

Mini-Review

VILIP-1

A Novel Regulator of the Guanylate Cyclase Transduction System in Neurons

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ABSTRACT

VILIP-1 belongs to the subfamily of Visinin-like proteins with its members VILIP-1, -2, -3, hippocalcin and neurocalcin δ which have been implicated in the modulation of a variety of intracellular signaling cascades. VILIP-1 has recently been characterized in more detail and an involvement in cyclic nucleotide signaling has been demonstrated. Thus, similar to other NCS proteins such as GCAPs and neurocalcin δ , VILIP-1 can influence the activity of guanylyl cyclase enzymes and the cGMP-signaling system of neurons in the nervous system.

INTRODUCTION

A variety of calcium-sensing proteins have been identified in the nervous system in addition to the ubiquitous calcium sensor protein calmodulin, reflecting the importance of the regulative function of calcium in nerve cells. These proteins have been termed neuronal calcium sensors (NCS).¹ Fourteen NCS protein family members constitute five major subfamilies including recoverins and GCAPs expressed in retinal neurons, and KChIPs, NCS-1/frequenins and VILIPs expressed in the CNS of various species.

The Visinin-like proteins (VILIPs), a Subfamily of Neuronal Calcium Sensor (NCS) Proteins. The visinin-like proteins VILIP-1, VILIP-2, VILIP-3, hippocalcin and neurocalcin δ show a high degree of amino acid identity between 67 and 94%.^{1,2} VILIPs are typically 191–193 amino acid residues long and have a M-G-X₃-S consensus sequence for N-terminal myristoylation. An EF-hand calcium-binding motif identifies them as members of the EF-hand superfamily of calcium-binding proteins. This review will focus on VILIP-1 which has been most extensively characterized so far.

STRUCTURE OF VILIP-1

VILIP-1 shares the structural topology of four EF-hand motifs (EF1-EF4) with all members of the NCS protein family. The proteins form a highly compact structure with a protein fold constituted by two pairs of EF-hand motifs. Upon binding of calcium, the proteins undergo a conformational change where EF1 and EF2 are relocated and the protein adopts a more extended conformation. The canonical calcium binding in EF-hands occurs by seven oxygen ligands in a pentagonal bipyramidal geometry³ and is constituted by the amino acid sequence motif D-X-D/N-X-D/N-X-Y-X₄-E (X is any amino acid). While Asp, Asn and Glu residues coordinate the calcium ion via their side chains, residue Y participates with its backbone carbonyl oxygen. The apical coordination position of the bipyramid is occupied by a water molecule. In most NCS proteins, due to deviations in the first repeat of the amino acid sequence required for canonical calcium binding, EF1 is rendered dysfunctional. Intriguingly, EF1 is the most variable part in the amino acid sequence of all members of the NCS protein family and might thus be a possible binding site for target proteins. While there is experimental evidence for an interaction of parts of EF1 and EF2 of GCAP1 with retinal guanylyl cyclase (GC),⁴ small stretches of hydrophobic amino acids following EF3 and EF4 might also participate in protein-protein interactions.

Structures of NCS proteins, including recoverin⁵⁻⁹ and neurocalcin δ ,¹⁰ have been solved by X-ray crystallography and NMR in the presence and absence of calcium. At the amino acid sequence level, VILIP-1 shares a high degree of identity with recoverin and neurocalcin δ which is the basis for prediction of the structure of VILIP-1 by a homology modeling approach. While recoverin has a dysfunctional calcium-binding site in EF4, VILIP-1 and neurocalcin δ both possess an intact calcium binding sequence motif in the fourth EF-hand. Thus, neurocalcin δ was our preferred template for homology modeling, but in the absence of a structure of apo-neurocalcin δ , we used the three-dimensional

structure of apo-recoverin (PDB entry 1IKU),⁶ in addition to the calcium-bound neurocalcin δ (PDB entry 1BJF),¹⁰ as template. In the absence of calcium, the different NCS proteins adopt highly similar conformations. However, in their calcium-bound forms, recoverin and neurocalcin δ differ significantly in their conformations, as evident from a root-mean-square deviation of 8.9 Å, as well as the presence of a calcium binding site in EF4. The homology model for VILIP-1 in the apo- and calcium-bound state is shown in Figure 1.

