Biochimica et Biophysica Acta 1848 (2015) 2385–2393

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Topological analysis of the Na^+/H^+ exchanger

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ARTICLE INFO

Article history:
Received 17 February 2015
Received in revised form 20 July 2015
Accepted 21 July 2015
Available online 26 July 2015

Keywords:
Na^+/H^+ exchanger
Membrane protein
Topology model
pH regulation

ABSTRACT

The mammalian Na^+/H^+ exchanger isoform 1 (NHE1) is a ubiquitously expressed integral membrane protein present in mammalian cells. It is made up of a hydrophobic 500 amino acid membrane domain that transports and removes protons from within cells, and a regulatory intracellular cytosolic domain made of approximately 315 amino acids. Determining the structure of NHE1 is critical for both an understanding of the Na^+/H^+ exchange mechanism of transport, and in the design of new improved inhibitors for use in treatment of several diseases in which it is involved. Differing models of the NHE1 protein have been proposed. The first model suggested by two groups proposes that amino acids 1–500 form a 12 transmembrane segment spanning region in which amino acids 1–127 form two transmembrane segments, and amino acids 315–411 form a single transmembrane segment with membrane associated segments. A second model based on the structure of the *Escherichia coli* Na^+/H^+ exchanger protein proposes an overall similar topology, but suggests amino acids 1–127 are removed as a signal sequence and are not present in the mature protein. It also suggests a different topology of amino acids 315–411 to form three transmembrane segments. We used cysteine scanning accessibility and examination of glycosylation of the mature protein to characterize the NHE1 protein. Our results demonstrate that the model of NHE1 is correct which suggests that amino acids 1–127 form two transmembrane segments that remain connected to the mature protein, and the segment between amino acids 315–411 is one transmembrane segment.

1. Introduction

The Na^+/H^+ exchanger (NHE) is a membrane transport protein ubiquitously present in living organisms. In mammals, its primary function is to protect cells from excess intracellular acid, which it achieves by catalyzing the electroneutral removal of a single intracellular H^+ in exchange for one extracellular Na^+. The ubiquitous isoform one (NHE1), was the first isoform discovered in 1989 [1,2]. Ten isoforms of NHE are currently known to exist (NHE1–10) with different tissue expression, cellular localization and physiological roles. Some types are mainly present on intracellular organelles (NHE6–9) while others NHE1–5, are mainly plasma membrane proteins. NHE1 is made up of 815 amino acid residues separated into two domains — an N-terminal transmembrane (TM) domain where ion transport is catalyzed and a C-terminal cytosolic domain that regulates the ion transport activity [3].

The major physiological role of NHE1 is regulation of intracellular pH but it is also involved in cell differentiation, cell proliferation, cell volume regulation, cytoskeletal organization and cell migration. In transformed cells, alkalinization mediated by NHE1 plays also an important role in the development of the transformed phenotype and this is prevented when NHE1 is inhibited [4,5]. NHE1 also plays a clear role in mammalian development. Mice with a NHE1 deletion have decreased postnatal growth and increased mortality, ataxia and epileptic seizures [6] and we have recently demonstrated that homozygous expression of a defective NHE1 gene in humans results in disease, with a phenotype including hearing loss and cerebellar ataxia [7].

The structure of the *Escherichia coli* Na^+/H^+ exchanger NhaA [8] and that of NapA from *Thermus thermophilus* has been elucidated [9]. Briefly, the crystal structure of NhaA [8] contained two groups of 6 transmembrane (TM) segments each had two three TM bundles. TMIV and TMX1 make a novel fold with extended non-helical regions that crossed and were thought to contain various charged residues important for ion binding and transport. That protein was in an acid locked state and not active however the structure of NapA was determined in an active state with significant differences from NhaA. A two domain, rocking bundle, alternating access model of sodium proton antiport was hypothesized [9] though elements of this model have been disputed [10]. The structure of another Na^+/H^+ exchanger, the archaeal Na^+/H^+ antiporter NhaP1, has been determined at 7Å resolution and it varies from NhaA with 13 membrane spanning TM segments instead of 12, but the 6 helix bundle structure is conserved and similar to that of NhaA [11].

