Steroids 77 (2012) 1160–1168

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Caveolin–Na/K-ATPase interactions: Role of transmembrane topology in non-genomic steroid signal transduction

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ARTICLE INFO

Article history:
Received 15 February 2012
Received in revised form 4 April 2012
Accepted 10 April 2012
Available online 9 May 2012

Keywords:
Na/K-ATPase
Caveolin
Protein topology
Progesterone
Ouabain
Meiosis

ABSTRACT

Progesterone and its polar metabolite(s) trigger the meiotic divisions in the amphibian oocyte through a non-genomic signaling system at the plasma membrane. Published site-directed mutagenesis studies of ouabain binding and progesterone–ouabain competition studies indicate that progesterone binds to a 23 amino acid extracellular loop of the plasma membrane α-subunit of Na/K-ATPase. Integral membrane proteins such as caveolins are reported to form Na/K-ATPase–peptide complexes essential for signal transduction. We have characterized the progesterone-induced Na/K-ATPase–caveolin (CAV-1)–steroid 5α-reductase interactions initiating the meiotic divisions. Peptide sequence analysis algorithms indicate that CAV-1 contains two plasma membrane spanning helices, separated by as few as 1–2 amino acid residues at the cell surface. The CAV-1 scaffolding domain, reported to interact with CAV-1 binding (CB) motifs in signaling proteins, overlaps transmembrane (TM) helix 1. The α-subunit of Na/K-ATPase (10 TM helices) contains double CB motifs within TM-1 and TM-10. Steroid 5α-reductase (6 TM helices), an initial step in polar steroid formation, contains CB motifs overlapping TM-1 and TM-6. Computer analysis predicts that interaction between antipathic strands may bring CB motifs and scaffolding domains into close proximity, initiating allostearic changes. Progesterone binding to the α-subunit may thus facilitate CB motif:CAV-1 interaction, which in turn induces helix–helix interaction and generates both a signaling cascade and formation of polar steroids.

1. Introduction

The surfaces of most cells are studded with microscopic, flask-shaped invaginations called caveolae (~70 nm average outer diameter) that can either open for receiving and/or releasing material, or close for processing and/or delivery to intracellular sites [1–3]. In addition to facilitating the entry or exchange of small molecules, caveolar proteins, called caveolins, form membrane aggregates of 12–14 peptides and participate in the sequestration of signaling molecules such as Src, EGFR, and Ras [1,2,4–6]. Liu et al. [7] were the first to report the presence of Na/K-ATPase in caveolae of various cell types, and to suggest that the caveolar pool of the enzyme may be responsible for the stimulus-induced signaling by Na/K-ATPase. Since then, there has been increasing interest in the differences between caveolar and non-caveolar Na/K-ATPase, and a number of studies have addressed signaling mechanisms attributed to the caveolar pool of enzymes [8–12].

Observing that the α-subunit of the Na/K-ATPase contains two caveolin binding motifs, Wang et al. [9] hypothesized that a caveolin–Na/K-ATPase complex forms in response to ouabain which facilitates ouabain-activated signal transduction in renal epithelial cells. Using a Glutathione *S*-transferase pull-down assay, Wang et al. [9] found that the α-subunit of Na/K-ATPase binds to the N-terminal region of CAV-1 isolated from LLC-PK1 cells. Further evidence suggested that a ouabain-induced Na/K-ATPase–caveolin–Src complex recruits and/or phosphorylates multiple plasma membrane proteins and initiates several different signaling cascades. More recently, using chemical crosslinking, co-immunoprecipitation, and immunodetection studies in pig kidney outer medullar membranes, Liu et al. [13] showed that, in contrast to the non-caveolar pool of Na/K-ATPase, the caveolar pool contained caveolin-1 oligomers, annexin-2 tetramers, and oligomers of the α,β,γ-protomers of Na/K-ATPase as a large multiprotein complex. Thus, the Na/K-ATPase present in caveolar and non-caveolar plasma membrane regions is associated with quite different arrays of proteins.

The α-subunit of the Na/K-ATPase acts as a cell surface receptor for the non-genomic action of steroids as diverse as progesterone [14,15] and ouabain [16] and also interacts with other integral membrane enzymes such as phosphatidylethanolamine

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http://dx.doi.org/10.1016/j.steroids.2012.04.012

N-methyltransferase and sphingomyelin synthase [17]. Our emphasis here is on the interaction between CAV-1 and the α-subunit and on the extension and refinement of our previous attempts to characterize progesterone binding to the α-subunit as part of the steroid signal transduction initiating the meiotic divisions in the oocyte. Helix–helix interactions between CAV-1 oligomers and the caveolin binding domains of the α-subunit [18] may explain the observed aggregation of CAV-1 and Na/K-ATPase within the caveolae, as well as the sequestration of peptide signaling molecules. As noted elsewhere [17], helix–helix interactions between two or more integral membrane proteins appear to regulate ligand-initiated response systems. A preliminary report of CAV-1 topology has been presented [19].

