MOLECULE PAGE

Complexin 2

Michelle A. Falkowski and Guy E. Groblewski

Department of Nutritional Sciences, University of Wisconsin, Madison, WI 53706

e-mail: mawest@wisc.edu


Official Symbol: Cplx2 and Name: complexin 2
Other Designations: CPX II; complexin II; complexin-2; synaphin-1
Chromosome: 17; Location: 17p14
Annotation: Chromosome 17, NC_005116.2 (16314592..16322938, complement)

1. General function

Complexins are a small family of proteins comprised of 4 members – complexin 1-4. Complexin 1, originally identified as synaphin, is a small 134 amino acid cytosolic protein that is expressed exclusively in neurons where it associates with soluble \( N \)-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes necessary for exocytosis (16,25). Complexin 2 is ubiquitously expressed and thought to function in an analogous manner in other secretory cells. Complexin 1 and 2 are 86% identical at the amino acid level (2). Moreover, despite considerable differences in their mRNA sequences, mouse, rat and human complexin 2 proteins are 100% identical (20). Complexin 3 and 4 were recently identified and evidence of their specific function is still unclear. Complexin 3 is expressed strongly in retina and certain regions of the brain including the hippocampus and thalamus, whereas complexin 4 is only expressed in retina (24).

Complexin 1 was originally proposed to clamp ternary SNARE complexes composed of vesicle associated membrane protein (VAMP) 2 on synaptic vesicles and syntaxin 1 and SNAP 25 on the plasma membrane at a pre-fusion step in the exocytic process thereby inhibiting neurotransmitter secretion (8,31). It was proposed that upon elevation of intracellular \( \text{Ca}^{2+} \), complexin 1 is displaced from the SNARE complex by the synaptic vesicle protein synaptotagmin 1, in turn, allowing the final stages of neurotransmitter release to commence (8). More recent studies have shown that in addition to this clamping activity, complexin 1 also directly modulates the final stages of SNARE complex formation to facilitate exocytosis (27,29).

Complexins are comprised of an N-terminal region (aa 1-29), accessory alpha-helix (aa 30-48), central alpha-helix (aa 49-70) and C-terminal region (aa 71-134) (Fig 1). In vitro vesicle fusion assays and mutational analysis in neurons indicate the N-terminus has a positive regulatory effect on vesicle fusion and activates synchronous \( \text{Ca}^{2+} \)-induced exocytosis (19,30). Conversely, the accessory alpha helix has a negative regulatory effect on vesicle fusion and restricts spontaneous exocytosis (19,30). The central alpha helix is essential for SNARE binding, whereas the
function of the C-terminus is unclear (30).

Figure 1. Schematic of complexin 1 and 2 functional domains. See text for details.

Complexin 1 binds with high affinity to the ternary SNARE complex and with lower affinity to the t-SNARE complex containing SNAP 25 and syntaxin 1 yet is unable to bind monomeric SNARE proteins (13,17,20,21,29). Complexin 1 binds the ternary SNARE complex as an antiparallel helix in the groove between the VAMP 2 and syntaxin helices (4,15,19,20,22,30). Complexin 1 has also been shown to function in neurons by simultaneously suppressing spontaneous vesicle fusion and activating fast Ca\(^{2+}\)-stimulated fusion (19). Additional evidence demonstrates that complexin 1 binding controls the force that trans-SNARE complexes apply onto opposing membranes to facilitate their fusion (19).

Complexin 2 has been studied in neural, chromaffin, mast and pancreatic acinar cells all of which support a functional role in Ca\(^{2+}\)-triggered exocytosis (3,6,18,26,27). Knockdown of complexin 2 expression in RBL-2H3 mast cells attenuated Ca\(^{2+}\)-dependent degranulation (27).

2. Specific function in exocrine pancreas

Expression of complexin 2 in rat pancreatic acinar cells was demonstrated by reverse transcription PCR, immunoblotting and immunofluorescence microscopy (6). Complexin 2 is a cytosolic protein that is not found on zymogen granule membranes but does accumulate along the apical membrane. GST-pulldown assays in acinar lysates demonstrate that complexin 2 interacts with VAMP 2, syntaxin 3 and syntaxin 4 but not VAMP8. Furthermore, complexin 2 colocalizes with VAMP 2 along the apical membrane and this association is significantly enhanced following CCK-8 stimulation (Fig 2).

Figure 2. Colocalization of complexin 2 and VAMP2 in apical membrane regions of pancreatic acini. Left and middle panels demonstrate the enhanced accumulation of complexin 2 at the apical plasma membrane following CCK-8 (100 pM) stimulation. Right panel shows quantification of multiple reconstructed z-series images from 3 separate tissue preparations of the % of total complexin 2 colocalized with VAMP2 at the plasma membrane. This data is reproduced from ref 6.

A functional role for complexin 2 in exocrine secretion was demonstrated by introducing recombinant wild-type and mutated forms of the protein into perfringolysin-O permeabilized acinar cells. High concentrations of recombinant complexin 2 inhibited Ca\(^{2+}\)-dependent amylase release and these effects were abolished by mutations that inhibited complexin 2 interactions...
with VAMP 2 (6). These results are consistent with studies examining complexin 1 exocytic function in neurons and suggest that high levels of complexin 2 stabilize trans-SNARE complex formation at a prefusion state, thereby inhibiting Ca\(^{2+}\)-stimulated secretion.

3. Tools to study complexin

A. Antibodies

Purified rabbit anti-complexin 1,2 antibodies (Synaptic Systems cat # 122 002 and 122 102) were successfully used in rat pancreas for immunoblotting and immunofluorescence (1,5,6).

B. cDNA clones

In 1995, McMahon and colleagues cloned in rat brain, complexin 1 and 2 and inserted full length complexin 1 or complexin 2 into pGEX-KG (20). Since then several mutations and truncations of complexin 1 have been produced and studied in brain (9,18,19,30).

C. Knockout mice

Complexin 1/2 double knockout mice die shortly after birth (23). However, single knockout mice however are viable.

Complexin 1 knockout mice are infertile (32). They are also characterized by abnormal early motor development, reduced exploratory behavior, profound ataxia, deficits in social behavior and reduced habitation yet have normal cognitive function (10,11,12,23).

Complexin 2 knockout mice show no overt phenotypic changes (23), yet they have cognitive and motor deficits (10). In the CA1 and CA3 regions of the hippocampus, complexin 2 knockout mice show altered long-term synaptic potentiation (7,14,28). However, synaptic transmission is not altered in the hippocampus of these mice (14).

D. siRNA

Complexin 2-specific siRNA are available from Santa Cruz (sc-41925, sc-41926).

4. References