Based on the experimental data on interaction between GCAP1 and retinal GC, residues putatively important for NCS protein:cyclase interactions can be mapped onto the VILIP-1 homology model. The identified surface-exposed residues line a groove on the “base” of VILIP-1 formed by EF3 and EF4. In the apo-form, this groove is capped by three basic amino acids (K28, K32, R83) located in EF1 and EF2. During conversion to the extended, calcium-bound form, these three residues are relocated to remote positions and the groove seems to be slightly compressed. Hypothetically, the VILIP-1 apo-form might bind a target peptide in this groove, and, upon addition of calcium and simultaneous conversion into the extended form, the subtle changes in the binding groove confer a conformational change onto the target protein without disassembly of the complex.

THE CALCIUM-MYRISTOYL SWITCH OF VILIP-1

Apart from KChIP2-4, which are not myristoylated at their N-termini, most NCS proteins can translocate to subcellular membrane compartments by a molecular mechanism termed calcium-myristoyl switch, which is dependent on calcium-binding and N-terminal myristoylation of the proteins.¹¹ The molecular mechanism of the switch has been analyzed in detail from tertiary structure data of recoverin which was analyzed in its calcium-bound and calcium-free myristoylated forms.^{5,12} The four EF-hand motifs in recoverin are arranged in a compact tandem array, with the myristoyl side chain buried in a hydrophobic pocket constituted by residues Ala9, Met13, Lys27, Trp30, Tyr31, Phe55, Phe56 from EF1, Phe82, Phe85, Ile86, Leu89 from EF2, and Trp103, Met107 from EF3. These residues are highly conserved in the NCS family of proteins and the structure of the hydrophobic cleft is similar in all family members. Binding of calcium in the motifs EF2 and EF3 induces a conformational change leading to the surface exposure of hydrophobic parts of the polypeptide and of the myristoyl side chain, thereby making these structures available for interaction with cellular membranes and/or target proteins. Biochemically, the existence of the calcium-myristoyl switch mechanism has been shown for all members of the VILIP-subfamily.¹³⁻¹⁶

Furthermore the switch has also been observed in living cells after increasing intracellular calcium concentration.¹⁷⁻²⁰ The reversible localization of signaling proteins and calcium sensors to distinct membrane compartments via the molecular mechanism of the calcium-myristoyl switch, has been postulated to be a signal transduction

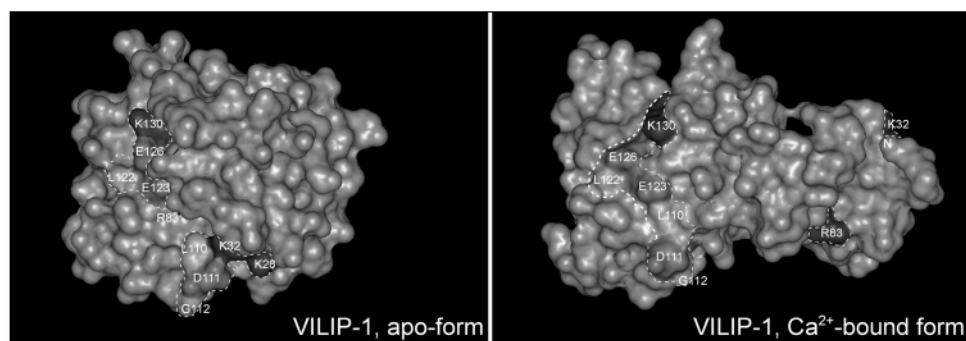


Figure 1. Potential target interaction sites on VILIP-1. Potentially interacting residues were mapped onto the VILIP-1 models of the apo- and the calcium-bound form using the available experimental data from GCAP1: retinal GC interactions.⁴ For the figure, both structures were aligned on EF-hand motifs EF3 and EF4. The identified surface-exposed residues implicated in GCAP1: retinal GC interactions are highlighted on the Connolly surface. The position of the N-terminus is marked with “N”. The figure was prepared with InsightII. Computation of the VILIP-1 homology models involved amino acid sequence alignments with recoverin (1IKU) and neurocalcin δ (1BJF) with the program MUSCLE.⁴⁹ Twenty independent homology models for the apo- as well as the calcium-bound form were calculated with the programme Modeller^{45,50} and the ones with the lowest energy were selected. The selected models were visually inspected with O⁵¹ and their overall geometry were scrutinized using PROCHECK.⁵²

mechanism for the selective activation of downstream signaling cascades, such as receptor signaling complexes and signal effector molecules.¹⁷⁻²¹ Following calcium stimulation VILIP-1 associates with the surface membrane and with trans-Golgi membranes in cell lines and in hippocampal neurons.¹⁷ In addition, endogenous VILIP-1 shows cell surface membrane association including membranes of axons and dendrites, which is in line with the proposed function of VILIP-1 as modulator of cell surface associated proteins.²²⁻³⁰

