Identification of SNARE proteins in fish—Tilapia

Oreochromis niloticus

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Abstract

SNARE proteins are a group of membrane-associated proteins involved in exocytosis, secretion and membrane trafficking events in eukaryotic cells. Research on SNARE protein biology has become a more attractive field in recent years which is applied to marine biology specifically to the fish Tilapia Oreochromis niloticus. Plasma membrane fractions of different tissues of Tilapia, including brain, liver-pancreas, intestine, skin and muscle were extracted and immuno-decorated with isoform-specific antibodies to the SNARE families and associated proteins. The presence of Syntaxins - 1A, 2 and 3, SNAP - 23 and SNAP - 25, VAMP - 2, Munc - 18 - 1 and Munc - 13 in the brain was identified which were differentially distributed in the other organ tissues of the fish Tilapia. The distinct distribution of SNARE and associated proteins will serve as the basis for further investigation into their special secretive function in these tissues of the fish.

Key words: SNARE proteins, exocytosis, secretion, fish

1 Introduction

Secretion is a basic function of all living organisms. In fish like in mammals, neurotransmitters, hormones and other macromolecules are secreted in several neuronal, neuroendocrine and exocrine organs and tissues located in brain, pancreas, gastrointestinal tract and kidney. Bond 1996, Shi et al. 2005. Fish also has some unique secretory glands like caudal neurosecretory tissue and corpuscles of Stannius which appear to influence water balance and osmoregulation. Between a fish body and its aqueous environment there exists a mucous layer secreted by the mucosa glands in the skin and this mucous layer is involved in fish locomotion, osmoregulation, interspecies chemical communication and immunological functions Lemaitre et al. 1996. It has been reported that the mucous secreted from the fish skin contains immunoglobulin, complements, C-reactive protein, lectin, lysozyme and haemolysins which serve as a barrier against pathogen of environment and disease prevention Yano 1996, Qiu et al. 2005, 2006.

The secretion and protein trafficking between
intracellular compartments of the cell are mediated by carrier vesicles that bud from one organelle and fuse selectively with the cognate membrane compartment. These vesicular transport processes require specialized proteins now identified as SNAREs: SNAP (soluble NSF attachment protein) proteins that regulate carrier vesicle transport and fusion with the highest degree of fidelity [Jahn and Hanson 1998; Rothman and Wieland 1996]. According to the SNARE hypothesis the cognate vesicle membrane bound SNARE proteins v-SNAREs and cognate target membrane proteins t-SNAREs assemble to form a ternary complex which brings the cognate membrane compartments in close proximity and upon disassembly by a fusogenic stimulus sufficient energy is released to induce membrane fusion [Jahn and Hanson 1998; Rothman and Warren 1994].

For secretion and exocytic fusion the key t-SNAREs are Syntaxin and synaptosomal – associated protein of 25 kDa SNAP-25 and v-SNARE is the vesicle associated membrane protein VAMP. The very specific pairing of distinct sets of v- and t-SNARE isoforms accounts for the high fidelity of not only distinct secretory events but also intracellular vesicular trafficking between donor and acceptor compartments [Jahn and Hanson 1998; McNieu et al. 2000]. Such fidelity of vesicular transport and fine control of membrane fusion are further ensured by a large number of associated proteins such as Munc13 sRab sSynaptotagmin s which assist in the assembly and disassembly of the respective membrane-fusing SNARE complexes [Jahn and Hanson 1998].

Although the VAMP the first member of SNARE family was discovered in the electric organ of the Torpedo [Trimble et al. 1988; Risinger et al. 1993; Risinger et al. 1998; Chen et al. 2006]. Although fish shows unique characteristics of animals in water it might share a similar secretory mechanism as the mammals. Whether and how SNARE proteins play a role in fish physiological and intracellular activities remain unknown. In the present study we have begun to determine the presence of SNARE proteins in Tilapia by isolating the membrane fraction from different organ tissues of this fish conducting Western blot analysis to identify the major SNARE and associated proteins. The findings will provide a basic profile of SNARE proteins involved in the regulation of different secretory processes in these tissues of the fish.

