnight.

For GST-SNARE complex, GST-H3 was incubated with excessive VAMP, SNAP-25N and SNAP-25C at 4°C overnight and the GST-SNARE complex was purified by glutathione-Sepharose chromatography. For SNARE-CFP complex, VAMP, syntaxin H3 domain, SNAP-25N-CFP and SNAP-25C were incubated at 4°C overnight and further purified by ion exchange chromatography. The concentrations of all proteins or complex were determined by BCA protein assay kit and the Bradford assay.

Pull-down Assays

All pull-down assays were carried out by immobilizing GST-SNARE complex on the glutathione-Sepharose beads. Immobilized fusion proteins were incubated with excessive purified soluble binding partners for 1–1.5 h at 4°C on a rotator. After washing four times with Buffer A supplemented with 0.5% Triton X-100, bound proteins were eluted from bead with loading buffer, subjected to 15% SDS-PAGE, and visualized by immunoblotting and Coomassie Blue staining.

Fluorescence Studies

All fluorescence studies were performed at room temperature using F-4500 fluorescence spectrophotometer (Hitachi) with a 150 μl sample in a 10×2 mm cuvette (*21*). The excitation and emission slit is 5 nm. Emission spectra were recorded at 433 nm excitation (range from 450 to 560 nm) of donor/acceptor pairs, acceptor and donor alone, and at 500 nm excitation (515–560 nm) of donor/acceptor pairs and acceptor alone.

For the FRET titration assay, the concentration of SNARE-CFP was fixed at 0.2 μM and the concentrations of YFP-CPXII mutants (CPXII 71, CPXII 77, CPXII 77A, CPXII 77E and CPXII) were gradually increased. For the competition assay, the concentrations of SNARE-CFP and YFP-CPXII 77 were kept constant at 0.4 μM and the concentrations of unlabelled complexin mutants (CPXII 77, CPXII 47 and CPXII 55) were varied. The FRET efficiency was calculated according to the formula: $I_T = 1 - I_D/I_D^0$, where I_D^0 and I_D were the SNARE-CFP fluorescence intensity at 475 nm in the absence and presence of acceptor, respectively. The FRET efficiencies were calculated and plotted against the concentration of YFP-complexin II or its mutants (for the FRET titration assay) or the concentration of unlabelled complexin II mutants (for the competition assay). The affinity of complexin to SNARE complex is taken as the concentration of complexin at which the 50% of maximum FRET efficiency remained.

RESULTS

Fragment 71–77 of CPXII is Indispensable for Complexin to Bind to the SNARE Complex

Complexin is a small, highly hydrophilic protein that plays an important role during neurotransmitter release. It performs its function by its interaction with the SNARE complex. Although the X-ray results showed that the fragment of CPX 48–70 is the domain that binds to the SNARE core complex, sequence alignment of complexins from different organism

shows that the flanking regions of fragment 48–70 are also highly evolutionarily conserved (Fig. 1).

According to the sequence alignment, we first designed four truncated complexin mutants, CPXII 71, CPXII 77, CPXII 47 and CPXII 55 (Fig. 2A). Pull-down assay revealed that CPXII 71 has a lower ability to bind to the SNARE complex than the other mutants (Fig. 2B). This is also supported by FRET titration assay (see Fig. 4B, compared YFP-CPX71 with YFP-CPXII and YFP-CPXII 77). Although the X-ray results showed that the fragment of CPX 48–70 is the domain that binds to the SNARE complex, our result showed that N-terminal deletion of complexin II (CPXII 47 and CPX II 55) can still interact with SNARE complex. In the case of C-terminal deletion, CPXII 77 binds to the SNARE complex similar to the full-length complexin II, while CPXII 71 does not, which indicates that fragment 71–77 of complexin is indispensable for CPXII 77 to bind to the SNARE complex. Previous NMR results showed that a stable alpha-helix is formed from residues 29–64, while residues 65–86 contain a substantial but lower population of alpha-helix (*15*). According to this, fragment 71–77 may be needed to maintain alpha-helix of CPX that is necessary to bind to the SNARE complex.

