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Mini-review

Biochemical and biophysical approaches to study the structure and function of the chloride channel (ClC) family of proteins

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ABSTRACT

The chloride channel (ClC) protein family comprises both chloride (Cl^-) channels and chloride/proton (Cl^-/H^+) antiporters. In prokaryotes and eukaryotes, these proteins mediate the movement of Cl^- ions across the membrane. In eukaryotes, ClC proteins play a role in the stabilization of membrane potential, epithelial ion transport, hippocampal neuroprotection, cardiac pacemaker activity and vesicular acidification. Moreover, mutations in the genes encoding ClC proteins can cause genetic disease in humans. In prokaryotes, the Cl^-/H^+ antiporters, such as ClC-ec1 found in *Escherichia coli* promote proton expulsion in the extreme acid-resistance response common to enteric bacteria. To date, structural and functional studies of the prokaryotic protein have revealed unique structural features, including complicated transmembrane topology with 18 α-helices in each subunit and an anion-coordinating region in each subunit. Several different approaches such as X-ray crystallography, NMR, biochemical studies, and molecular dynamics simulations have been applied to the study of ClC proteins. Continued study of the unique structure and function of this diverse family of proteins has the potential to lead to the development of novel therapeutic targets for neuronal, renal, bone, and food-borne diseases.

1. Introduction

Chloride (Cl^-) is the most abundant ion in nature. It is transported by several distinct families of chloride transporting proteins including chloride channels (ClC), ligand gated GABA and glycine receptors, cystic fibrosis conductance transmembrane regulators, cation chloride transporters, and chloride/bicarbonate exchangers. These proteins resides in both the plasma membrane and in the membranes of intracellular organelles, and contribute to a variety of essential processes. Members of the ClC protein family in particular, which includes both Cl^- channels and chloride/proton (Cl^-/H^+) antiporters, participate in many different physiological functions within prokaryotic and eukaryotic organisms. In this review we focus on recent structural and functional insights pertaining to the ClC proteins in particular.

The first ClC protein to be identified was ClC-0, found in the electric organ of the *Torpedo marmorata*, also known as the tropedo fish [1,2]. Since then, ClC proteins have been identified in numerous species of bacteria, plants, and mammals. In the first part of this review, we discuss the mammalian ClC proteins, which have been shown to be vital to cellular function and to play a role in human disease. However, the mechanisms underlying their roles are not well understood. Much of what we know about ClC proteins, their structures and their function, comes from the study of prokaryotic ClC proteins. Thus, in the second part of this review, we discuss the prokaryotic ClC proteins, specifically ClC-ec1, the protein found in *Escherichia. coli* (*E. coli*) that plays a critical role in the bacteria's ability to survive in highly acidic environments for short periods of time. Finally, we review several different approaches currently used to study the structure of ClC-ec1.

2. The mammalian ClC family

2.1. Function and distribution

To date, there are nine known members of the human ClC family. These are categorized into three groups based on their sequence homology (Fig. 1) [1]. The first group comprises the Cl^- channels: ClC-1, ClC-2, and the kidney-specific chloride channels ClC-Ka and ClC-Kb [1]. All four of these proteins are found predominantly in the plasma membrane where they function as gated

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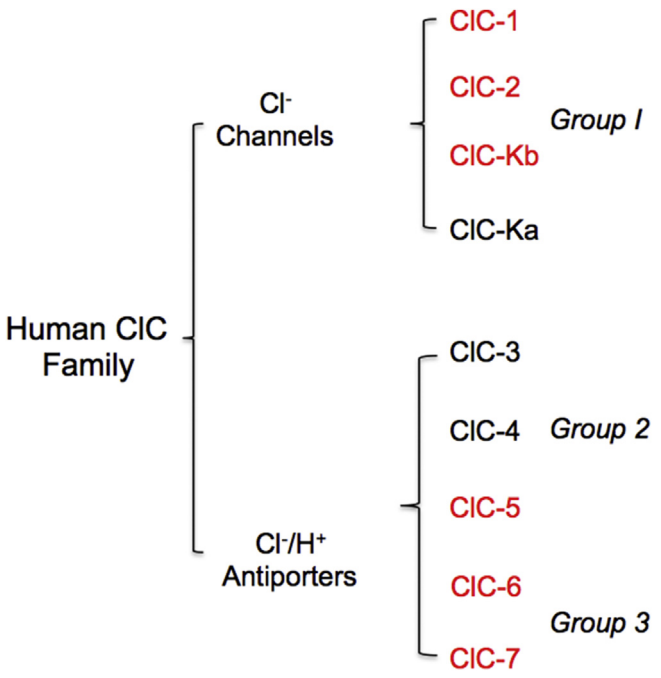

Fig. 1. Human ClC channels and antiporters. The nine members of the ClC protein family are divided into three subfamilies based on their sequence homology (1). The first subfamily consists Cl^- channels while the second and the third consist of Cl^-/H^+ antiporters. Protein shown in red have been associated with human genetic diseases (Table 1).

Cl^- channels and act to stabilize the membrane potential or to mediate epithelial transport [1].