VILIP-1 EFFECT ON VARIOUS NEURONAL SIGNALING CASCADES

Previous results suggested VILIP-1 as a modulator of multiple target proteins leading to a ‘pleiotropy’ of physiological action. VILIP-1 has been shown to modulate nicotinic acetylcholine receptors²⁵ and integrin receptors.²⁷ It has also been implicated in regulation of adenylyl cyclase activity^{22,23,26-28} and activity of the related soluble and the membrane-localized guanylyl cyclases.^{24,29,30}

VILIP-1 influences guanylyl cyclase B signaling in neurons. An influence of VILIP-1 on nonretinal guanylyl cyclases, namely the receptor GC-A and -B and soluble cyclases, has been observed in vitro.²⁴ VILIP-1 affects cGMP-levels in several transfected neural cell lines, including C6, NG-108, PC12 and Neuro2A cells. Following stimulation of receptor GCs with natriuretic peptides or the soluble GC with nitric oxide an enhanced cGMP-accumulation was observed in all stably VILIP-1-transfected cell lines. Interestingly, the effect on soluble and particulate GCs depends on the subcellular localization of VILIP-1. The membrane-localized wild type VILIP-1 mainly affects the particulate receptor cyclases GC-A and GC-B, whereas the myristoylation mutant of VILIP-1, localized in the cytosol, influences the soluble cyclase enzyme. Thus, membrane-localized VILIP-1 was shown to modulate the membrane-localized natriuretic peptide receptor guanylyl cyclase GC-A and -B.^{24,29,30} The effect of VILIP-1 on cGMP-signaling was also found in primary cerebellar cultures, where VILIP-1 specifically affected neuronal GC-B but not glial GC-A.²⁴ Furthermore, in primary hippocampal neurons VILIP-1 colocalized with GC-B in many but not all cultured neurons, and also significantly enhanced CNP-stimulated

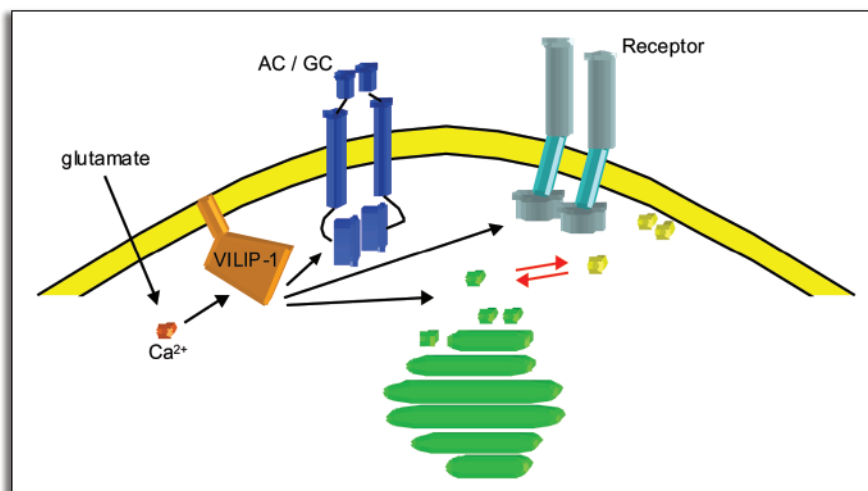


Figure 2. VILIP-1 is a calcium-dependent modulator of intracellular signaling cascades. VILIP-1 has been identified as a modulator of nicotinic acetylcholine receptor (nAChR), adenylyl cyclase (AC) and guanylyl cyclase (GC) signaling. VILIP-1-expression increases cGMP-accumulation in transfected cell lines and acts as modulator of receptor guanylyl cyclase B in primary neurons. Expression of membrane localized VILIP-1 increases the surface expression of the receptor GC-B in primary neurons by influencing clathrin-dependent receptor recycling. Similar to VILIP-1, neurocalcin δ and hippocalcin have been identified as modulators of adenylyl (olfactory adenylyl cyclase) and guanylyl cyclase (olfactory and retinal guanylyl cyclase) enzymes.