Eliminating the Positive Charges in the fragment 71–77 impaired the Ability of Complexin to Bind to the SNARE Complex

Our above result shows that fragment 71–77 of CPXII is necessary for complexin to bind to the SNARE core complex. However, according to the sequence alignment, this fragment is conversed in different organisms, especially contains three conserved, positively charged Lys (Fig. 1, second box), thus we want to know whether these positively charged residues have a special role for complexin to bind to the SNARE complex. From Fig. 3, we can see that substituting three highly conserved Lys with Ala (CPXII 77A) can weaken the interaction between complexin and the SNARE complex. However, if the three conserved Lys were substituted by Glu, the binding ability of CPXII 77E to the SNARE complex was greatly reduced (Fig. 3, lane 5).

To further confirm the different binding abilities of complex II mutants to the SNARE complex, we compared the interaction between complexin mutants and SNARE core complex using FRET titration assay. In this experiment, the concentration of SNARE-CFP complex was kept constant at 0.2 μM while the concentrations of YFP-CPXII mutants were gradually increased. As shown in Fig. 4B, with the gradually increased concentration of YFP-CPXII or its mutants, the FRET efficiency becomes higher and higher. Moreover, the ability of CPXII 77 to bind to the SNARE complex is the same as that of CPX II with the affinity about 60 nM. However, CPXII 71 is almost unable to bind to the SNARE complex according to the FRET efficiency, which agrees well with the result by pull-down assay. Although GST-SNARE can pull down CPXII 77A, eliminating the positive charges in the fragment of 71–77 (CPXII 77A) reduces the ability of complexin II to bind to SNARE complex, with the affinity of about 150 nM. Moreover, changing the positive charges to negative charges (CPXII 77E) further inhibits the interaction between complexin and the SNARE complex. All above results suggest that the positive charges in the fragment 71–77

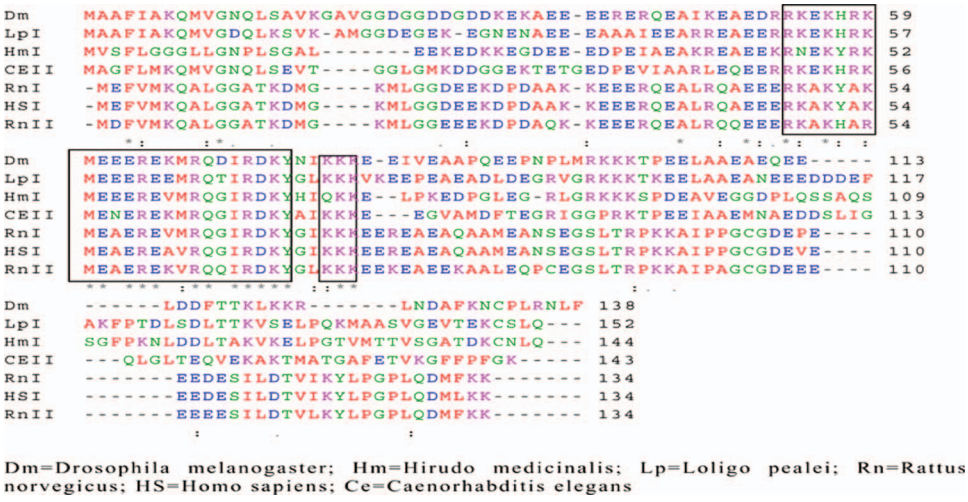

Figure 1. Sequence alignment of complexins from different organisms using Cluster W (http://www.ebi.ac.uk/clustalw). Accession numbers of sequences are as follows: Hm-CPX1 = AJ512832, Lp-CPX1 = BAB62069, Dm-CPX1 = AAF69518, Ce-CPX2 = AAG23387, Rn-CPX1 = NP_074055, Rn-CPX2 = NP_446330, Hs-CPX1 = NP_006642. The first box indicates the site that directly binds to the SNARE complex according to the crystal structure; the second box is a segment highly conserved among all complexins.

