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Membrane binding of Neuronal Calcium Sensor-1 (NCS1)

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ABSTRACT

Neuronal Calcium Sensor-1 (NCS1) belongs to the family of Neuronal Calcium Sensor (NCS) proteins. NCS1 is composed of four EF-hand motifs and an N-terminal myristoylation. However, the presence of a calcium–myristoyl switch in NCS1 and its role in the membrane binding are controversial. The model of Langmuir lipid monolayers is thus used to mimic the cell membrane in order to characterize the membrane interactions of NCS1. Two binding parameters are calculated from monolayer measurements: the maximum insertion pressure, up to which protein binding is energetically favorable, and the synergy, reporting attractive or repulsive interactions with the lipid monolayers. Binding membrane measurements performed in the presence of myristoylated NCS1 reveal better binding interactions for phospholipids composed of phosphoethanolamine polar head groups and unsaturated fatty acyl chains. In the absence of calcium, the membrane binding measurements are drastically modified and suggest that the protein is more strongly bound to the membrane. Indeed, the binding of calcium by three EF-hand motifs of NCS1 leads to a conformation change. NCS1 arrangement at the membrane could thus be reshuffled for better interactions with its substrates. The N-terminal peptide of NCS1 is composed of two amphiphilic helices involved in the membrane interactions of NCS1. Moreover, the presence of the myristoyl group has a weak influence on the membrane binding of NCS1 suggesting the absence of a calcium–myristoyl switch mechanism in this protein. The myristoylation could thus have a structural role required in the folding/unfolding of NCS1 which is essential to its multiple biological functions.

1. Introduction

Calcium signaling plays a crucial role in cell functions [1–3]. Indeed, calcium is an intracellular messenger which is involved in multiple biological processes such as neuronal plasticity and neurotransmission [4–7]. The diversity of cell responses to calcium is provided by a family of EF-hand calcium-binding proteins, including the subfamily of Neuronal Calcium Sensor (NCS) proteins [8–10]. The NCS family has 14 members composed of four EF-hand motifs (two α helices connected by a short loop region) and an N-terminal myristoylation consensus sequence.

Among the proteins of the NCS family, Neuronal Calcium Sensor-1 (NCS1) is expressed in most neuron types, as well as in developing heart muscle cells and mast cells [11–13]. NCS1 is mostly localized at the membrane and is involved in several functions such as the facilitation of neurotransmitter release [14–17], the regulation of the lipid kinase activity of phosphatidylinositol 4-kinase β [18–21] and the modulation of potassium channels [22]. NCS1 is a 22 kDa myristoylated protein and has four EF-hand motifs, among which EF1 is cryptic and hence does not bind calcium. The three-dimensional structure of non-myristoylated NCS1 in the presence of calcium has previously been determined (Fig. S1) [23]. The binding of calcium by EF-hands 1–3 induces a conformational change of the protein leading to the extrusion of the C-terminal segment and the exposure of a large hydrophobic crevasse [23,24]. This conformational change allows the protein to bind several substrates via its hydrophobic crevasse [25–29].

The calcium–myristoyl switch is a molecular regulation mechanism leading to the extrusion of the myristoyl group out of the hydrophobic crevasse in the presence of calcium [30–35]. The presence of a calcium–myristoyl switch was proven in several NCS proteins such as recoverin, VILIP-1, VILIP-3 and hippocalcin. However, the three-dimensional structure of non-myristoylated NCS1 in the presence of calcium does not allow to confirm the presence of this mechanism [23]. Furthermore, the presence of a calcium–myristoyl switch in NCS1 is controversial.

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Fig. 1. Sequences alignment of human NCS1 (orange), fission yeast Ncs1 (pink) and budding yeast Frq1 (blue). The motif responsible of the extrusion of the myristoyl group in the presence and absence of calcium is represented in red [29] (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Nuclear Magnetic Resonance (NMR), fluorescence, and equilibrium calcium-binding measurements suggest that the NCS1 homolog from budding yeast (Frq1) may possess a calcium–myristoyl switch [36]. This suggestion is supported by the determination of the NMR structure of the NCS1 homolog from fission yeast (Ncs1) [28]. Indeed, the Ncs1 myristoyl group is buried into a cavity in the absence of calcium and extruded in the presence of calcium. However, the sequences of NCS1, Ncs1 and Frq1 are not similar and could lead to structural differences (Fig. 1). Indeed, fission yeast Ncs1 and budding yeast Frq1 are respectively 69.5% and 60.0% identical to mammalian NCS1. Moreover, a structural analysis allowed to identify a motif of 6 residues within the N-terminal segment of NCS1 which lock the myristoyl group in an exposed conformation, even in absence of calcium [29]. This 6 residues motif (highlighted in red in Fig. 1) is very different for each species. This difference could explain the presence of a calcium–myristoyl switch for Frq1 and Ncs1, differently from their human homolog NCS1. A model for NCS1 function has highlighted a constitutive membrane association independent of the presence of calcium [37]. Moreover, calcium is not required for its membrane association *in vitro* and in neural cell lines, suggesting the absence of a calcium–myristoyl switch [37,38]. Other publications use these different results to confirm or deny the presence of a calcium–myristoyl switch in NCS1 [10,27,39–41]. Despite the numerous studies of NCS1, the presence of a calcium–myristoyl switch remains questionable and its exact mechanism is yet to be determined.