2. Experimental

The present study uses our published data on: (1) the induction of the meiotic divisions in amphibian oocytes by progesterone and progesterone polar metabolites [20], (2) progesterone–ouabain competition for a binding site on the external surface of isolated plasma membranes from *Rana pipiens* oocytes [14], and (3) the site-directed mutagenesis studies of ouabain binding to sheep and human Na/K-ATPase α1-subunits by other investigators (e.g. [21]). The amino acid sequences of the caveolins and the α-subunit of Na/K-ATPase from several vertebrates, as well as the enzymes in Table 2, were obtained from the ExPASy Proteonomic Server of the Swiss Institute of Bioinfomatics (www.expasy.org/UniprotKB).

2.1. Secondary structure predictions

Caveolin secondary structures were predicted by both the PSI-PRED v. 3.0 [22] and NetSurfP v. 1.1 methods [23]. Helical wheel representations were visualized using the applet written by Edward K. O'Neil and Charles M. Grisham. This applet is accessible at: (http://cti.itc.Virginia.EDU/~cmgDemo/wheel/wheelApp.html). The TMhit method [24] predicts helix–helix interactions within the same molecule based on residue contacts.

2.2. Transmembrane helix predictions

The positions of transmembrane helices was predicted using: (1) the TOPCONS algorithm [25] (http://topcons.cbr.su.se/), (2) the ConPred II server [26] (http://bioinfo.si.hirosaki-u.ac.jp/~ConPred2/), (3) the SVMtop method [27]; (bio-cluster.iis.sinica.edu.tw/~bioapp/SVMtop/index_adv.php/), (4) the MEMSAT-SVM server [28] (http://psipred@cs.ucl.ac.uk), (5) SOSUI ver. 1.1 [29] (http://bp.nuap.naoya~u.ac.ip/sosui/), (6) the MemBrain method [30] (http://chou.med.harvard,edu/bioinf/MemBrain/) and (7) Phobius [31]. Chem 3D Ultra v. 11 (Cambridgesoft, Cambridge Scientific Computing, Cambridge, MA) was used to visualize the 3D structure of the transmembrane helices.

3. Results and discussion

3.1. Secondary structure of plasma membrane CAV-1

Fig. 1 compares the secondary structures of CAV-1 from pig (*Sus scrofa*) kidney (Accession #Q6RVA9) as predicted using NetSurfP (A, upper left), MEMSAT-SVM (B, upper right) and PSI-PRED (C, bottom). All three methods predict an extensive helix-rich region in the C-terminal half of the protein. β-Strand regions are juxtaposed to the helical region in both the N- (residues 84–98) and C-terminal region (residues 168–176) using NetsurfP analysis [23]. A large β-strand region is also apparent near the N-terminus. A comparison of the relative surface accessibility of CAV-1 using NetsurfP indicates that the lowest surface accessibility occurs in the region with the highest helical content (T_{91}–F_{137}). (Relative surface accessibility reflects the percentage of the surface area of a given amino acid in peptide linkage that is inaccessible to the solvent (water).) Since CAV-1 is an integral membrane protein [1–4], the data suggest that the helical region in the C-terminal half of CAV-1 (residues 99–167) contains one or more transmembrane helical structures.

3.2. CAV-1 transmembrane topology

Consensus prediction of membrane protein topology analysis using the MEMSAT-SVM method indicates that CAV-1 contains two transmembrane helices corresponding to a helix-turn-helix configuration (B, Fig. 1). Both the N- and C-terminal ends of CAV-1 are cytoplasmic. TM-1 occurs between $CAV_{92–116}$ whereas TM-2 is located between $CAV_{119–143}$; the separate transmembrane helices being linked by two amino acids (I_{117}, Y_{118}) at the extracellular surface. The relative positions of TM-1 and TM-2 in the CAV-1 peptide are indicated above the N-terminal part of the helical region illustrated in Fig. 1.

Table 1
Comparison of the topology of pig kidney caveolin 1 (Accession #Q6RVA9) using different web servers for predictingtransmembrane regions.

Transmembrane prediction servers[a]	N-terminal position	TM-1	External loop	TM-2	C-terminal position
TOPCONS	in	97–117	118	119–139	in
MEMSAT3	in	92–116	117–118	119–143	in
ConPred II	in	99–116	117	118–135	in
SVMtop 1.0	in	96–114	115–116	117–135	in
SOSUI	in	82–104	105–110	111–133	in
MemBrain	ND[b]	91–126	127–130	131–160	ND

[a] See Section 2
[b] Not defined.

Table 2
Enzymes activated by progesterone in isolated plasma–vitelline membranes from *Rana pipiens* oocytes.

Enzyme	Major product	Maximum activation	TM helices	CB motifs	Refs.
PE-*N*-methyltransferase (Q9UBM1)	Phosphatidylcholine	~60 s	5	0	[17]
Sphingomyelin synthase (Q8NHU3)	Sphingomyelin	~3 min	6	2	[17]
Sphingomyelinase (P17405)	Ceramide	~10 min	1	3	[46]
Phospholipase D1 (Q153393)	Phosphatidic acid	~0.5 h	1	3	[48]
PI-phospholipase C (P19174)	DAG & IP3	2–3 min	1	2	[48]
Steroid 5α-reductase (Q6DF23)	5α-Pregnane-3αol,20-one	4–5 h	6	3	[45]