While significant progress has been made describing the prokaryotic Na^+/H^+ exchanger NhaA structure and on some other primitive types,

Abbreviations: BCECF-AM, 2′,7′-bis(2-carboxyethyl)-5(6) carboxyfluorescein-acetoxymethyl ester; CHO, Chinese hamster ovary; HA, hemagglutinin; NHE1, Na^+/H^+ exchanger type 1 isoform; pHi, intracellular pH; WT, wild type.

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http://dx.doi.org/10.1016/j.bbamem.2015.07.011
0005-2736/

only limited progress has been made on deciphering the structure of the mammalian isoforms of the protein. Mammalian Na^+/H^+ exchangers have little homology to NhaA and a 1:1 stoichiometry, in contrast to the 2:1 stoichiometry of NhaA. The NHE1 isoform is the only mammalian type with significant progress made in direct examination of its structure. While the entire protein is resistant to large scale overexpression and crystallization, we have been able to examine the structure of TM segments by NMR [12–18]. These studies have revealed interesting characteristics of the TM segments that are similar to some segments of NhaA. However, they cannot give a complete picture of the structure of the protein without a better understanding of the overall topology of the protein.

Wakabayashi et al. [19] used cysteine scanning accessibility experiments to make an initial analysis of the topology of NHE1. They suggested a 12 TM model based on the accessibility of the residues tested with the N- and C-terminus in the cytoplasm. They proposed two intracellular loops, between TMs IV–V (amino acids 176–190) and VIII–XI (316–338), which contained amino acids that were accessible extracellularly but were suggested to be intracellular and part of re-entrant loops (Fig. 1A, B). Amino acids 341–362 formed TM segment IX, and extracellular loop 5 that was thought to be associated with the membrane. An alternate 3D model of NHE1 was later proposed based on the structure of NhaA as a template [20]. This model also contained 12 TM helices but had several notable differences. It did not include the first two helices of the model of the Wakabayashi model (1–125), which were thought to be removed by cleavage (Fig. 1A). TMIX (339–359) was assigned as two short helices and a re-entrant segment between TMIX-X is reassigned as TMIX (374–398) (Fig. 1C). This rearrangement of the re-entrant loop placed EL5 (360–410), which had numerous extracellularly accessible residues [19], on the inside of the membrane. They suggested that this loop could be near the pore of the protein, accounting for this accessibility. The last three TMs (411–505) are the same in both models (Fig. 1). More recently Nygaard et al. [21] proposed a model of NHE1 that was based on the NhaA model and on the work of Wakabayashi et al. [19]. The model of Nygaard et al. [21] has also proposed a 12 transmembrane structure of NHE1. The overall two dimensional configuration of this model is similar to that of the Wakabayashi model with the main differences in the membrane segments being the beginning and end of some of the helices. Most of the beginnings and ends of the transmembrane segments were very similar (TM's I–VIII, and X–XII), varying by 2–5 amino acids while TMIX was amino acids 339–359 and 333–353 in these two models, respectively [19,21] (the models were recently reviewed by [22]). Nygaard et al. attempted to verify one part of their model using electron paramagnetic resonance, but their final model suggested that the charged side chain of D172 is critical to NHE1 function and it has been shown that mutation of this residue to N or Q did not impair NHE1 function [23]. Clearly a greater refinement of existing models of NHE1 is necessary and more direct characterization of the structure of NHE1 is necessary. The controversy in the area has continued [24,25].

In the present study, we examined the accessibility of numerous amino acid residues of the mature NHE1 protein, to distinguish between these three models. We studied the regions that are more controversial, the N-terminal amino acids 1–127 and the segment containing amino acids 315–411. Further, we used immunoprecipitation of the mature surface protein and an analysis of the N-linked glycosylation of the protein to study the N-terminal transmembrane segments. The results are consistent with amino acid residues 1–127 forming two transmembrane segments that remain connected to the mature protein, and the segment between amino acid residues 315–411 forming one transmembrane segment as proposed by Wakabayashi et al.'s [19] model.

