Biochimica et Biophysica Acta 1818 (2012) 1706–1716

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Flexibility of the PDZ-binding motif in the micelle-bound form of Jagged-1 cytoplasmic tail

Matija Popovic [a], Ventsislav Zlatev [a], Vesna Hodnik [b], Gregor Anderluh [b,c], Isabella C. Felli [d], Sándor Pongor [a,*], Alessandro Pintar [a,*]

[a] Protein Structure and Bioinformatics Group, International Centre for Genetic Engineering and Biotechnology (ICGEB), AREA Science Park, Padriciano 99, I-34149 Trieste, Italy
[b] Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia
[c] Laboratory for Biosynthesis and Biotransformation, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia
[d] Magnetic Resonance Center and Department of Chemistry, University of Florence, Via Luigi Sacconi 6, I-50019 Sesto Fiorentino, Italy

ARTICLE INFO

Article history:
Received 9 November 2011
Received in revised form 13 March 2012
Accepted 14 March 2012
Available online 21 March 2012

Keywords:
Notch signaling
Membrane/cytoplasm interface
Phosphorylation
NMR
Surface plasmon resonance
Circular dichroism

ABSTRACT

Human Jagged-1, one of the ligands of Notch receptors, is a transmembrane protein composed of a large extracellular region and a 125-residue cytoplasmic tail which bears a C-terminal PDZ recognition motif. To investigate the interaction between Jagged-1 cytoplasmic tail and the inner leaflet of the plasma membrane we determined, by solution NMR, the secondary structure and dynamics of the recombinant protein corresponding to the intracellular region of Jagged-1, J1_tmic, bound to negatively charged lysophospholipid micelles. NMR showed that the PDZ binding motif is preceded by four α-helical segments and that, despite the extensive interaction between J1_tmic and the micelle, the PDZ binding motif remains highly flexible. Binding of J1_tmic to negatively charged, but not to zwitterionic vesicles, was confirmed by surface plasmon resonance. To study the PDZ binding region in more detail, we prepared a peptide corresponding to the last 24 residues of Jagged-1, J1C24, and different phosphorylated variants of it. J1C24 displays a marked helical propensity and undergoes a coil–helix transition in the presence of negatively charged, but not zwitterionic, lysophospholipid micelles. Phosphorylation at different positions drastically decreases the helical propensity of the peptides and abolishes the coil–helix transition triggered by lysophospholipid micelles. We propose that phosphorylation of residues upstream of the PDZ binding motif may shift the equilibrium from an ordered, membrane-bound, interfacial form of Jagged-1 C-terminal region to a more disordered form with an increased accessibility of the PDZ recognition motif, thus playing an indirect role in the interaction between Jagged-1 and the PDZ-containing target protein.
© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Ligands to Notch receptors are type I membrane spanning proteins. They all share a poorly characterized N-terminal region, a DSL (Delta/Serrate/Lag-2) domain, a series of tandem epidermal growth factor-like repeats, a transmembrane segment, and a unique cytoplasmic tail of ~100–150 amino acids [1]. Five different ligands to Notch receptors have been identified in mammals, three orthologs (Delta-1, -3 and -4) of *Drosophila* Delta and two orthologs (Jagged-1 and -2) of *Drosophila* Serrate. Although the molecular mechanisms of ligand specificity are still unclear, evidence from *in vivo* studies suggests that each ligand exerts non-redundant effects. Gene knock-out of Jagged-1 [2] or Delta-1 [3], heterozygous deletion of Delta-4 [4], or homozygous mutants in Jagged-2 [5] all lead to severe developmental defects and embryonic lethality in mice. There is no significant sequence similarity shared among the intracellular region of the different ligands [6], apart from the identical PDZ binding motif (ATEV) found at the C-terminus of Delta-1 and Delta-4. Neither Delta-3 nor Jagged-2 presents a PDZ recognition motif whereas the cytoplasmic tail of Jagged-1 contains a different C-terminal PDZ interacting motif (EYIV). Jagged-1 was shown indeed to interact in a PDZ-dependent manner with afadin (AF6), a protein located at cell–cell adherens junctions [7,8]. The cytoplasmic tail of Notch ligands is also a target for ubiquitination and ligand endocytosis. Both Mind bomb 1 [9–11] and a myrystoylated, membrane-anchored form of Neuralized-like 1 [12] have been proposed to be involved in

Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] sodium salt; DMPS, 1,2-dimyristoyl-sn-glycero-3-[phospho-L-serine] sodium salt; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate-d_6 sodium salt; HSQC, heteronuclear single quantum correlation; LMPC, 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholine; LMPG, 1-myristoyl-2-hydroxy-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] sodium salt; LPPC, 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine; LPPG, 1-palmitoyl-2-hydroxy-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] sodium salt; PDZ, domain present in PSD-95, Dlg, and ZO-1/2; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) sodium salt; SDS, sodium dodecyl sulfate; SPR, surface plasmon resonance; TFE, 2,2,2-Trifluoroethanol

* Corresponding authors. Tel.: +39 040 3757354; fax: +39 040 226555.
E-mail addresses: pongor@icgeb.org (S. Pongor), pintar@icgeb.org (A. Pintar).

0005-2736/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2012.03.012

mono-ubiquitinylation of Jagged-1 in mice. Finally, there is compelling evidence that Notch ligands, much alike Notch receptors, undergo ectodomain shedding and regulated intramembrane proteolysis. The proteolytic processing of Jagged-1 is mediated by the ADAM17 metalloprotease [13,14] and by the presenilin/γ-secretase complex [13]. Although the fate of the cleaved intracellular region is still unclear, it is possible that it acts as a membrane-tethered transcriptional co-activator. The C-terminal fragment of Jagged-1 comprising part of the transmembrane segment and the intracellular region expressed in COS cells was shown to localize mainly in the nucleus, and to activate gene expression through the transcription factor activator protein 1 (AP1/p39/*jun*) enhancer element [13].

Depending on the environment, the intracellular region of Jagged-1 is then supposed to exist in at least two distinct forms, as a membrane-tethered protein located at the interface between the membrane and the cytoplasm and as a soluble nucleocytoplasmic protein. In a previous study [15], we showed that a recombinant protein corresponding to the cytoplasmic tail of human Jagged-1 (J1_tmic) is mainly disordered in solution, but has a distinct propensity to adopt a helical conformation in the presence of TFE. J1_tmic also gains secondary structure upon binding to the negatively charged surface of SDS micelles, or to vesicles made of negatively charged phospholipids such as DMPS and DMPG, which are prevalent components of the inner layer of the plasma membrane in eukaryots. On the contrary, the structure of J1_tmic is not affected by the presence of vesicles formed by a zwitterionic phospholipid like DMPC, suggesting that the negative charge density at the surface of SDS micelles or lipid vesicles is required to promote binding and secondary structure formation. We also showed that helix formation is strongly pH-dependent, with a sharp increase in the helical content from pH ~7 to ~6. As J1_tmic contains six endogenous histidines, we suggested that protonation of one or more of the histidines is promoting helix formation or extension.

The partial folding of the cytoplasmic domain of Jagged-1 accompanied by its association with the inner side of the cell membrane may have relevant effects on the function of Jagged-1 in Notch signaling. For instance, it would selectively mask certain residues that are potential targets for post-translational modifications such as phosphorylation, ubiquitinylation, or O-glycosylation with β-N-acetylglucosamine. In a similar way, it would mask or expose selected binding motifs with respect to binding partners. The partial folding and association of the intracellular region of Jagged-1 with the membrane is also expected to reduce the accessibility of the PDZ binding motif. To get more insight into the interaction between Jagged-1 cytoplasmic tail and the membrane, we studied a model system made of the recombinant protein corresponding to the cytoplasmic tail of Jagged-1 (J1_tmic) and negatively charged lysophospholipid micelles used to mimic the interface between the plasma membrane and the cytosol. We applied solution NMR methods to determine the secondary structure and dynamics of J1_tmic bound to micelles. Furthermore, we hypothesized that phosphorylation of selected residues close to the PDZ binding motif might alter the affinity for the negatively charged inner layer of the cytoplasmic membrane and possibly change the conformational properties of Jagged-1 C-terminal region. To test this hypothesis, a series of peptides corresponding to the C-terminal sequence of Jagged-1 (JAG1_HUMAN, residues 1195–1218) and phosphorylated at different positions was synthesized, and their conformational properties studied by far-UV circular dichroism.

