Available online at www.sciencedirect.com
ScienceDirect
Computational Biology and Chemistry 31 (2007) 378–383
Computational Biology and Chemistry
www.elsevier.com/locate/compbiolchem

Brief communication

Structural analysis of SNARE motifs from sea perch, *Lateolabrax japonicus* by computerized approaches

Kui Chen [a,b,c], Xiaohang Huang [b,*]

[a] *Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China*
[b] *The First Institute of Oceanography, State Oceanic Administration, Qingdao 266061, China*
[c] *Graduate University, Chinese Academy of Sciences, Beijing 100039, China*

Received 24 November 2006; accepted 10 August 2007

Abstract

Three cDNA sequences encoding four SNARE (*N*-ethylmaleimide-sensitive fusion protein attachment protein receptors) motifs were cloned from sea perch, and the deduced peptide sequences were analyzed for structural prediction by using 14 different web servers and softwares. The "ionic layer" structure, the three dimensional extension and conformational characters of the SNARE 7S core complex by using bioinformatics approaches were compared respectively with those from mammalian X-ray crystallographic investigations. The result suggested that the formation and stabilization of fish SNARE core complex might be driven by hydrophobic association, hydrogen bond among R group of core amino acids and electrostatic attraction at molecular level. This revealed that the SNARE proteins interaction of the fish may share the same molecular mechanism with that of mammal, indicating the universality and solidity of SNARE core complex theory. This work is also an attempt to get the protein 3D structural information which appears to be similar to that obtained through X-ray crystallography, only by using computerized approaches.

Keywords: Sea perch; SNARE proteins; Motif; 7S core complex; Protein structure analysis

1. Introduction

Membrane fusion is the fundamental cellular process by which exocytosis and endocytosis occur. Among the most important and widely studied proteins participating in this secretion pathway are soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptors, or SNAREs. These membrane-associated proteins have become, after more than a decade of intensive biochemical and molecular biological endeavors, the most intensively studied protein machinery involved in intracellular trafficking in eukaryotic cells (Söllner et al., 1993a,b; Ferro-Novick and Jahn, 1994; Woodman, 1997; Jahn and Sdhof, 1999; Joseph and John, 2003; Jahn and Scheller, 2006).

The SNARE proteins are divided into several small conserved families, for example synaptobrevin/VAMP family, SNAP-25/23 family and Syntaxin family with a growing member of isoforms (Gerst, 1999). Each SNAREs conserve SNARE domain(s) or motif(s), a sequence of 60–70 amino acids in length to assemble into a four-helix bundles during SNARE protein interaction (Weimbs et al., 1997). According to classical SNARE hypothesis, intracellular synaptic membrane fusion requires the parallel arrangement of four SNARE motifs, one from vesicle-associated VAMP (v-SNARE) and three from target-localized SNAP-25 (two motifs) and Syntaxin (one motif) (*t*-SNARE). Within one complex (termed as *trans*-SNARE complex or SNAREpin), four SNARE motifs bridge two opposite membranes into close proximity (Sutton et al., 1998; Daniel and Frederick, 2003). This is believed to be the critical step for membrane fusion and the principal fusogens of the secretion pathway (Juan and Benjamin, 2004).

The micro topology and wispy organization of the fusion synaptic complex has been deduced by X-ray crystallography (Sutton et al., 1998; Antonin et al., 2002). In the central four-helix-bundle axis is the 16 layers structure, including 7 upstream layers (layers −1 to −7) and 8 downstream layers (layers +1 to +8) extended from central ionic 0 layer which is formed by three key glutamine residue from *t*-SNARE and one arginine residues from v-SNARE. The layers flanking the ionic 0 layer are composed of leucine, isoleucine and valine residues, obeying the packing roles of parallel, tetrameric leucine-zipper

* Corresponding author. Tel.: +86 532 88969292; fax: +86 532 88969292.
E-mail address: xiaohanghuang@fio.org.cn (X. Huang).

1476-9271/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.compbiolchem.2007.08.002

proteins. These layers of SNARE motifs are knitted together to form α-helices by hydrophobic interactions in the core as well by several disperse hydrogen-bonding or salt-bridging side chain interactions on the solvent-exposed surface (Sutton et al., 1998; Fasshauer et al., 1998; Chen and Scheller, 2001; Bracher et al., 2002).