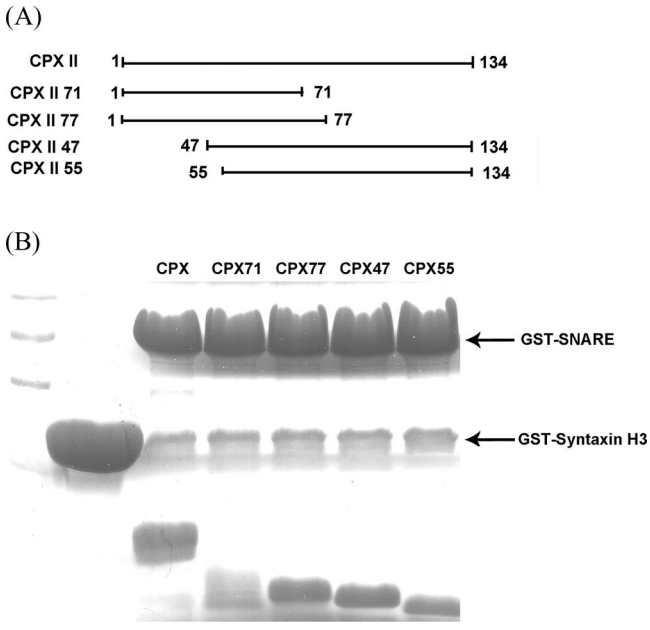

Figure 2. Segment 71–77 is indispensable for complexin's interaction with the SNARE complex. (A) Schematic diagram of truncated complexin II mutants from rat. (B) The interaction between the SNARE complex and CPXII or its mutants. Immobilized GST-SNARE complex were incubated with excessive complexin II or its mutants (CPXII 71, CPXII 77, CPXII 47 and CPXII 55) at 4°C for 1.5 h followed by washing extensively four times. The elution was subjected to SDS-PAGE and visualized by Coomassie Blue staining.

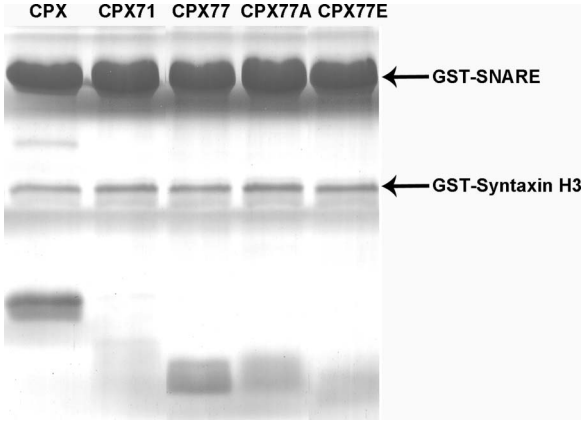

Figure 3. Eliminating the positive charges in the segment 71–77 impairs the ability of complexin to bind to the SNARE complex. Immobilized GST-SNARE complexes were incubated with excessive complexin II or its mutants (CPXII 71, CPXII 77, CPXII 77A and CPXII 77E) at 4°C for 1.5 h followed by washing extensively four times. The elution was subjected to SDS-PAGE and visualized by Coomassie Blue staining.

are involved in the interaction between complexin II and the SNARE complex.

The N-terminal Deletion of CPXII Disrupts the Stability of the Complexin/SNARE Complex

As mentioned above, both CPXII 47 and CPXII 55 can be pulled down from the GST-SNARE complex (Fig. 2, lane 5 and 6), however, we do not know whether the N-terminal deletion of CPXII would affect the ability of complexin II to bind to SNARE complex. Because previous work showed that the ability of CPXII 77 binding to SNARE complexin is almost consistent with that of the full-length CPXII (Fig. 4), thus, we compared the binding ability of CPXII 47 and CPXII 55 to SNARE complex with CPXII 77 by the FRET competition assay. In the following series of experiments, we kept the concentrations of YFP-CPXII 77 and SNARE-CFP constant at 0.4 μM, while the concentrations of CPXII 47, CPXII 55 and CPXII 77 were varied. In the absence of unlabelled complexin II mutants, YFP-CPXII 77 and SNARE-CFP bind to each other with a remarkable FRET signal. With increasing concentration of unlabelled CPXII mutants, they competed with YFP-CPXII 77 to bind to SNARE core complex, which resulted in reduced FRET signals. According to the different inhibition abilities by different complexin II mutants, we compared the ability of complexin II mutants to bind to the SNARE complex. As shown in Fig. 5, compared with CPXII 77, the inhibition ability of CPXII 47 is weakened by N-terminal deletion. However, deletion of N-terminal 55 amino acid residues almost completely abolished the ability of CPXII 55 to inhibit the interaction between YFP-CPXII 77 and SNARE-CFP. These results indicate that although CPXII 47 and CPXII 55 can interact with GST-SNARE complex by the pull-down assay, the N-terminal deletion of complexin weakens their interaction with SNARE complex and disrupts the stability of complexin/SNARE complex.