2 Materials and methods

2.1 Membrane protein sample preparation

Living adults Tilapia Oreochromis niloticus were purchased from the local market and triplicate experiments were conducted in the cold room. Membrane fractionation was carried out in a manner we had previously described [Huang et al. 2001]. In brief brain liver-pancreas intestine skin and muscle were excised from several individuals with a body mass of over 450 g homogenized with a Waring blender six strokes in an ice cold buffer pH 7.4 containing 0.3 mol/dm³ sucrose 0.01% soybean trypsin inhibitor 2 μmol/dm³ β-mercaptoethanol and 1 mmol/dm³ PMSF at 1:10 the ratio of mass to volume. The resulting homogenates were fractionated by a series of centrifugations. A low-speed centrifugation 3 000 r/min 10 min removed the cell debris nuclei and then the supernatants were centrifuged at 14 000 r/min for 15 min to remove mitochondria and other large cell organelles. The resulting supernatants were collected and centrifuged at 26 000 r/min for 2 h on a Beckman L8 –
70M ultracentrifuge using a Ti50 fixed angle rotor all at 4°C. The resulting pellets containing the purified plasma membrane fractions were collected dissolved in the sucrose buffer and quantified for protein concentration. Membrane fractions were characterized by using alkaline phosphodiesterase I as the marker enzyme for plasma membrane.

2.2 Western blotting

The purified membrane fractions were loaded on to a 15% SDS polyacrylamide gel separated by electrophoresis and then transferred to a PVDF membrane. Isoforms specific antibodies were used for immunoblot analysis. Monoclonal antibodies against SNAP – 25, SMI – 25, Sternerberger Monoclonal Lutherville MD Syntaxin – 1A, Sigma St. Louis, Munc – 13 and Munc – 18 were from commercial sources as indicated. Rabbit polyclonal anti-Syntaxin – 2 and VAMP – 2 antibodies were the general gifts from Vesa Oikonen U. of Helsinki, Finland and William Trimble Hospital for Sick Children Toronto Canada. SNAP – 23 antibody was generated by ourselves Gaisano et al. 1996. The mouse brain membrane fraction was used for positive control and pre-immune sera in replacement of primary antibodies were used for negative control. SNARE proteins were identified by incubating the blots with a battery of indicated primary antibodies 1:1 000 dilution for 2 h and appropriate HRP conjugated secondary antibodies for 1 h at room temperature and visualized by enhanced chemiluminescence Amercham ECL. The specific bands were quantified by densitometric scanning of the blots with Bandscan 5.0 software Drets 1978.

3 Results and discussion

Since the amounts of protein loaded on the gel are identical we are able to compare the relative abundance and distribution of these proteins in the different tissues shown in Fig. 1. Our experiments showed that the Tilapia brain contains the protein isoforms corresponding to all neuronal and “ non-neuronal exocytotic SNARE proteins reported in the mammals. These proteins had the same molecular mass as those from the mouse brain and Western blots confirmed their specificities with corresponding antibodies Gaisano 2000 Gaisano et al. 1996. SNARE proteins SNAP – 25 Syntaxins and VAMP – 2 except SNAP – 23 were more abundant in the brain than in the other tissues examined. The SNARE associated proteins Munc – 13 and Munc – 18 were only detected in the Tilapia brain. The negative controls did not show any signal after repeating the same procedures of immunoblot analysis data not shown.

In the other tissues t-SNARE SNAP-25 showed a weak signal in the liver-pancreas in addition to the brain and was undetectable in the rest of non-neuronal tissues as would be expected. This tissue distribution of SNAP – 25 could be attributed to its role in neurotransmitter release in the brain and insulin secretion in the islets within liver-pancreas in fish. SNAP – 23 was abundantly detectable in all non-neuronal tissues notably in liver-pancreas and comparably in abundance to the brain levels. Although liver and pancreas are separated in mammals they are integrated into a single “ hepatopancreas” organ in some teleost fish Bond 1996. The role of SNAP – 23 in fish should therefore be similar to its exocrine and neuroendocrine secretions characterized in the mammalian pancreas and fat cells Huang et al. 2001 Thurmord and Pessin 2001.

At the gene level SNAP – 25 was cloned from zebrafish and in situ hybridization localized its expression only in the brain tissue. The nuclear acid sequence of this gene in fish shares more than 80% similarity to its counterpart in mammals Risinger et al. 1993 Risinger et al. 1998 indicating its
Sequential conservation and suggesting a similar function in fish as in mammals.