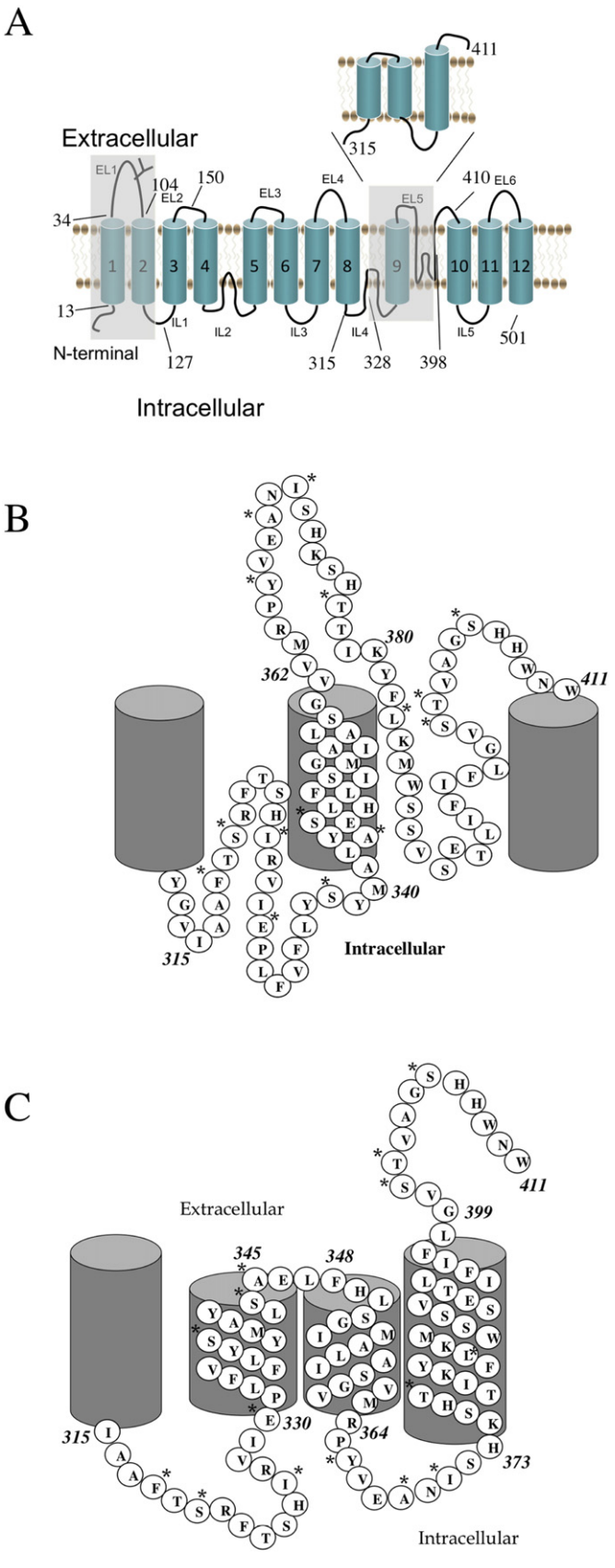

Fig. 1.

Fig. 1. Models of topology of the NHE1 protein. A, Schematic diagram of transmembrane domain of NHE1 protein. Transmembrane segments 1–12 are illustrated together as suggested by Wakabayashi et al. [19,21] (model #1). Shaded areas are regions in dispute in model #2 [20]. Transmembrane segments 1–2 were suggested to be deleted in model #2. An alternate topology of amino acid residues 315–411 is illustrated above from model #2. B and C show detailed alternative topologies of amino acid residues 315–411 models #1 and #2, respectively. EL, extracellular loop; IL, intracellular loop. * indicates amino acid mutated to Cys in the present study.