DISCUSSION

Complexin is a small hydrophilic protein family that plays an important role during the calcium-dependent fast neurotransmitter release. As a cytoplasmic protein, complexin performs its function through interaction with the SNARE complex (*1, 12, 19, 20*). In solution, complexin displays lower alpha-helix content in the N-terminal region and lacks a well defined tertiary structure (*12*). However, crystal structure of complexin/SNARE complex revealed that complexin binds to the SNARE complex in an anti-parallel alpha-helix conformation to the groove between syntaxin and VAMP through the fragment 47–70 (*17, 18*). Thus, what are the roles of the flanking regions of complexin?

One role of the N-terminal region may be to maintain the alpha-helix of complexin. NMR results had shown that residues 29–64 of complexin contains a stable alpha-helix

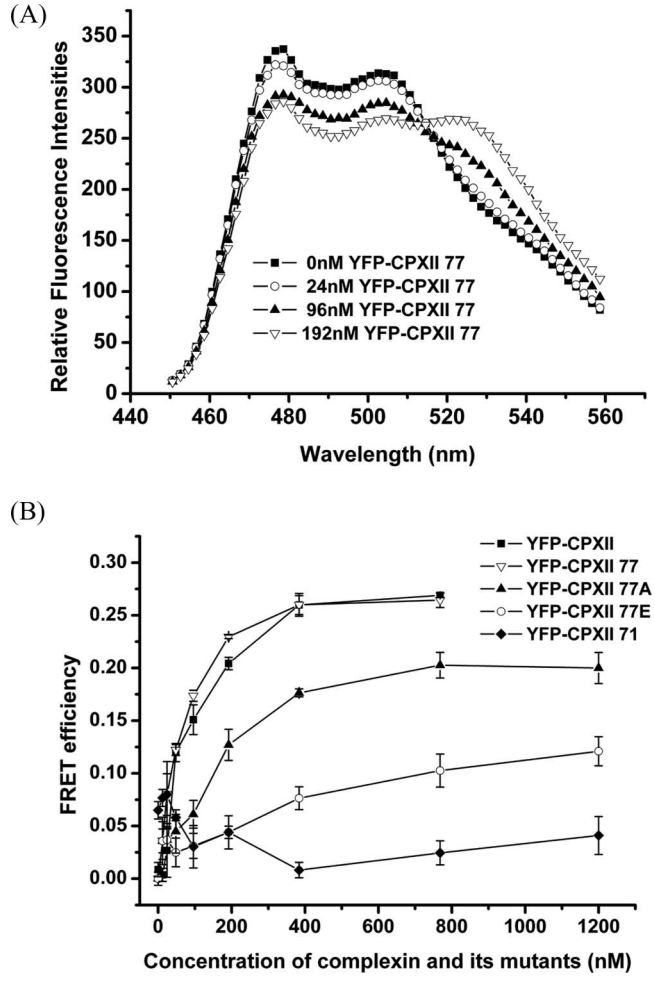

Figure 4. Comparison of the ability of complexin II mutants to bind to the SNARE complex using FRET titration assay. (A) Representative FRET spectra of the interaction between YFP-CPXII 77 and SNARE-CFP. In this assay, the concentration of SNARE-CFP was kept constant at 0.2 μM and the concentration of YFP-CPXII 77 was gradually increased. All experiments were performed at room temperature with 433 nm excitation. FRET spectra of the interactions between SNARE-CFP and YFP-complexin II, YFP-CPXII 71, YFP-CPXII 77A, YFP-CPXII 77E are not shown. (B) Comparison of the binding abilities of complexin II and its mutants to the SNARE complex. The efficiency of FRET in each case was plotted against the concentration of YFP-complexin II or its mutants. The experiments were repeated at least three times.