Fig. 1. SNARE proteins Munc-13 and 18 proteins from different tissues of the Tilapia were revealed by Western blot. The tissues examined include: (1) brain, (2) liver-pancreas, (3) intestine, (4) skin, and (5) muscle. This is a representative of three separate experiments. Specific bands from three experiments were quantified by densitometric scanning of the blots and summarized as percentage of densitometry reading of the brain tissue (means ± SEM).

t-SNARE Syntaxin-1A was detected in all tissues of Tilapia very rich in brain relatively abundant in the liver-pancreas and less in the other tissues. Syntaxin-2 was presented in all tissues except the muscle. Syntaxin-3 was only detected in brain and liver-pancreas. The Syntaxin-1 through 4 are the isoforms mediating exocytosis among 16 members of Syntaxin family (Bennett et al., 1992; Gerst, 1999) and we have first confirmed their presentation in fish. Syntaxin-1A was most abundant in neuronal and neuroendocrine tissues in mammals (Bennett et al., 1992) and was so in Tilapia. The non-neuronal Syntaxin isoforms Syntaxin-2 and 3 were found to mediate apical exocytosis in epithelial cells of mammals, particularly the exocrine pancreas (Gaisano et al., 1996; Hansen et al., 1999). Here in fish we also confirmed Syntaxin-2 and 3 in the tissues which uniformly contain secretory epithelial cells and both Syntaxins were undetectable in muscle as expected. The distinctive distribution of Syntaxins is likely to mediate more specific exocytotic targeting within the different epithelial secretory cells in the tested tissues of the fish, particularly the liver-pancreas and intestine intestinal mucosa.
Note that in the intestine of this teleost pancreatic islets are also dispersed and several peptides were found in the intestinal mucosal cells which are involved mainly in regulating secretions or motility of digestive tract [Bond 1996].

v-SNARE VAMP – 2 was abundant in the liver-pancreas and less in the intestine and somewhat abundant in the skin and muscle. Of the two exocytotic VAMP proteins VAMP – 1 is found almost exclusively in the neuron Trimble et al. 1988 whereas VAMP – 2 is very broadly distributed Gerst 1999. Consistently we found VAMP – 2 in all tissues of Tilapia examined. As the major v-SNARE in fish VAMP – 2 is likely to participate in both regenerative secretion and intracellular transport events by its interaction with the major t-SNARE. Since the VAMP – 2 is relatively abundant in skin it would also be intriguing to examine how VAMP – 2 is distributed in the mucous glands of the fish skin and whether they play a role in mucus secretion involved in multiple functions in the fishes.

At the gene level VAMP – 2 gene was cloned from Torpedo and sea perch Trimble et al. 1988 Chen et al. 2006. Its DNA sequence is conservative and shares 70% identity with the mammalian VAMP – 2. VAMP – 2 mRNA was detected in ten tissues of sea perch. Among these tissues the brain and blood were the most abundant tissues whereas the muscle tissue was the least one Chen et al. 2006.

Munc – 13 and Munc – 18 were presented only in the brain. These proteins specifically modulate the SNARE complex assembly by their actions on Syntaxin – 1A convert Syntaxin – 1A conformation from a closed form to an open one to interact with its cognate SNARE proteins SNAP – 25 and VAMP – 2 Richmond et al. 2001. Here we found these SNARE associated proteins to be present only in the brain. Perhaps additional non-neuronal isoforms of Munc – 13 Brose et al. 2000 and Munc – 18 isoforms Huang et al. 2002 may be present in the non-neuronal tissues of the fish whose functions should be to modulate the assembly of the non-neuronal SNARE complexes Gaisano 2000.

SNARE proteins are thus important mediators of the regulated exocytosis in neurons and non-neuronal cells. In the present study the major members of the mammalian SNARE proteins were detected in several tissues of Tilapia. Interestingly to note SNAP – 23 VAMP – 2 and Syntaxin – 1A were found in the fish muscle which we have used as a non-secretory tissue control in this study. The muscle contains glucose transporter proteins which require the SNARE complex to get the muscle membrane surface in mammals Thurmond and Pessin 2001. Since the amount of SNAP – 23 and VAMP – 2 was relatively abundant in the Tilapia muscle their distinctive functions in fish muscle should be further investigated.

Our study reveals that the putative SNARE proteins SNAP – 25 VAMP – 2 and Syntaxin families which form the exocytotic ternary complexes in mammals Rothman and Warren 1994 were present in all tissues of fish indicating the secretion in fish shares the same molecular mechanism as in mammals. Applying the insights gained from the study of SNARE proteins in mammalian cells could now be extended to the fish to begin to elucidate the secretory function of each of these proteins in the different tissue-cell types of the fish and how their function could be altered in disease.

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References


