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Dynamics of saxitoxin binding to saxiphilin c-lobe reveals conformational change ☆

Penny Lewis[a], Ingrid Fritsch[a], Robert E. Gawley[a,*], Ralph Henry[b], Alicia Kight[b], Jackson O. Lay Jr., Rohana Liyanage[c], Jeanne McLachlin[b]

[a]*Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR 72701, USA*
[b]*Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701, USA*
[c]*Statewide Mass Spectrometry Facility, University of Arkansas, Fayetteville, AR 72701, USA*

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Abstract

Thermodynamic parameters (ΔG, ΔH, ΔS, ΔC_p) have been determined to evaluate the dynamics of binding of saxitoxin to the c-lobe of saxiphilin. We have developed an improved method to rapidly express and purify recombinant saxiphilin c-lobe, and fully characterized it by mass spectrometry for the first time. Surface plasmon resonance (SPR) was used to characterize the interaction between saxitoxin and immobilized c-lobe. At 298 K, c-lobe binds saxitoxin with $K_D = 1.2$ nM, $\Delta H^\circ = -11.7 \pm 0.8$ kcal/mol, and $\Delta S^\circ = 1.17 \pm 0.07$ cal/mol K. Analysis of ΔC_p of toxin association at several temperatures suggests that hydrophobic forces contribute to the binding event. Additionally, changes in 8-anilino-1-naphthalene sulfonic acid (ANS) fluorescence upon binding to c-lobe in the presence and absence of saxitoxin support a conformational change in c-lobe upon saxitoxin binding.

Keywords: Saxitoxin; Saxiphilin; PST; Paralytic shellfish toxins; Ligand binding; ΔC_p, ANS

1. Introduction

Saxitoxin is a bisguanidinium alkaloid that is the parent structure of the paralytic shellfish toxins, or PSTs, and is the only marine natural product that has been declared a chemical weapon (Hall et al., 1990; Llewellyn, 2006). PSTs are biosynthesized by several species of dinoflagellates in marine waters and by five genera of freshwater cyanobacteria, making them ubiquitous in a variety of environments (Llewellyn, 2006; Oshima et al., 1990). These poisons are neurotoxins that act by blocking ion currents in most, but not all, isoforms of voltage-gated sodium channels (Bricelj et al., 2005; Kao, 1981). Extreme cases of paralytic shellfish poisoning result in respiratory paralysis (Acres and Gray, 1978). Saxitoxin is the most toxic of the PSTs, and, as such, has been the most studied.

Saxiphilin is a serum protein that was originally isolated from bullfrogs, but has also been found in other organisms such as amphibians, fish, arthropods, and reptiles (Llewellyn et al., 1997). Saxiphilin

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*Corresponding author. Tel.: +1 479 575 6933; fax: +1 479 575 5178.
E-mail address: bgawley@uark.edu (R.E. Gawley).

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is related to the transferrin class of proteins; although it does not bind iron, it binds saxitoxin very strongly. Bullfrog saxiphilin binds saxitoxin from pH 5.5 to 9, with K_D~0.2 nM at 0 °C and neutral pH (Llewellyn et al., 1997; Llewellyn and Moczydlowski, 1994; Mahar et al., 1991). Saxiphilin sequesters the toxin in the blood stream, precluding its binding to sodium channels. Perhaps, saxiphilin evolved in these species as a survival mechanism in environments where saxitoxin and other PSTs are present, although it may have another, unknown, role in ectothermic animals in which it is found (Llewellyn, 2006). Saxiphilin binds other PSTs with varying affinity, but the affinity does not appear to correlate strongly with toxicity relative to saxitoxin (Llewellyn, 2006).

Morabito et al. (1995) found that the saxitoxin-binding site is located in the saxiphilin carboxy-terminal domain (hereinafter referred to as "c-lobe"). Successful expression of saxiphilin has been accomplished by Morabito et al. in insect cells transfected with a baculovirus expression vector containing the saxiphilin-coding sequence. Krishnan et al. (2001) expressed the 361 amino acid c-lobe using the same approach and showed that carboxy-terminal Flag and hexahistidine (His_6) tags, which aided in immunodetection and affinity purification, do not hinder c-lobe binding to saxitoxin in solution.

As part of a program to develop a device for the detection of paralytic shellfish toxins using a displacement assay, we required quantities of saxiphilin c-lobe. We therefore developed an improved method for the production of recombinant c-lobe using lipid-mediated transfection. To characterize the protein, we employed unequivocal mass spectral characterization of the entire sequence of the recombinant c-lobe. We then used surface plasmon resonance (SPR) spectroscopy to study the dynamics of binding of saxitoxin to c-lobe that had been immobilized on a surface. We find that c-lobe that has been immobilized through an exposed lysine, via a biotin–streptavidin construct, maintains high affinity for saxitoxin and can be used to study the dynamics of toxin binding at several temperatures. We note that the free energy of binding of immobilized c-lobe is similar to that found with native saxiphilin in free solution. More importantly, changes in heat capacity (ΔC_p), fluorescence enhancements, and wavelength shifts of 8-anilino-1-naphthalenesulfonic acid (ANS) reveal a previously unnoticed conformational change in c-lobe upon binding of saxitoxin.

2. Materials and methods

2.1. Preparation of polyclonal antibody to saxiphilin c-lobe

Rabbit polyclonal antibody to c-lobe with His_6 tag was prepared by Cocalico Biologicals against purified c-lobe inclusion bodies expressed by *Escherichia coli*. The c-lobe coding sequence was codon optimized for expression in *E. coli* and synthesized by GeneArt Inc. (Toronto, Ontario, Canada) with the addition of an NcoI restriction site at the 5′ end and a HindIII site at the 3′ end for ligation into appropriately restricted pET-22b (Novagen, EMD Biosciences). The sequence was confirmed by GeneArt, and is included as Supplementary Material. All cloned sequences were verified by DNA sequencing (Molecular Resource Laboratory, University of Arkansas for Medical Sciences, Little Rock, AR). The ligated plasmid was transformed into *E. coli* strain BL21 Star (Invitrogen) and expression was induced with isopropyl-β-D-thiogalactoside to a final concentration of 1 mM at an optical density of 0.66 (OD_{600}). After overnight expression at 21 °C, c-lobe present as insoluble inclusion bodies was isolated as described by Cline et al. (1993) and further purified by 12.5% SDS-PAGE followed by electroelution of Coomassie blue-stained c-lobe excised from the gel.

2.2. Saxiphilin c-lobe expression and purification in insect cells

High Five™ cells (Invitrogen) were cultured as adherent cells at 27 °C in serum-free Express Five™ insect cell media (Gibco) supplemented with 20 mM L-glutamine and penicillin–streptomycin solution (10 units/mL penicillin, 0.01 mg/mL streptomycin). DNA coding for c-lobe, with the coding sequence for signal peptide at the 3′ end, was synthesized by GenScript Corporation (Piscataway, NJ) using codon optimization for high-level expression in insect cells. The sequence was verified by GenScript Corporation, and is included as Supplementary Material. A coding sequence was added to the 3′ end of the c-lobe DNA resulting in addition of Flag and His_6 tags at the c-terminus of the recombinant protein (DYKDDDDKIVGGHHHHHH; underlined residues serve as a spacer between the two tags). A SacI restriction site at the 5′ end and an XbaI restriction site at the 3′ end were included to facilitate ligation into appropriately restricted