This publication aims to study the membrane binding of NCS1 in the presence and absence of calcium and its myristoyl group in order to solve the current controversy. Furthermore, little information on the NCS1 membrane binding is available. NMR assignments revealed that structural changes occur for NCS1 in the presence and absence of the membrane [42]. Moreover, fluorescence measurements have been performed with two different lipid vesicles (palmitoyl-oleoyl phosphoserine POPS and palmitoyl-oleoyl phosphocholine POPC) and suggest that NCS1 binds POPS vesicles in a calcium dependent way [24]. Finally, immunocytochemical data show that a small quantity of NCS1 is found in detergent-resistant membrane (DRM) obtained after Triton X-100 extraction [43]. However, the influence of the composition of phospholipids on the membrane binding of NCS1 is not yet described. In this publication, Langmuir lipid monolayers model is used to mimic the cell membrane and to determine the membrane binding parameters of NCS1 in several conditions.

First, the membrane binding behavior of myristoylated NCS1 (mNCS1) has been studied in the presence of calcium with different phospholipid monolayers. These data allowed to characterize the influence of the composition of phospholipids on the protein binding. The same experiments were then performed in the absence of calcium in order to understand the influence of the conformational change on its binding. The behavior of the non-myristoylated NCS1 (nmNCS1) was then studied in order to highlight the effect of the myristoyl group in the presence or absence of calcium. Finally, a peptide similar to the NCS1 N-terminal segment was used to better understand its role in the protein membrane binding.

2. Material and methods

2.1. Material

The deionized water used for the preparation of buffer solutions was from a Barnstead Nanopure system (Barnstead, Dubuque, IA). Its resistivity and surface tension at 20 °C were respectively 18.2 MΩ × cm and 72 mN/m. Phospholipids were from Avanti Polar Lipids (Alabaster, Al): dipalmitoyl phosphocholine (DPPC), distearoyl phosphocholine (DSPC), dioleoyl phosphocholine (DOPC), didocosahexaenoyl phosphocholine (DDPC), dipalmitoyl phosphoethanolamine (DPPE), distearoyl phosphoethanolamine (DSPE), dioleoyl phosphoethanolamine (DOPE), didocosahexaenoyl phosphoethanolamine (DDPE), dipalmitoyl phosphoserine (DPPS), distearoyl phosphoserine (DSPS), dioleoyl phosphoserine (DOPS) and didocosahexaenoyl phosphoserine (DDPS). $CaCl_2$, Hepes and mercaptoethanol were from Sigma (St. Louis, MO). KCl and EGTA were from the Laboratoire Mat (Québec, QC). The solutions composed of DO and DD acyl chains were prepared in chloroform at a concentration of 0.1 mg/mL and stored under argon in the presence of the anti-oxidant BHT (5 μg/mL) at −20 °C. All the other chemicals were used as received.

2.2. Expression and purification of NCS1

mNCS1 and nmNCS1 were overexpressed and purified as previously described [44,45]. The N-terminal peptide was synthesized by solid-phase procedures using standard 9-fluorenyl methoxy carbonyl/disopropyl carbodiimide (Fmoc/DIC) chemistry [46]. The N-terminal glycine was myristoylated at the final step of the synthesis using myristic anhydride and standard coupling procedures [46].

2.3. Measurement of the binding parameters

Surface pressure (Π) was measured by the Wilhelmy method using the DeltaPi4 microtensiometer and a 500 μL glass trough (2 cm^2) from Kibron Inc. (Helsinki, Finland). Before each experiment, the glass troughs were rinsed with tap water and then brushed with a Q-Tip in the presence of ethanol. These washings were repeated 3 times before a large rinsing with deionized