2. Materials and methods

2.1. Protein expression and purification

The recombinant protein corresponding to the cytoplasmic tail of human Jagged-1, starting from the putative presenilin/γ-secretase cleavage site and with the transmembrane cysteine mutated to alanine (residues 1086–1218 of JAG1_HUMAN; C1092A; see Supporting Material, Fig. S1) was expressed in BL21(DE3) *Escherichia coli* (Novagen) cells from a codon-optimized synthetic gene and purified in two steps by immobilized metal ion affinity chromatography followed by RP-HPLC, as described [15]. For the preparation of the ^{15}N- and of the ^{15}N, ^{13}C-labeled proteins, cells were grown in a minimal medium containing 6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 0.5 g/L NaCl, 0.12 g/L $MgSO_4$, 0.01 g/L $CaCl_2$, 1.7 g/L yeast nitrogen base without amino acids and ammonium sulfate (Difco), and 100 μg/mL ampicillin, supplemented with 0.5 g/L $^{15}NH_4Cl$, 5 g/L D-glucose or 0.5 g/L $^{15}NH_4Cl$, 5 g/L U-$^{13}C_6$ D-glucose for the single and doubly labelled proteins, respectively. Expression and purification of the labelled proteins were carried out as described above. The purified proteins were analyzed by LC–MS to confirm their identity.

2.2. NMR sample preparation

The protein stock solutions were prepared dissolving the freeze-dried labeled proteins in sodium phosphate buffer (20 mM), pH 6.1. Lysophospholipid stock solutions were prepared dissolving the 1-Myristoyl-2-Hydroxy-*sn*-Glycero-3-[phospho-*rac*-(1-glycerol)] sodium salt (LMPG) or 1-Myristoyl-2-Hydroxy-*sn*-Glycero-3-Phosphocholine (LMPC) (Avanti Polar Lipids Inc., Alabaster, AL, USA) in sodium phosphate buffer (20 mM, pH 6.1). The mixed micelle (LMPC/LMPG, 80/20 mol/mol) lysophospholipid stock solution was prepared dissolving the lysophospholipids in $CH_3OH/CHCl_3$, mixing the appropriate volumes of the two, gently drying the solution, and redissolving the mixture in sodium phosphate buffer, pH 6.1. Samples for NMR spectroscopy were prepared mixing the appropriate volumes of stock solutions, and adding D_2O (10 vol.%).

The final protein concentration was ~0.1 mM for the reference sample dissolved in buffer only, ~0.5 mM for the ^{15}N-labeled samples in the presence of LMPG (80 mM), LMPC (80 mM) or LMPC/LMPG (80 mM), and 0.9 mM for the ^{15}N, ^{13}C-labeled protein in the presence of LMPG (150 mM). Assuming a micelle aggregation number of ~120, the protein/micelle molar ratio was ~0.75. An additional sample with a protein concentration of ~0.33 mM and a LMPC/LMPG (80/20 mol/mol) concentration of 113 mM, which corresponds to a protein/micelle ratio of 0.35 was also prepared. From an average micelle aggregation number of ~120, a MW of 478.5 for each LMPG molecule, and a 1:1 stoichiometry, the calculated MW of the complex would be ~73 kDa.

2.3. NMR spectroscopy

NMR data were acquired at 298 K. Preliminary and ^{15}N relaxation experiments were acquired on a Bruker Avance 500 MHz spectrometer equipped with a TXI triple resonance cryoprobe using the ^{15}N-labeled protein. For assignment purposes, standard HNCACB, CBCA(CO)NH, HNCACO, HNCO and HN(CA)NNH experiments were acquired on Bruker Avance 700 and 800 MHz spectrometers equipped with cryogenic probeheads optimized for ^{1}H detection. The protonless experiments CON, (H)CBCACON and (H)CBCANCO [16–18] were acquired on a Bruker Avance 700 MHz equipped with a cryogenic probehead optimized for ^{13}C direct detection. $^{3}J_{HNH\alpha}$ coupling constants and exchange rates of backbone amides with solvent were estimated from HNHA [19] and CLEANEX-PM [20] experiments, respectively. Data were processed using XwinNMR and analyzed with the CCPN program ANALYSIS (http://www.ccpn.ac.uk). Chemical shifts were referenced to DSS. Deviations of Cα, C′, Hα, and Cβ chemical shifts from random coil values [21] were used to determine secondary structure regions. R_1 and R_2 relaxation rates were estimated from fitting a three parameter exponential decay to experimental intensities of HN cross peaks acquired with delays of 0.010, 0.150, 0.540, 1.00, 2.50 s for R_1 and 17, 68, 153, 204, 271 ms for R_2 experiments. ^{1}H–^{15}N steady-state

Download English Version:

https://daneshyari.com/en/article/10797347

Download Persian Version:

https://daneshyari.com/article/10797347

Daneshyari.com