doi:10.1016/j.jmb.2010.05.013 J. Mol. Biol. (2010) 400, 448–462

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Structure of the Cytosolic Portion of the Motor Protein Prestin and Functional Role of the STAS Domain in SLC26/SulP Anion Transporters

Elisa Pasqualetto[1,2]†, Rosa Aiello[1,2]†, Lorenzo Gesiot[1], Greta Bonetto[1,2], Massimo Bellanda[1] and Roberto Battistutta[1,2]*

[1]*Department of Chemical Sciences, University of Padua, via Marzolo 1, 35131 Padua, Italy*

[2]*Venetian Institute for Molecular Medicine, via Orus 2, 35129 Padua, Italy*

Received 24 February 2010; received in revised form 4 May 2010; accepted 7 May 2010
Available online 21 May 2010

Prestin is the motor protein responsible for the somatic electromotility of cochlear outer hair cells and is essential for normal hearing sensitivity and frequency selectivity of mammals. Prestin is a member of mammalian solute-linked carrier 26 (SLC26) anion exchangers, a family of membrane proteins capable of transporting a wide variety of monovalent and divalent anions. SLC26 transporters play important roles in normal human physiology in different tissues, and many of them are involved in genetic diseases. SLC26 and related SulP transporters carry a hydrophobic membrane core and a C-terminal cytosolic portion that is essential in plasma membrane targeting and protein function. This C-terminal portion is mainly composed of a STAS (*s*ulfate *t*ransporters and *a*nti-sigma factor antagonist) domain, whose name is due to a remote but significant sequence similarity with bacterial ASA (*a*nti-*s*igma factor *a*ntagonist) proteins. Here we present the crystal structure at 1.57 Å resolution of the cytosolic portion of prestin, the first structure of a SulP transporter STAS domain, and its characterization in solution by heteronuclear multidimensional NMR spectroscopy. Prestin STAS significantly deviates from the related bacterial ASA proteins, especially in the N-terminal region, which—although previously considered merely as a generic linker between the domain and the last transmembrane helix—is indeed fully part of the domain. Hence, unexpectedly, our data reveal that the STAS domain starts immediately after the last transmembrane segment and lies beneath the lipid bilayer. A structure–function analysis suggests that this model can be a general template for most SLC26 and SulP anion transporters and supports the notion that STAS domains are involved in functionally important intramolecular and intermolecular interactions. Mapping of disease-associated or functionally harmful mutations on STAS structure indicates that they can be divided into two categories: those causing significant misfolding of the domain and those altering its interaction properties.

Keywords: prestin; motor protein; STAS domain; SLC26 anion transporters; SulP transporters

Edited by R. Huber

Introduction

Prestin (SLC26A5) is a membrane protein that is abundantly expressed in outer hair cells (OHCs) of the inner ear.[1] It is localized in the basolateral membrane and is considered the motor protein responsible for the OHC somatic electromotility that increases hearing sensitivity and frequency selectivity in mammals.[2–7] Several mammalian cell lines heterologously expressing prestin show properties

*Corresponding author. E-mail address: roberto.battistutta@unipd.it.

† E.P. and R.A. contributed equally to this work.

Abbreviations used: SLC26, solute-linked carrier 26; OHC, outer hair cell; NLC, nonlinear capacitance; 3D, three-dimensional; DLS, dynamic light scattering; SeMet, selenomethionine; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy; β-OG, β-octyl-glucoside; PDB, Protein Data Bank; ESRF, European Synchrotron Radiation Facility; TOCSY, total correlation spectroscopy.

unique to OHC, in particular changes in shape and a voltage-driven nonlinear capacitance (NLC), the electrical signature of prestin function. It has been shown that prestin can form oligomers, either dimers[8] or tetramers,[9,10] that most probably are located at the level of the 11-nm protein particles that constitute a substantial portion of the OHC lateral membrane.[11]

Prestin is one of 11 members of mammalian solute-linked carrier 26 (SLC26) anion exchangers, a family of membrane proteins capable of transporting a wide variety of monovalent and divalent anions.[12–14] SLC26 transporters play important roles in normal human physiology in different tissues, and many of them are involved in genetic deseases.[15] SLC26A2 (DTDST) is responsible for dystrophic dysplasia, SLC26A3 (DRA) is responsible for congenital chloride diarrhea, and SLC26A4 (pendrin) is responsible for autosomal-recessive hearing loss and goiter. Prestin is supposed to be responsible for nonsyndromic hearing loss in humans.[16] Little is known about the relationship between structure and function for these proteins.