cGMP accumulation following transient transfection with the VILIP-1 cDNA. Heterologous expression of VILIP-1 led to a clathrin-dependent increased surface expression and/or attenuation of GC-B internalization in hippocampal neurons. The increased expression of VILIP-1 thereby enhances basal as well as CNP-stimulated cGMP-accumulation. Interestingly, VILIP-1 also affects clathrin-dependent recycling of the transferrin receptor, indicating that VILIP-1 is a general modulator of membrane trafficking.³⁶

A hypothesis on the mechanism of the effect of VILIP-1 on guanylyl cyclase B signaling in neurons. Other NCS proteins like the KChIPs and NCS-1 influence surface expression of Ca^{2+} and potassium channels.^{31,32} KChIP1 influences post ER trafficking of Kv4 potassium channels.^{19,31} NCS-1 interacts with ARF1 to control trans-Golgi network-plasma membrane trafficking of the Kv4 potassium channel.³³ Various other NCS proteins can interact with molecules connected to membrane trafficking, including neurocalcin which interacts with α - and β -clathrin and β -2-adaptin.^{34,35} Furthermore, in an approach to identify proteins linked to clathrin-mediated trafficking, clathrin-coated vesicles from rat brain were isolated and associated proteins were analyzed. VILIP-1 was found to be associated with vesicles that were also enriched with clathrin heavy and light chain. Other vesicle-associated proteins include the α , β , γ , μ -adaplin subunits of the AP-2 complex which functions at the plasma membrane, and the AP-1 complex which functions at the trans-Golgi network.³⁶ These data implicate VILIP-1 to be involved in clathrin-dependent membrane trafficking processes such as endo- and/or exocytosis. A role of VILIP-1 in membrane transport and receptor trafficking has been suggested earlier by its localization to raft membranes³⁷ and the Golgi,¹⁷ membrane compartments known to be involved in endo-/exocytosis.³⁸ VILIP-1 influences rhoA activity, a modulator of the cortical cytoskeleton,²² and it was shown that it can interact with the cortical actin cytoskeleton itself,^{15,17} which is also involved in the endo-/exocytosis process. These results are in line with a proposed role of VILIP-1 as regulator

of post-Golgi membrane trafficking.³⁰ VILIP-1 is not only able to increase cell surface expression of the GC-B receptor,³⁰ but also influences receptor recycling of transferrin³⁰ and the $\alpha 4\beta 2$ nicotinic acetylcholine receptor.²⁵ As shown in detail for the example of GC-B, VILIP-1 appears as a general regulator of clathrin mediated events throughout the cell.

SUMMARY

Recent data implicate VILIP-1 as a physiological modulator of the neuronal CNP signaling pathway, the receptor GC-B and the second messenger cGMP. The colocalization of VILIP-1 with GC-B in the soma and dendritic membranes of hippocampal neurons suggests that VILIP-1 might influence GC activity under physiological conditions in the hippocampus in vivo. In the context with the Ca^{2+} -dependent localization of VILIP-1 at the trans-Golgi network and dendritic membrane specializations in hippocampal neurons¹⁷ it is conceivable that GC-B and VILIP-1 may influence neuronal signaling in dendrites of hippocampal neurons in a Ca^{2+} - and cGMP-dependent manner.³⁰ Thus VILIP-1 might influence cGMP-

dependent neuronal processes, including different forms of synaptic plasticity,^{39,40} and learning and memory.⁴¹ Interestingly, it was shown that other proteins of the VILIP subfamily influence cyclases. Neurocalcin δ was found to interact with and directly influence related guanylyl cyclases, namely the retinal and the olfactory GCs.⁴²⁻⁴⁵ Moreover, hippocalcin was also found to inhibit olfactory adenylyl cyclase but activate olfactory guanylyl cyclase.⁴⁶ Thus, besides the GCAPs, additional NCS proteins including VILIP-1, hippocalcin and neurocalcin δ can influence guanylyl cyclase family members. A major function of members of the VILIP-subfamily of NCS proteins might therefore be the modulation of cyclase enzymes in neurons, similar to the related GCAP family regulating guanylyl cyclases of photoreceptor cells.^{47,48}

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