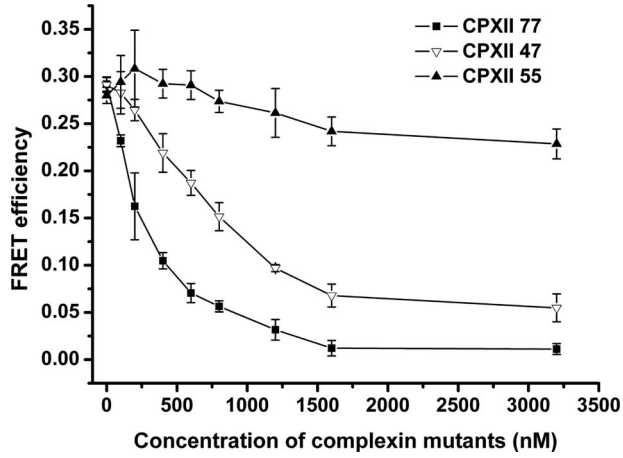

Figure 5. N-terminal deletion of complexin disrupts the stability of complexin/SNARE complex. In this experiment, the concentrations of SNARE-CFP and YFP-CPXII 77 were kept constant at 0.4 μM, and unlabelled CPXII 77, CPXII 47 or CPXII 55 was added gradually. In each case, the fluorescence spectra were recorded from 450 – 560 nm with 433 nm excitation at room temperature. The efficiency of FRET at each concentration was plotted against the concentration of unlabelled complexin II mutants. The experiments were repeated at least three times.

while a substantial but less stable alpha-helix is formed from residues 65 – 86 (*12*). The stable alpha-helix in the N-terminal may help the C-terminal alpha-helix to bind to the SNARE complex. Our results directly confirmed this: although CPXII 47 and CPXII 55 can bind to the GST-SNARE complex by the pull-down assay, N-terminal deletion of complexin (CPXII 47 and CPXII 55) weakens the binding ability of complexin to the SNARE complex and disrupts the stability of complexin/SNARE complex, which may be because deletion disrupts the integrity of alpha-helix at the N-terminal end of complexin. The integrity of alpha-helix in the C-terminal region is also important, because deletion of the last 65 residues (CPXII 71) almost abolished its binding to the SNARE complex while deletion of the last 57 residues (CPXII 77) did not. In addition, substituting the conserved positively charged residues with neutrally or negatively charged residues in the fragment 71 – 77 (CPXII 77A and CPXII 77E) remarkably inhibited their binding to the SNARE complex, which suggests that the positive charges in the fragment 71 – 77 are involved in the interaction between complexin and the SNARE complex. However, this conclusion seems to be inconsistent with the crystal structure of complexin/SNARE complex. Because, according to the crystal structure, this fragment does not directly interact with the SNARE complex. Nevertheless, crystal structure of SNARE/complexin complex also revealed that although complexin binds to the SNARE complex through fragment 47 – 70 in an anti-parallel manner, after G71, the following several residues of complexin pack back in anti-parallel conformation to its own helix (*18*). In addition, on the corresponding position of the SNARE complex, negatively charged residues are enriched, which may attract the positively charged residues in the fragment 71 – 77 of complexin and help complexin properly interact with the

SNARE complex (see supplementary figure – online version only). Thus the most possible explanation for this apparent contradiction is that this fragment indeed does not directly bind to SNARE and that the charges in this fragment could influence complexin's interaction with the SNARE complex by electrostatic interaction.

Besides the possibilities discussed above, there are potentially other functions for this fragment. For example, complexin III and IV have farnesylation at the C-terminal, which helps their targeting to the membrane (*4*). Further studies are needed to discover whether there are other roles for complexin flanking regions.

ACKNOWLEDGEMENTS

This research was supported by grants from National Natural Science Foundation of China and National Basic Research Program of China (2004CB720005).