SLC26 transporters are part of the large and ubiquitous family of sulfate permease SulP,[17–19] whose members are present in archaea, bacteria, fungi, plants, and animals. These proteins carry a hydrophobic core with a variable membrane topology and a C-terminal cytosolic domain (around 240 amino acids long in prestin) that is essential in plasma membrane targeting and protein function.[20–22] In prestin, deletions, mutations, or replacement with analogous C-terminal portions of related SLC26 transporters (pendrin or PAT-1) have been reported to be lethal to function.[23] As in other SLC26 and SulP transporters, the prestin C-terminal part is mainly composed of a STAS (*s*ulfate *t*ransporters and *a*nti-sigma factor antagonist) domain, whose name is due to a remote but significant sequence similarity with bacterial spoIIAA [ASA (*a*nti-sigma factor *a*ntagonist)] proteins.[24] Bacterial ASAs, which are key components in the mechanism that induces sporulation in response to nutrient deficiency,[25] are structurally well characterized both by NMR spectroscopy and by X-ray crystallography.[26–31] No experimental three-dimensional (3D) structures are available yet for the STAS domains of SLC26 or SulP transporters.

The location of the STAS domain in SLC26, as reported in proteins and domains databases, is predicted essentially based on multiple sequence alignments with bacterial ASAs (Fig. 1).[24] In transporters, there are long extensions both at the C-terminus and at the N-terminus (toward the transmembrane region) whose function and structural role are not clear. This and the low sequence similarity between the SLC26 STAS domains and the bacterial ASA proteins (identity around 10–15%) imply that the exact boundaries of the transporters STAS domain are not certain, at least from a structural point of view. Secondary structure predictions on the transporters STAS domain and the functional characterization of the SLC26A3 (DRA) STAS domain[34] indeed suggest that the domain can extend farther than that of the ASA proteins, in particular at the N-terminus. Given this uncertainty in the N-terminal and C-terminal ends, we designed and studied different variants of STAS domains in an attempt to identify the protein with the correct ends for structural characterization. Here we present and discuss the 3D crystal structure at 1.57 Å resolution and the characterization in solution of two closely related variants, providing the first structural characterization of an SLC26 STAS domain at atomic level that allows an understanding of important structure–activity relationships. We propose that this structure can be considered a general template for many SLC26 anion transporters and, more widely, for SulP transporters.

Results

Production and structure determination

In search of proteins amenable to crystallographic and NMR studies, we designed, cloned, produced in *Escherichia coli*, and characterized 10 different variants of prestin STAS based on multiple sequence alignments, secondary structure and crystallization predictions, and limited proteolysis analysis. Some constructs turned out to be insoluble when expressed in *E. coli*, and others had a strong tendency to aggregate in solution.[35] For the transporters STAS domains, an insertion, called the "variable loop," of different lengths (from around 35 to 135 residues in SLC26) is predicted between helix α1 and strand β3 (secondary structure nomenclature for ASAs).[24] Prestin STAS constructs without the variable loop, which is predicted to be mostly disordered, showed a better behavior in solution, with circular dichroism (CD) spectra typical of well-folded proteins (Fig. 2a). In particular, two of these variants resulted as monodisperse monomers in solution, as indicated by dynamic light scattering (DLS) measurements (Fig. 2b), and were suitable for structural studies. These variants comprise the C-terminal domain of *Rattus norvegicus* prestin starting at residue 505 and ending at residues 718 and 727, respectively. The variable loop is deleted between positions 564 and 636, where a dipeptide (GlySer in our variants) mimicking the bacterial ASAs was introduced to connect the two halves, in particular the predicted helix α1 and strand β3 (Fig. 1). The short variant ([505–563]GS[637–718]) was crystallized in the presence of 2 M ammonium sulfate and 5% polyethylene glycol 400. Optimized crystals diffracted at around 1.6 Å resolution, but attempts to solve the structure by molecular replacement using bacterial ASAs as templates failed. Then we produced and crystallized a selenomethionine (SeMet) derivative and solved the structure by single anomalous dispersion method, using the anomalous signal of one selenium atom for a 143-