The remaining proteins, classified as group 2 and 3, are electrogenic Cl^-/H^+ antiporters, whose distribution and physiological functions are distinct from the Cl^- channel proteins [1,3–5]. These five proteins (ClC-3, ClC-4, and ClC-5 in group 2; and ClC-6 and ClC-7 in group 3) are predominantly found in intravascular membranes of the endosomal-lysosomal pathway and are only expressed to a limited extent at the plasma membrane [1]. Currently, little is known about the physiological function of ClC-3, ClC-4, and ClC-6. ClC-3 knockout mice exhibit retinal and neuronal degeneration indicating that ClC-3-mediated acidification of synaptic compartments may play a significant role in neuronal stability [6–8]. Moreover, studies in cultured fibroblasts show that ClC-4 is essential for proper trafficking and function of the transferrin receptor [9,10].

The two best-characterized Cl^-/H^+ antiporters so far are ClC-5 and ClC-7. ClC-5 is predominantly expressed in the proximal tubule of the kidney, where it is localized to apical endosomes. ClC-3, ClC-4, and ClC-5 cooperate with the vacuolar-type proton-ATPase (V-ATPase) to facilitate the acidification of endosomes [6,11,12]. Endosomal acidification mediated by group 2 ClC proteins is essential to promote ligand/receptor dissociation, enable early-to-late endosomal maturation, and activate hydrolytic enzymes [13–15]. The precise mechanism of ClC-mediated acidification remains controversial. One proposed scenario is that antiporters may mediate the entry of Cl^- into the endosome, thereby dissipating the positive charge generated by the V-ATPase [6,11,12,15,16]. The group 3 antiporter proteins, ClC-6 and ClC-7, are thought to facilitate protein degradation by regulating Cl^- ion concentration to ensure optimal function of the hydrolytic enzymes of late endosomes and lysosomal compartments [17].

Evidence of a role for ClC proteins in human disease comes from genetic studies linking mutations in these proteins to the occurrence of many hereditary diseases. For example, malfunction of ClC-5 leads to Dent's disease, an X-chromosome-linked disorder associated with proteinuria and the development of kidney stones. Mutations in ClC-7 can cause impaired bone resorption and are linked with several variants of osteopetrosis (Table 1) [18]. Clearly, mammalian ClC channels and antiporters play a vital role in cellular function, but details of the mechanisms involved remain elusive. Clarification of these processes requires a thorough understanding of the behavior of ClC proteins at the molecular level. However, to date most of the molecular level work has focused on prokaryotic proteins, as they are relatively easy to express and purify in milligram quantities compared to their eukaryotic counter parts. Clearly, more studies are needed in order to fully appreciate the role that ClC proteins play in mammalian physiology and pathophysiology.

2.2. ClC channels: structure and gating properties

The structural organization of ClC proteins varies somewhat between protein types found in prokaryotes and eukaryotes, but several key features are conserved. All ClC proteins have a transmembrane catalytic domain and most (all eukaryotic types and a few prokaryotic types) have a cytoplasmic regulatory domain [19]. The cytoplasmic domain appears to be involved in diverse regulatory mechanisms, including oligomerization, protein sorting, channel gating, and ligand binding [20]. In the eukaryotic proteins, the cytoplasmic domain is crucial for protein function [21,22], as mutations in this domain are associated with hereditary diseases, such as myotonia, Dent's disease, osteopetrosis, and Bartter syndrome [23].

Insights into the conductance and gating properties of group 1 ClC proteins came from electrophysiological experiments studying the eukaryotic muscle-type channels ClC-0 (a ClC protein variant found in *T. marmorata*) and ClC-1 [24]. The gating mechanisms of ClC-0 have also been studied via analysis of the behavior of single channels reconstituted in artificial lipid membranes [24]. This work revealed the surprising result that the channel has two equally spaced, binominally distributed conductance states, indicative of two identical, independently-gated pores [24]. Additionally, this study showed that individual gates act on each pore separately, while a separate common gating mechanism can affect both pores simultaneously. We now know that gate opening and closing is determined by three factors: pH, Cl^- concentration, and membrane potential, where pore opening is favored by low pH, high Cl^- concentration, and depolarization of the membrane. While the channel is more likely to open at more depolarized membrane potentials, movement of Cl^- ions into the transmembrane electric field during the opening step can locally alter membrane potential, thereby shifting the voltage-dependence of channel gating [25]. This permeant ion concentration-dependent gating mechanism is profoundly different from that found in classical voltage-gated cation channels. In those channels, gating is rendered voltage-dependent by a “voltage sensor” domain containing several positively charged residues that move within the transmembrane electric field [26].

In ClC channels, the structural determinant of the individual gating process is a single glutamate (E) residue that alternates between occupying the extracellular site for Cl^-, thereby prohibiting ion flow in the closed state, or moving away from this site allowing Cl^- permeation through the selectivity filter when the channel is open. Less is known about the specific structural determinants of the common gating mechanism in this channel. Several experiments have suggested that additional conformational changes underlie the concerted closing of both pores during common gating [27–29]. Biochemical studies have indicated that mutations at the dimer interface of the catalytic domains [30], as well as those occurring in the cytoplasmic domains can influence the functioning