Although the first SNARE protein, VAMP, was identified in electric ray (*Torpedo marmorata*) (Trimble et al., 1988), according to the present knowledge, few articles dealing with SNARE in marine teleostean has been published (Chai et al., 2006). In this work, various bioinformatic approaches including biological web servers *Pfam*, *NCBI/BLASTN*, *BLOCK* (Henikoff and Henikoff, 1996; Henikoff et al., 1998), *PROF* version, *PHD* version (Rost et al., 1994), Self-Optimized Prediction Method with Alignment (*SOPMA*) (Geourjon and Deléage, 1995), *Protein-Modeling* (Schwede et al., 2003) and computer programs *Primer Premier* (Singh et al., 1998), *Vector NIT Suite, DNASIS MAX*, *OMIGA* (Kramer, 2001) and *DNASTAR/Protean* (Plasterer, 1997) have been introduced for gene target-cloning, protein structural predications, hydrophobicity profile analysis, dimensional extension and conformational characterization of the synaptic SNARE motifs from marine teleostean, sea perch. The comparative analysis of "ionic layer" structure between sea perch and mammal model revealed that the SNARE four-helix-bundle of the fish may share the similar characters with those of mammal at molecular level, indicating the universality and solidity of SNARE hypothesis (Rothman,1993).

2. Materials and Methods

2.1. Target Cloning of Sea Perch SNARE Motifs

Bioinformatic details of the mammalian Syntaxin-1 were obtained from *Pfam* database (Sanger Institute, UK, Table 1). Homogeny search for Syntaxin-1 was performed by running programs blastp and tblastx (Table 1) in the NCBI/*GenBank* database. The alignments of various Syntaxin-1 protein sequences in FASATA format were submitted in *BLOCKS/Block Maker* server and the SNARE domains within them were localized by using "GIBBS" method. Based on obtained information, primer pairs, *syx1sp* and *syx1ap*, which precisely span SNARE motif in Syntaxin-1, were designed by using software Primer Premier (Table 1). And the primer sequences of SNAP-25 (*snsp* and *snap*) and VAMP-2 (*vsp* and *vap*) were designed in the similar way. cDNA template was reverse-transcripted from brain tissue mRNA of sea perch by using Oligo-d(T)18 primer and M-MLV reverse transcriptase (Promega). PCR were conducted separately using different pair of primers mentioned above. During each amplification, the annealing temperature was 59.0 °C (*syx1sp* and *syx1ap*), 55.8 °C (*snsp* and *snap*) and 54.1 °C (*vsp* and *vap*), respectively. Amplified fragments were cloned into pMD18-T vector (TaKaRa) and sequenced afterwards.

2.2. Secondary Structure Predication

The deduced amino acid sequences of SNARE motifs in Syntaxin-1, SNAP-25 and VAMP-2 cloned from sea perch were input and the secondary structure predication was curried out separately by running computer programs OMIGA, DNASTAR/Protean and DNASIS MAX (Table 1) with the assistance of Chou–Fasman method (Chou and Fasman, 1978). Also, the sequences were copied into analysis platform of web servers *SOPMA*, *PROF and PHD* (Table 1) and 1D structural predication was performed. All calculations were programmed with their default parameters.

2.3. Three-dimensional (3D) Structure Predication

The peptide sequence of sea perch SNAP-25 motif was submitted into web server *ExPAsy* (Table 1), then the sequence document was converted into PDB format by running *SWISS-MODEL* program. And the 3D structure of protein was displayed using computer software *Vector NIT Suite*. The 3D structures of SNARE motifs in sea perch VAMP-2 and Syntaxin-1 were processed in the same way.

Table 1
Web servers and softwares used in this study[a]

Web server and software	Task	Website
Pfam (Protein family)	Homogeny search	www.sanger.ac.uk/software/pfam/
NCBI/BLAST	Homogeny search	ncbi.nlm.nih.gov/BLAST/
BLOCKS/Block Maker	Motifs location	blocks.fhcrc.org/blockmkr/make_blocks.html
PROF 2000_06	Secondary structure predication	Predictprotein.org
PHD 1996.1	Secondary structure predication	Predictprotein.org
SOPMA	Secondary structure predication	npsa-pbil.ibcp.fr/NPSA/npsa_sopma.html
SWISS-MODEL	3D structure predication	http://swissmodel.expasy.org
Primer Premier 5.0	Primer pairs design	
MegAlign 5.0	Sequences alignment analysis	
BioEdit 4.8.5	Sequences alignment analysis	
Omiga 2.0	Secondary structure predication	
DNASIS MAX 1.0	Secondary structure predication	
Protean 5.0	Secondary structure predication	
Vector NIT Suite 8.0	3D structure graphic conversion	

[a] Software names are in boldface.