REFERENCES

1. McMahon, H. T., Missler, M., Li, C., and Sudhof, T. C. (1995) Complexins: cytosolic proteins that regulate SNAP receptor function. *Cell* **83**, 111 – 119.
2. Takahashi, S., Yamamoto, H., Matsuda, Z., Ogawa, M., Yagyu, K., Taniguchi, T., Miyata, T., Kaba, H., Higuchi, T., Okutani, F., et al. (1995) Identification of two highly homologous presynaptic proteins distinctly localized at the dendritic and somatic synapses. *FEBS Lett*. **368**, 455 – 460.
3. Ono, S., Baux, G., Sekiguchi, M., Fossier, P., Morel, N. F., Nihonmatsu, I., Hirata, K., Awaji, T., Takahashi, S., and Takahashi, M. (1998) Regulatory roles of complexins in neurotransmitter release from mature presynaptic nerve terminals. *Eur. J. Neurosci*. **10**, 2143 – 2152.
4. Reim, K., Wegmeyer, H., Brandstatter, J. H., Xue, M., Rosenmund, C., Dresbach, T., Hofmann, K., and Brose, N. (2005) Structurally and functionally unique complexins at retinal ribbon synapses. *J. Cell Biol*. **169**, 669 – 680.
5. Takahashi, S., Ujihara, H., Huang, G. Z., Yagyu, K. I., Sanbo, M., Kaba, H., and Yagi, T. (1999) Reduced hippocampal LTP in mice lacking a presynaptic protein: complexin II. *Eur. J. Neurosci*. **11**, 2359 – 2366.
6. Reim, K., Mansour, M., Varoqueaux, F., McMahon, H. T., Sudhof, T. C., Brose, N., and Rosenmund, C. (2001) Complexins regulate a late step in Ca^{2+}-dependent neurotransmitter release. *Cell* **104**, 71 – 81.
7. Itakura, M., Misawa, H., Sekiguchi, M., Takahashi, S., and Takahashi, M. (1999) Transfection analysis of functional roles of complexin I and II in the exocytosis of two different types of secretory vesicles. *Biochem. Biophys. Res. Commun*. **265**, 691 – 696.
8. Tokumaru, H., Umayahara, K., Pellegrini, L. L., Ishizuka, T., Saisu, H., Betz, H., Augustine, G. J., and Abe, T. (2001) SNARE complex oligomerization by synaphin/complexin is essential for synaptic vesicle exocytosis. *Cell* **104**, 421 – 432.
9. Archer, D. A., Graham, M. E., and Burgoyne, R. D. (2002) Complexin regulates the closure of the fusion pore during regulated vesicle exocytosis. *J. Biol. Chem*. **277**, 18249 – 18252.
10. Schaub, J. R., Lu, X., Doneske, B., Shin, Y. K., and McNew, J. A. (2006) Hemifusion arrest by complexin is relieved by Ca(2+)-synaptotagmin I. *Nat. Struct. Mol. Biol*. **13**, 748 – 750.
11. Giraudo, C. G., Eng, W. S., Melia, T. J., and Rothman, J. E. (2006) A clamping mechanism involved in SNARE-dependent exocytosis. *Science* **313**, 676 – 680.
12. Pabst, S., Hazzard, J. W., Antonin, W., Sudhof, T. C., Jahn, R., Rizo, J., and Fasshauer, D. (2000) Selective interaction of complexin with the neuronal SNARE complex. Determination of the binding regions. *J. Biol. Chem*. **275**, 19808 – 19818.
13. Chen, Y. A., and Scheller, R. H. (2001) SNARE-mediated membrane fusion. *Nat. Rev. Mol. Cell. Biol*. **2**, 98 – 106.
14. Weber, T., Zemelman, B. V., McNew, J. A., Westermann, B., Gmachl, M., Parlati, F., Sollner, T. H., and Rothman, J. E. (1998) SNAREpins: minimal machinery for membrane fusion. *Cell* **92**, 759 – 772.
15. Sutton, R. B., Fasshauer, D., Jahn, R., and Brunger, A. T. (1998) Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 A resolution. *Nature* **395**, 347 – 353.
16. Hayashi, T., McMahon, H., Yamasaki, S., Binz, T., Hata, Y., Sudhof, T. C., and Niemann, H. (1994) Synaptic vesicle membrane fusion complex: action of clostridial neurotoxins on assembly. *Embo J*. **13**, 5051 – 5061.
17. Chen, X., Tomchick, D. R., Kovrigin, E., Arac, D., Machius, M., Sudhof, T. C., and Rizo, J. (2002) Three-dimensional structure of the complexin/SNARE complex. *Neuron* **33**, 397 – 409.
18. Bracher, A., Kadlec, J., Betz, H., and Weissenhorn, W. (2002) X-ray structure of a neuronal complexin-SNARE complex from squid. *J. Biol. Chem*. **277**, 26517 – 26523.
19. Pabst, S., Margittai, M., Vainius, D., Langen, R., Jahn, R., and Fasshauer, D. (2002) Rapid and selective binding to the synaptic SNARE complex suggests a modulatory role of complexins in neuroexocytosis. *J. Biol. Chem*. **277**, 7838 – 7848.
20. Hu, K., Carroll, J., Rickman, C., and Davletov, B. (2002) Action of complexin on SNARE complex. *J. Biol. Chem*. **277**, 41652 – 41656.
21. Liu, J., Guo, T., Wei, Y., Liu, M., and Sui, S. F. (2006) Complexin is able to bind to SNARE core complexes in different assembled states with distinct affinity. *Biochem. Biophys. Res. Commun*. **347**, 413 – 719.